ISSN: 2594-0252

# AGRO PRODUCTIVIDAD

## Morphology and biochemistry of achacha (*Garcinia humilis* (Clusiaceae) (Vahl) C.D. Adam) fruits harvested at three consumption ripeness stages

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Colegio de Postgraduados

# Contenido

Año 16 • Volumen 16 • Número 6 • junio, 2023

AGRICULTURA
SECRETARÍA DE AGRICULTURA Y DESARROLLO RURAL

AGRO PRODUCTIVIDAD

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Año 16, Volumen 16, Número 6, junio 2023, Agro productividad es una publicación mensual editada por el Colegio de Postgraduados. Carretera México-Texcoco Km. 36.5, Montecillo, Texcoco, Estado de México. CP 56264. Tel. 5959284427. www.colpos.mx. Editor responsable: Dr. Jorge Cadena Iñiguez. Reservas de Derechos al Uso Exclusivo No. 04-2017-031313492200-203. ISSN: 2594-0252, ambos otorgados por el Instituto Nacional del Derecho de Autor. Responsable de la última actualización de este número, M.C. Valeria Abigail Martínez Sias. Fecha de última modificación, 26 de julio de 2023.

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# Organic food consumer behavior in Hermosillo, Sonora, Mexico

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**ABSTRACT**

**Objective**: To describe and characterize the behavior of organic food consumers in Hermosillo, Sonora, in order to identify whether this location constitutes a potential market for said products.

**Design/Methodology/Approach**: We asked consumers at points of purchase to answer a questionnaire. Then we analyzed the resulting data with descriptive and inferential statistics.

**Results**: There is a market segment acquainted with organic products and willing to buy them. This segment is unsatisfied with the local offer of fruits and vegetables. The purchase of organic food increases among consumers who care about their diet and health, and who show positive attitudes toward the environment.

**Study limitations/Implications**: The study focuses on a specific geographic area. Therefore, extrapolation of results must be done cautiously.

**Findings/Conclusions**: Overall, there is a potential local demand for organic fruits and vegetables. Adequate marketing and segmentation strategies are necessary to address the identified consumer profile so that traders and farmers can position their products in the market.

**Keywords**: Consumer behavior, Organic food purchasing, Lifestyles.

**Citation**: Romero-Valenzuela, D. M., Camarena Gómez, B. O., & Camarena-Gómez D. M.J. (2023). Organic food consumer behavior in Hermosillo, Sonora, Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i4.2501

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: September 19, 2022.
**Accepted**: February 02, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 3-9.



## INTRODUCTION

Conventional agriculture meets a large part of the world's food demand. This type of agriculture prioritizes monoculture, the use and development of technical agricultural implements, and the employment of synthetic inputs—mainly fertilizers, pesticides, and herbicides (Leaños Luna, 2006; Pichardo, 2006). Although conventional agricultural practices increase productivity (Ortega, 2008), they have been linked to human health damage and numerous environmental problems: wherever this agriculture takes place, it impacts ecosystems and biodiversity (FAO, 2018; WWF, 2018).

The state of Sonora, located in northwestern Mexico, has a remarkable agricultural production. In 2020 it contributed to the production of 44 agricultural products, reaching one of the first three places in production volume for 14 of them and contributing 7% of the value of agricultural production in Mexico (SIAP, 2020). Several researchers have

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analyzed possible environmental consequences derived from agricultural production in the state. The presence of various agrochemicals/pesticides in different environmental matrices (water, soil, and food) as well as in biological samples of the resident population (blood, semen, breast milk) has been proven, even in the case of agricultural fields abandoned for more than ten years (Albert, 2006; Camarena-Gómez *et al.*, 2020; Cantú *et al.*, 2019; Cejudo *et al.*, 2012; Leal *et al.*, 2014; Silveira-Gramont *et al.*, 2018).

To attend to this situation, alternative types of agricultural production are emerging, more respectful of environmental balances and safer for human health. One of these alternatives is organic agriculture (WHO and FAO, 2007). However, moving from one agricultural production model to another is not easy. Demand is one of the main reasons that motivate producers to transition from conventional to organic agriculture (Arias, 2015). Studying the consumer's perspective and behavior toward the organic food sector is therefore relevant. We must note that research in this area is extensive and diverse. The findings establish that purchase intent is related to aspects such as quality, care for the environment, and concern for good health (Bernabéu *et al.*, 2004; De Moura *et al.*, 2012; De Olde *et al.*, 2020; Gutiérrez *et al.*, 2012). Moreover, various factors affect the supply and demand of organic products: the lack of awareness about these products, their overprice compared to conventional foods, and a limited supply or scarce availability (Higuchi, 2015; Rodríguez *et al.*, 2002; Stolz *et al.*, 2011).

In Sonora, we identified at least ten institutions that produce organic food —a production destined mostly for the export market (Gómez *et al.*, 2008). In this context, studying the local demand for organic food and the factors that drive it can provide the necessary incentives for farmers in the region. On the one hand, farmers may feel encouraged to dedicate part of their production to the local market; on the other, conventional producers may want to consider transitioning to another agricultural production model.

Our objective is to describe and characterize the behavior of organic food consumers and, based on this, to find out whether there is a potential market for this production in Hermosillo, Sonora. We intend to do this through the description of their purchases, their willingness to pay, lifestyles, and attitudes toward the environment. With the information generated in this document, we intend to highlight benchmarks on which to focus marketing strategies to position such products in a potential market for organic foods.

## MATERIALS AND METHODS

To fulfill the main objective of our research, we designed a questionnaire aimed at consumers over 18 years old. We obtained the sample using a probabilistic simple random sampling and applying the formula for infinite populations. The sampling error was 6%, and the confidence level was 95.5%. We applied a total of 277 questionnaires at points of purchase where organic products are offered (supermarkets and specialized stores) in the city of Hermosillo, Sonora.

Once we completed the fieldwork, we coded the information and elaborated a database with the IBM SPSS Statistics 25 software. Finally, we proceeded to conduct descriptive and bivariate analysis.

## RESULTS AND DISCUSSION

The socio-demographics show that 71.8% of the sample are women. Of all participants, 70.7% are between 23 and 52 years old. The monthly family income ranges between 10,000 and 25,000 pesos in 64.9% of the cases. Of all participants, 54.4% have higher education; 54.1% are married; 38.9% have one or two children, while 38.2% have none.

Regarding consumption habits, 87% of the participants claimed to know what an organic product was, and 77.6% had bought one. Around 60.8% of consumers buy organic food at least once every two weeks. The points of purchase where they buy these products are supermarkets and specialized stores (37.8% and 34.0%, respectively). In addition, we must note that the most purchased organic foods are vegetables (22.4%) and fruits (13.8%), followed by other processed foods.

However, 44.8% of consumers indicated that they were not satisfied with the current offer of organic fruits and vegetables, mainly because there are not enough places that sell them (45.8%) or due to a lack of variety (15.6%). Consumers buy organic fruits and vegetables because they consider their quality to be superior (30.4%) and appreciate their health benefits (25.7%).

Price is a relevant variable in studies focused on organic certified foods, since their cost is higher than that of products without said certification. Of all participants, 92.5% stated that they would opt for organic fruits and vegetables if their price were the same as that of conventional produce. Were the price higher, only 39.4% would continue to buy them.

To characterize the attitudes of organic food consumers toward the environment, we analyzed the differences between people who had bought organic food in the last twelve months and those who had not. The analysis shows significant differences between the two categories. In the sphere of social awareness, the group that acquired organic products showed more concern regarding the need to prevent environmental degradation (4.59) and stated that the current societal order is destroying nature (4.52). In the sphere of individual awareness, organic food consumers are more willing to collaborate in conservation activities (3.97) and to use selective garbage containers (3.68) (Table 1).

Likewise, we found different lifestyles to produce contrasting behaviors between people who buy organic products and those who do not. Organic food consumers showed higher values regarding their concern for a balanced diet, a good health, and an active and balanced lifestyle (Table 2). In the food category, we found that organic food consumers frequently eat more fruits and vegetables (4.61), maintain a moderate intake of meats (3.91), try to eat foods without additives (3.86), and are mindful of the industrialized foods they consume (3.84). In the health category, we can observe that they are more agreeable to a periodic health check (3.78) and a number of them practice vegetarianism (2.32). In the category of active lifestyles, we noticed that they usually read product labels (4.12), exercise regularly (3.47), and collaborate with an NGO (3.97). Finally, in the balanced lifestyle category, we found that they seek to balance work with private life (4.09), lead an orderly and methodical life (4.01), try to reduce stress (3.96), and visit the dentist regularly (3.46).

**Table 1**. Attitudes toward the environment and purchase of organic products.

| Lifestyles related to attitudes toward the environment | | Purchased organics in the last 12 months | | | |
|---|---|---|---|---|---|
| **Category** | **Statements** | **Yes** | **No** | **F** | **U de Mann-Whitney** |
| Social awareness | If the necessary measures are not taken, the deterioration of the environment will be irreversible. | 4.59 | 4.15 | 10.457 | 0.000* |
| | Today's civilization is destroying nature. | 4.52 | 4.28 | 2.764 | 0.048* |
| | I am concerned about the consequences of human activity on climate change, and I act accordingly. | 4.29 | 4.13 | 1.182 | 0.248 |
| Individual consciousness | I collaborate in environmental conservation tasks. | 3.97 | 3.59 | 4.88 | 0.005* |
| | I throw garbage in selective containers. | 3.68 | 3.07 | 8.25 | 0.004* |
| | I prefer to consume recycled products. | 3.22 | 3.09 | 0.327 | 0.685 |

Note: We use a five-point scale, where 5 equals total agreement and 1 total disagreement.
We performed the Kolmogórov-Smirnov test of normality; all the items had a value of 0.000. Items with a significance lower than 0.050 are considered abnormal.
*Significant association of less than 5% according to the Mann-Whitney U test.
Based in Fraj and Martinez (2002). Prepared by the authors based on the answers to the questionnaires.

**Table 2**. Lifestyles and purchase of organic products.

| Lifestyles | Statements | Purchased organics in the last 12 months | | | |
|---|---|---|---|---|---|
| | | **Yes** | **No** | **F** | **Sig.** |
| Feeding | I frequently eat fruits and vegetables. | 4.61 | 4.04 | 21.5 | 0.000* |
| | I control my salt intake. | 4.04 | 3.83 | 1.463 | 0.234 |
| | I eat red meat in moderation. | 3.91 | 3.42 | 8.303 | 0.014* |
| | I try to eat food without additives. | 3.86 | 3.52 | 4.043 | 0.076** |
| | I try not to eat industrialized foods. | 3.84 | 3.44 | 5.311 | 0.064** |
| Health | I practice a vegetarian diet. | 2.32 | 1.76 | 6.137 | 0.007* |
| | Periodically, I voluntarily check my health. | 3.78 | 3.11 | 12.035 | 0.001* |
| | I visit the dentist regularly. | 3.46 | 2.8 | 12.04 | 0.001* |
| Active | I read product labels. | 4.12 | 3.19 | 31.92 | 0.000* |
| | I exercise regularly. | 3.47 | 2.57 | 20.97 | 0.000* |
| | I collaborate with NGOs . | 3.97 | 3.59 | 8.36 | 0.004* |
| | I belong to an association for the defense of nature. | 1.87 | 1.48 | 3.4 | 0.075** |
| Balance | I try to balance work and private life. | 4.09 | 3.81 | 3.25 | 0.088** |
| | I try to reduce stress. | 3.96 | 3.56 | 5.32 | 0.068** |
| | I try to lead an orderly and methodical life. | 4.01 | 3.48 | 13.06 | 0.002* |

Note: We use a five-point scale, where 5 equals total agreement and 1 total disagreement.
We performed the Kolmogórov-Smirnov test of normality; all the items had a value of 0.000. Items with a significance lower than 0.050 are considered abnormal.
*, ** Significant association of less than 5% and 10% according to the Mann-Whitney U test.
Based in Fraj and Martinez (2002). Prepared by the authors based on the answers to the questionnaires.

Considering the results described above, we can observe that organic consumers in Hermosillo, Sonora, practice healthy lifestyles and have positive attitudes toward caring for the environment.

When comparing our findings with the results of other studies examining consumer behavior toward organic products, we were able to observe some similarities. Such is the case of the quality that consumers perceive in organic foods, an element that is a strong motivator for purchase (Basha *et al.*, 2015; Escobar *et al.*, 2015; Hempel, 2016). Likewise, health benefits constitute a leading purchase motivator, which is reflected in healthy lifestyles. Organic food consumers tend to practice healthy eating and review their health status (Bostan *et al.*, 2019; Chattopadhyay and Khanzode, 2019; Eisinger-Watzl *et al.*, 2015; Farías, 2018; Kranjac *et al.*, 2017; Thais, 2018). Coinciding with Chiciudean *et al.* (2019) and Kranjac *et al.* (2017), the results show that fresh fruits and vegetables are organic foods in demand. Consumers choose them mainly for health concerns and because they contain fewer chemicals. The limitations for their purchase are usually high prices and insufficient distribution channels.

Finally, compared to those who do not buy organic food, people who usually buy it showed more positive attitudes toward the environment (individual and collective awareness).

## CONCLUSIONS

Our findings allowed us to characterize the consumers of organic products in Hermosillo, Sonora, Mexico, through the analysis of behavior, lifestyle, and attitudes toward the environment. The results confirm the existence of a market segment that consumes organic products in Hermosillo, Sonora. This market segment is unsatisfied with the local offer and represents an opportunity for businesses in the city, as well as for farmers in the region, who can feel motivated by buyers to transition to an organic agricultural production scheme. This research broadens our knowledge of consumer behavior regarding organic products. Sonoran consumers are driven to buy organically certified foods, especially fruits and vegetables, due to the quality and health benefits they perceive in them. In addition, these consumers have healthier lifestyles and more positive attitudes toward environmental conservation. We have established the existence of a potential market for organic fruits and vegetables in Hermosillo, Sonora. This latent demand can be expanded with appropriate marketing strategies that support traders and farmers to position their products in the market. To achieve this, providing information about the behaviors that characterize consumers of organic products and their purchase motivations is crucial. Efforts must therefore focus on the following: 1) Reinforcing contents related to caring for the environment; for example, displaying information regarding the production stages of the goods, such as the reduction of adverse effects on the environment derived from avoiding synthetic inputs (compared to conventional production). 2) Highlighting attributes related to health, *e. g.* the reduction of health risks linked to the continued use of agrochemicals in the conventional production process. 3) Segmenting the population according to lifestyle characteristics regarding food, concern for good health, and the willingness to sustain an active and balanced life.

We must note that this study is limited to a specific geographical area, so that extrapolating the results should be done cautiously. Our research provides some guidelines for further exploration. It is possible to expand the geographical scope and the extent of analysis, in addition to integrating more variables, such as consumers' health and innovations in organic products, among others.

## ACKNOWLEDGEMENTS

The authors would like to thank the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for the support granted for conducting this research.

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Colegio de Postgraduados

# Embryo development after ICSI, using spermatozoa from bovine testicular tissue treated with three membrane-destabilizing agents

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**ABSTRACT**

**Objective**. To determine the differences in the embryo development of bovine oocytes fertilized with frozen/thawing (F/T) spermatozoa or with the intracytoplasmic sperm injection (ICSI) of F/T, spermatozoa from fresh testicular tissue (FTT), and cryopreserved testicular tissue (CTT), using three spermatozoa membrane-destabilizing agents.

**Methodology**. Four treatments were used. Treatment (TRT-1): *In vitro* fertilization (IVF) with F/T. TRT-2: ICSI with F/T. TRT-3: ICSI with FTT. TRT-4: ICSI with CTT. The spermatozoa membranes were destabilized with Triton X-100 (TX), Lysolecithin (LL), and Heparin-Glutathione (Hep-GSH). Embryonic division was recorded at 48 h and grade 1 and 2 blastocysts (BL) were recorded 8 days (D8) after the fertilization. The means were compared using Fisher's least significant difference method.

**Results**. At D8, the blastocysts formation between ICSI treatments (F/T 13±3, FTT 6±3, and CTT 6±3, $p>0.05$) were lower than control (IVF 23±5). There was a lower cleavage at 48 h using Hep-GSH than when LL and TX were used (35±5 *vs.* 50±5 and 56±5, $p<0.05$). Embryo division at 48 h obtained better results with the ICSI+F/T and LL treatment, while the highest blastocyst percentage at D8 was obtained using TX.

**Conclusions**. Blastocysts can be produced through ICSI, using spermatozoa from fresh or cryopreserved testicular tissue. The spermatozoa treated with TX and LL produced a higher percentage of BL than the spermatozoa treated with Hep-GSH. Further experiments should be carried out using spermatozoa obtained from different sources, in order to improve embryo development after the ICSI.

**Keywords**: ICSI, vitrification, testicular tissue, oocytes, bovine, fertilization.

**Citation**: Lepe-Anasagasti, I. E., Canseco-Sedano R., Zárate-Guevara, O. E., Pardio-Sedas V. T., Domínguez-Mancera B., Cruz-Romero, A., Rosendo- Ponce, A., Vega-Murillo, V. E., & Contreras-Hernandez G. (2023). Embryo development after ICSI, using spermatozoa from bovine testicular tissue treated with three membrane-destabilizing agents. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2507

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: February 08, 2023.
**Accepted**: March 23, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 11-18.



## INTRODUCTION

*In vitro* embryo production (IVP) has benefited genetic improvement and it has also been a research model for the embryo development of several species (Diaza *et al*., 2013). IVP includes *in vitro* maturation (IVM), in vitro fertilization (IVF), and *in vitro* embryo culture (IVC). Every step is equally important for the production of embryos with a high implantation potential (Consensus Group, 2020). Devroey and Steirteghem (2004) pointed out that the intracytoplasmic sperm injection (ICSI) enables fertilization, even when there are few spermatozoa or they lack motility. This is the case when the spermatozoa come from bad quality testicular spermatozoa or semen.

Image by Steffen Erbe at Pixabay

The *in vitro* fertilized oocytes percentage in cattle is usually high; however, the fertilization rate using ICSI is lower than in other species (Canel *et al*., 2017; Canel *et al*., 2018; Ressaissi *et al*., 2021). Some of the events that can cause a drop in fertilization include defective sperm head decondensation, inadequate sperm capacitation, and the integrity of the spermatozoa membrane (Rho *et al*., 1998, Sekhavati *et al*., 2012, Arias *et al*., 2015, Águila *et al*., 2017). One of the main problems with defective sperm head decondensation is the inconsistent reduction of the disulfide double bond of the protamine in the spermatozoa nucleus that takes place after the fertilization (Malcuit *et al*., 2006).

Sutovsky *et al*. (1997) and De Matos and Furnus (2000) reported that the glutathione (GSH) is a reducing agent of the disulfide double bond and plays a fundamental role in the defective sperm head decondensation of the spermatozoon and the formation of the pronucleus during fertilization. Meanwhile, heparin (Hep) accepts and releases protamine into the DNA, consequently, helping the decondensation of chromatin (Delgado *et al*., 1999 and Romanato *et al*., 2003).

Another factor that can damage the cattle embryo development produced with ICSI is the following: when whole spermatozoa are injected in the oocyte, the enzymes in the acrosome are released into the ooplasm and can damage the organelle. Various strategies have been evaluated in order to remove the acrosomal membrane and consequently to facilitate the DNA decondensation and the formation of the male pronucleus. Zambrano *et al*. (2016) reported that the destabilization of the spermatozoa plasm membrane and the release of the acrosomal content through the use of Lysolecithin (LL) and Triton X (TX-100) improved development, without impacting the quality of cattle embryos produced with ICSI.

The above mentioned experiments used ejaculated spermatozoa. However, there is scarce information about the use of these destabilizing agents for the treatment of fresh or cryopreserved sperm cells from testicular tissue. Therefore, the objective of this study was to determine the fertilization rate and the development of cattle oocytes fertilized with ICSI, using testicular spermatozoa treated with Hep-GSH, LL, and TX.

## MATERIALS AND METHODS

### Sampling collection

The testicles were obtained from a slaughterhouse. They were dissected in their middle area, obtaining approximately 1.0 g of tissue. This sample was kept in a HTF medium, supplemented with 10% human serum albumin until it was used or cryopreserved. The cryopreserved semen of high-fertility bulls was donated by Genética Bovina de México S.A. de C.V.

### *In vitro* maturation of oocytes

The 3-6 cm wide follicles were aspirated (Seita and Kashiwazaki, 2009; Gupta *et al*., 2016) and placed in a BO-HEPES medium, supplemented with 2 $\mu$l/mL of heparin (HP, PiSA, Guadalajara, México). Cumulus oocyte complexes (COC) with dark and homogeneous cytoplasm and at least 2 cellular layers of the cumulus were selected for IVM (Hansen, 2013). The selected COC were washed with BO-HEPES and were transferred to a BO-IVM medium (IVF Bioscience®, Monterrey, NL, Mexico). They were incubated for 23 h, at 38.5 °C, in an atmosphere with 6% $CO_2$ and 100% humidity. Maturation was evaluated using the cumulus cell expansion, cytoplasm appearance, zona pellucida integrity, and the presence of a polar body (Gupta *et al.*, 2016).

**Preparation of the spermatozoa and in vitro fertilization (IVF)**

After the IVM, the COCs were placed in BO-INF droplets (IVF Bioscience®, Monterrey, NL, Mexico) and were cultivated at 38.5 °C, in an atmosphere of 6% $CO_2$ and 100% humidity. Regarding sperm capacitation, the fertilization straws were defrosted for 45 s in water at 37 °C. The semen was centrifuged at 400 G for 5 minutes (Hansen, 2013). The sediment was reconstituted in 200 $\mu$l of BO-IVC (IVF Bioscience®, Monterrey, NL, Mexico) and the concentration was adjusted to $6\times10^6$ spermatozoa/mL. Ten $\mu$L of this semen dilution were added to each BO-IVF droplet (5 oocytes per droplet). Afterwards, they were cultivated during 22 h, at 38.5 °C, in an atmosphere of 6% $CO_2$, 5% $O_2$ (balanced with nitrogen), and 100% humidity (Gupta *et al.*, 2016).

**Testicular tissue (TT) cryopreservation**

The TT was diluted with Tryladil® (Minitube, Querétaro, Mexico). They were aspirated with 0.25-ml French straws, sealing the straws with polyvinyl chloride dust (PVC). The straws were kept at a 5-8 °C temperature for 4 h. Subsequently, they were put in nitrogen vapor for 20 min, before being finally immersed in liquid nitrogen.

**Testicular tissue (TT) defrosting**

The straws were defrosted for 1 minute using water at 37 °C. The TT portions were washed in HTF-Hepes (In Vitro Care®, Frederick, MD, EUA) at 37 °C. Subsequently, they were placed in a HTF medium supplemented with 10% synthetic serum substitute (SSS) (Irvine Scientific®, García, NL, Mexico). Afterwards, the TT portions were cut using 22" gauge needles and their content were extracted pressing the seminiferous tubules (ST) against the bottom of a Petri dish. The ST content (20 $\mu$l) was placed in a Petri dish; it was covered with mineral oil and placed in a Diaphot 300 inverted microscope (Nikon®, New York, NY, USA), set at 200X, in order to locate the spermatozoa.

**Spermatozoa treatment**

**Heparin (Hep) -Glutathione (GSH)**

The frozen-defrosted spermatozoa and the spermatozoa from fresh or cryopreserved TT were placed in a BO-SEMEN PREP medium (IVF Bioscience®, Monterrey, NL, Mexico), supplemented with 80 $\mu$M Hep and 15 mM GSH, in 20-$\mu$L droplets covered for 20 h with mineral oil, at 38.5 °C, in 6% $CO_2$, before the ICSI process (Canel *et al.*, 2018).

**Lysolecithin (LL) and Triton X-100 (TX-100)**

The spermatozoa were incubated in BO-SEMEN PREP, supplemented with 0.05% LL or TX (Sigma-Aldrich, Toluca, Mexico). The spermatozoa were stirred for 1 min in a vortex (Zambrano *et al.*, 2016). Once all the treatments had ended, the spermatozoa were centrifuged at 400 g for 5 min and the sediment was reconstituted with 200 $\mu$L of BO-SEMEN PREP.

**Intracytoplasmic sperm injection (ICSI)**

The ICSI was carried out using an inverted microscope equipped with a micromanipulator and ICSI needles (Zambrano *et al.*, 2016). The injected oocytes were activated placing them in an HTF-SSS medium, supplemented with 7% ethanol for 5 min. Subsequently, the oocytes were washed 10 times in a BO-IVC medium and were cultivated in the same medium at 38.5 °C, in 6% $CO_2$, 5% $O_2$ (balanced with $N_2$), and 100% humidity.

**Embryo culture**

After a 20 h period of fertilization, the presumed zygotes of the control treatment (conventional IVF) were washed with BO-IVC and were cultivated in 30 $\mu$L droplets at 38.5 °C, in 6% $CO_2$, 5% $O_2$ (balanced with $N_2$), and 100% humidity. The injected oocytes were placed in cultivation right after the ICSI and the activation were carried out (Zambrano *et al.*, 2016).

**Embryo development evaluation**

Forty-eight hours after the fertilization (early cellular division), the divided embryos were transferred to fresh 30 $\mu$L BO-IVC drops and were cultivated for 144 h in groups of 5. Embryo development was evaluated at 120 and 144 h after the first evaluation. The final scores were recorded according to the AETA classification (Barfield, 2015).

### Statistical analysis

The data were analyzed using a general lineal model, assuming a binomial distribution for the dependent variables (embryo development in D2 or D7-8). The model included a Probit function for the main effects (destabilizers) and their interactions.

## RESULTS AND DISCUSSION

Regarding TRT-1 (IVF conventional treatment), the percentages recorded for embryo division and blastocyst development were 61% and 23%, respectively. These results are similar to those obtained by Rizos *et al.* (2001), who recorded 77% (embryo division) and 44% (blastocyst development) results.

The statistical analysis showed significative differences in the embryo development at D2; however, this was not the case in D7-8 for the embryos produced by ICSI. Therefore, the spermatozoa source did not have a significative impact on embryo development until the blastocyst stage (after the ICSI). This would seem to be the first experiment aimed to compare fresh or cryopreserved testicular tissue (TT) spermatozoa in cattle. ICSI —carried out using TT spermatozoa— is a treatment frequently used to deal with human infertility. Additionally, embryos produced by ICSI, using testicular spermatozoa from men that suffer cryptozoospermia or necrozoospermia, had a better implantation and pregnancy

**Table 1**. Means of least square and standard error (SE) for the cellular division in D2 and embryo development in D7-8, indicating the right standardization of the IVF technique to produce *in vitro* embryos.

| Source of spermatozoid | Ovocyte (n) | % Cellular division 48 h | % Development D8 |
|---|---|---|---|
| FIV (control) | 64 | $61\pm6^{a}$ | $23\pm5^{a}$ |
| ICSI F/T | 113 | $57\pm5^{a}$ | $13\pm3^{b}$ |
| ICSI FTT | 96 | $43\pm5^{b}$ | $6\pm3^{b}$ |
| ICSI CTT | 92 | $41\pm5^{b}$ | $6\pm3^{b}$ |

[a,b] means with different superscripts are different ($P<0.05$). F/T=Frozen-defrosted; FTT=Fresh testicular tissue; CTT=cryopreserved testicular tissue.

rates than the embryos produced by ICSI using ejaculated spermatozoa (Negri, 2014; Kang *et al.*, 2018). Some authors have proposed that spermatic DNA does not completely condense when entering the epididymis and, therefore, is more sensitive to oxidative stress. If, for whatever reason, oxygen reactive species are found in the epididymis, the DNA of the sperm will be damaged, causing a low fertilization rate. In this case, the immature spermatozoa of the seminiferous tubules would have greater chances to fertilize an oocyte and produce a viable embryo, since they have not been exposed to oxidative stress (Kang *et al.*, 2018).

In this experiment, when TX was used as membrane-destabilizing agent, 56% of the oocytes recorded cellular division 48 h after the ICSI (Table 2). These results are lower than the control group (61%), higher than the Hep-GSH group (35%), and similar to the LL group (50%).

Zambrano *et al.* (2016) reported a 66% division rate, 72 h after the ICSI. The discrepancy between the results of this experiment and the findings of those authors could be the result of the moment in which the embryo development was evaluated (48 *vs.* 72 h) and the influence of the oocyte activators (ethanol vs cycloheximide-ionomycin). In order to carry out the ICSI, the acrosomal membrane enters the injected oocyte, which can cause damage and a low development rate. LL has had positive effects, removing the acrosomal membrane of the spermatozoa of different species (Kang *et al.*, 2018).

There were no significative statistical differences in the interaction of the destabilizing agent with the sperm source (Table 3). Previously, Seita and Kashiwazaki (2009) reported a 10% blastocyst development in rats, when their spermatozoa were treated with LL. These

**Table 2**. Means of the least square and standard error (SE) for embryo division on D2 and embryo development on D7-8 (destabilizing agent).

| Agent destabilizing | Cellular division D2 | Development D 7-8 |
|---|---|---|
| FIV | $61\pm6^{a}$ | $23\pm5^{a}$ |
| ICSI-Hep-GSH | $35\pm5^{c}$ | $5\pm2^{b}$ |
| ICSI-LL | $50\pm5^{b}$ | $6\pm3^{b}$ |
| ICSI-TX | $56\pm5^{b}$ | $14\pm3^{b}$ |

[a,b] means with different superscripts are different ($P<0.05$). Hep-GSH=heparin-glutathione; LL=lysolecithin; TX=Triton X-100.

**Table 3**. Means of the least square and standard error (SE) for the treatment × embryo development* interaction, after the ICSI.

| Source of spermatozoid | Destabilizing | Ovocyte (n) | División celular of 48 h | Development to D 7 y 8 |
|---|---|---|---|---|
| F/T | Hep-GSH | 34 | 47±9 [a,b,c] | 12±6 [a,b] |
| F/T | LL | 38 | 63±8 [a] | 11±5 [a] |
| F/T | TX | 41 | 61±8 [a] | 17±6 [a,b] |
| FTT | Hep-GSH | 30 | 27±8 [b] | 3±3 [a] |
| FTT | LL | 32 | 56±9 [a,c] | 6±4 [a] |
| FTT | TX | 30 | 47±9 [a,b,c] | 10±5 [a,b] |
| CTT | Hep-GSH | 31 | 32±8 [b,c] | 3±3 [a] |
| CTT | LL | 31 | 32±8 [b,c] | 3±3 [a] |
| CTT | TX | 34 | 59±8 [a] | 15±6 [a,b] |
| FIV | | 64 | 61±6[a] | 23±3[b] |

* There were no significative differences (P>0.05). F/T=Frozen-Defrosted; FTT=Fresh testicular tissue; CTT=Cryopreserved testicular tissue; Hep-GSH=heparin-glutathione; LL=lysolecithin; TX=Triton X-100.

results match the findings of this study for the ICSI using F/T spermatozoa treated with LL (10% blastocyst development).

LL not only had a positive effect as membrane-destabilizing agent on the F/T spermatozoa, but also on the spermatozoa from the FTT or CTT: (56, 6, 32 y 3% for early cleavage and blastocyst formation for FTT and CCT respectively). This is a potential opportunity to produce viable cattle embryos from testicular spermatozoa that have not reached their final maturation and have not developed motility. Additionally, this tissue can be cryopreserved and thawed, before the spermatozoa are extracted. Subsequently, they can be treated with membrane destabilizing agents and the oocytes can be fertilized with ICSI, in order to produce cattle blastocysts. The capacity of these blastocysts to achieve a complete term gestation when transferred to the uterus of a recipient cow remains to be elucidated.

Under the conditions of this experiment, Hep-GSH seems to be a less efficient spermatozoa membrane-destabilizing agent than TX or LL. When the ICSI was carried out using F/T spermatozoa treated with Hep-GSH, the percentage of embryos that recorded early cellular division in D2 (47.0%) was lower than the results obtained by Sekhavati *et al.* (2012; 74-83%). However, our result was similar to that of Canel *et al.* (2017; 60.8%). This situation can be the result of the time during which the spermatozoa were incubated with Hep-GSH in this study (20 h), compared with the incubation time with GSH (7 h) reported by Zambrano *et al.* (2016). Perhaps 20 h was an excessive period, resulting in an increase of the spermatic DNA fragmentation, which affected embryo division.

Finally, the percentage of embryos that reached blastocyst stage from those that cleaved at 48 h was similar to that published in other studies (Canel *et al.*, 2018; Sekhavati *et al.*, 2012). This situation suggests that the prolonged exposition of the spermatozoa to Hep-GSH could impact the embryo division, but not the blastocyst development.

Further studies are required to improve the efficiency of these techniques for two main reasons. On the one hand, this technique could be used to obtain testicular tissue from genetically valuable bulls that have to be slaughtered for one reason or another and whose sperm could not have been previously frozen. On the other hand, results like the ones obtained in this study open the possibility of applying this technique to preserve endangered species.

## CONCLUSIONS

Cattle spermatozoa from fresh or cryopreserved testicular tissue can be used to fertilize an oocyte by ICSI and can promote development until the blastocyst stage. Under the conditions of this experiment, TX and LL were better membrane-destabilizing agents than Hep-GSH. Further experiments with spermatozoa from different sources are required, in order to improve embryo development after the ICSI.

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Colegio de Postgraduados

# The impact of extensive grazing in the behavior of soluble sugars in *Prosopis laevigata* (Humb. & Bonpl. ex Willd.) M.C. Johnst. trees

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**Citation**: Armijo-Nájera, G., Valenzuela-Núñez, L. M., García-De la Peña, C., Veliz Deras, F. G., Hernández-Herrera, J. A., Carrillo Moreno, D. I. (2023). *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2512

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: September 29, 2022.
**Accepted**: February 13, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 19-23.



**ABSTRACT**

**Objective**: To evaluate the impact of extensive grazing in the accumulation of soluble sugars in *Prosopis laevigata* trees, whose leaves and fruits are directly consumed by cattle.

**Design/Methodology/Approach**: The *ejido* Emiliano Zapata in Durango was the study area. Stem and root samples were collected from a stand of extensive grazing and a stand without cattle. The sampling was carried out in three growth stages: March (flowering), June (fruition), and October (leaf fall). The samples were frozen in liquid nitrogen and were lyophilized. Afterwards, they were ground and 10 mg of dry matter were weighted in microtubes. The total soluble sugars (TSS) concentration was determined following the Van Handel methodology, using a spectrophotometer at 625 nm. The statistical analysis was carried out using an ANOVA and the Tukey's test.

**Results**: In March, the grazing area had lower TSS concentrations during regrowth than the area without grazing, both at root and stem levels.

**Study Limitations/Implications**: The intensity of grazing and the pasture rotation should be regulated to favor carbohydrate accumulation in trees, which is required for the formation of the meristematic tissues.

**Finding/Conclusions**: Extensive grazing has an impact on the synthesis and accumulation of TSS in mesquite trees. Therefore, the consumption of branches, leaves, and fruits decreases TSS concentrations in the stem and the root.

**Keywords**: vegetative storage, carbohydrates, mesquite tree, herbivory.

## INTRODUCTION

The survival of trees is linked to the ordered and periodical accumulation of photosynthetic products and their related compounds (*e.g.*, carbohydrates, fats, and nitrogen compounds). These elements are accumulated during favorable periods and are mainly stored in winter. Subsequently, they are transported inside the plant to generate growth and reproduction

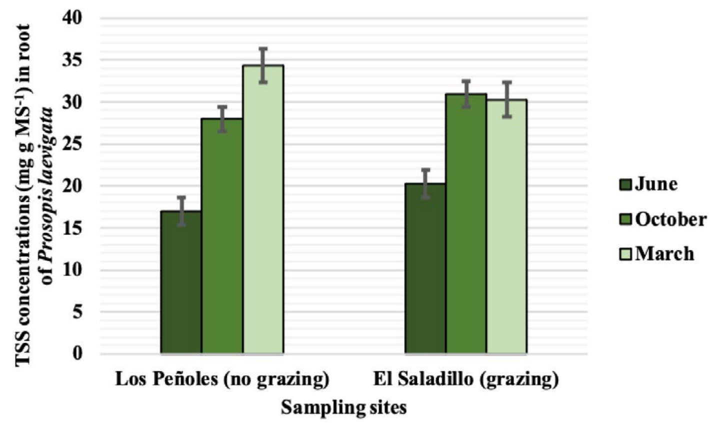
Image by Emilian Robert Vicol at Pixabay during the flowering and fruition stages (Kramer and Kozlowski, 1979; Magel *et al.*, 1997). Carbohydrates are essential for the survival of vegetables. They regulate the physiology of trees in the face of droughts, grazing, or other environmental stress factors (Liu *et al.*, 2019; Pinkard 2018; Tixier *et al.*, 2019; Furze *et al.*, 2019). Therefore, TSS are non-structural carbohydrates, whose function is to provide the carbon and the energy that trees require to maintain their metabolism during winter. They are also needed after the defoliation of the dormant season, because in early spring they form meristematic tissues, which in their turn form new tissues. Consequently, they are responsible for vegetative growth (Hennion *et al.*, 2019; Valenzuela-Núñez *et al.*, 2019). The objective of this study was to evaluate the impact of intensive grazing in the accumulation of total soluble sugars in *P. laevigata* trees, whose leaves and stems have been directly consumed by cattle.

## MATERIALS AND METHODS

The study was carried out in the ejido Emiliano Zapata, Cuencamé, Durango, located at 24° 25' 53.09" N and 103° 50' 41.28" W, at 2,020 m.a.s.l. Stem and root samples were obtained from four trees per pasture. The places chosen for the sampling were El Saladillo (extensive grazing) and Los Peñoles (without grazing). The sampling was carried out in three growth stages of *P. laevigata* trees (March=flowering; June=fruition; and October=leaf fall). The samples were frozen with liquid nitrogen and were lyophilized (Labconco Freezone Triad Freeze Dry Systems®). Afterwards, they were ground (Pulverisette 15 Fritsch®) and 10 mg of dry matter were weighted in microtubes. The total soluble sugars (TSS) concentration was determined following the Van Handel methodology (1968), using a spectrophotometer at 625 nm (Thermo scientific® Genesys 20) and taking sucrose as standard. The statistical analysis consisted of a mean comparison test (Tukey's test with a significance level of $P \leq 0.05$), using the SSPS® Statistics 20.0 package (IBM®, 2018).

## RESULTS AND DISCUSSION

The TSS concentration in the root of *P. laevigata* showed that the area subjected to grazing had differences in TSS concentrations during March and June ($F=11.741$; g.l.=11; $p \leq 0.001$), while, in the area without grazing, the TSS concentrations were different during the three months under study: March had the highest TSS concentration ($F=179.86$; g.l.=11; $p \leq 0.001$). This difference in the behavior of the accumulation of TSS —both in the phenological stage (Figure 1) and between areas (Table1)— can be the result of direct grazing, because the shoots of the tree have less time to produce new photosynthetic structures (*e.g.*, branches and leaves). This situation causes a decrease in the synthesis and accumulation of TSS that guarantees regrowth at the beginning of next year's sprouting (Piper and Fajardo 2014; Klein *et al.*, 2016). Additionally, they supply the demand of TSS for the development of new tissues during March, at the moment of regrowth and flowering (Hoch 2015). The grazing area had lower TSS concentrations (both in root and stem) than the area without grazing, at the moment of regrowth (March). These results match the findings of Kossola *et al.* (2001) and Endrulat *et al.* (2016), who reported that browsing and herbivory decrease the carbohydrates concentration in trees without chlorophyll functions.



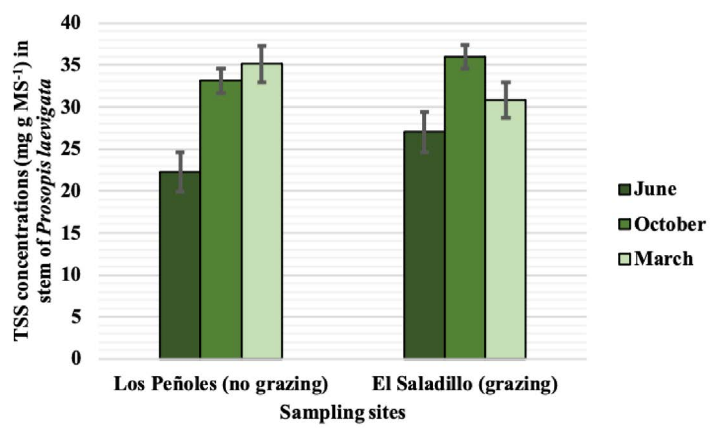




**Figure 1**. Total soluble sugars (TSS) concentrations in root (first image) and stem (second image) of *P. laevigata* from two areas of Durango, Mexico. One of the areas was used for extensive grazing (El Saladillo), while the other (Los Peñoles) was not used for grazing during the three months of the study. a,b,c: different letters indicate significative monthly differences between organs in each area (Tukey $p \leq 0.05$).

**Table 1**. Total soluble sugars (TSS) concentration in the root and stem of *P. laevigata* trees, in two areas of Durango, Mexico. One of the areas was used for extensive grazing (El Saladillo), while the other (Los Peñoles) was not used for grazing during the three months of the study.

| Phenological stage | Los Peñoles (no grazing) | El Saladillo (grazing) |
|---|---|---|
| Root | | |
| March (flowering) | $34.28 \pm 3.40^{a,A}$ | $30.28 \pm 3.34^{b,B}$ |
| June (fructification) | $16.98 \pm 4.93^{a,A}$ | $20.25 \pm 4.62^{b,B}$ |
| October (leaf fall) | $27.95 \pm 7.15^{a,A}$ | $30.95 \pm 5.39^{a,B}$ |
| Stem | | |
| March (flowering) | $35.11 \pm 2.78^{a,A}$ | $30.80 \pm 3.81^{b,B}$ |
| June (fructification) | $22.27 \pm 6.19^{a,A}$ | $27.04 \pm 6.73^{b,B}$ |
| Octuber (leaf fall) | $33.11 \pm 4.22^{a,A}$ | $35.97 \pm 2.40^{a,B}$ |

a, b: Different low-case letters indicate significative monthly differences between organs.
A, B Different capital letters indicate significative monthly differences between areas ($p \leq 0.05$).

However, in June (the maximum vegetative growth season), the grazing area had higher TSS concentrations, both in root and stem, than the area without grazing. This behavior is the response of trees whose branches, leaves, and fruits are consumed and matches the results of Palacio *et al.* (2012), Piper *et al.* (2015), and Puri *et al.* (2015). Additionally, Piper *et al.* (2015), Puri *et al.* (2015), and Schmid *et al.* (2017) pointed out that, during the maximum vegetative growth season, trees subjected to herbivory tend to store carbohydrate in the reserve organs as a priority. This behavior was also observed in this study for *P. laevigata*, during the maximum vegetative growth season (June).

Analyzing which sugars are transported from the stem and the root is important. Additionally, in the case of the fruits and seeds of mesquite, sucrose is the sugar that is transported inside young plants and functions as a reserve for the growing of seedlings (Gallão *et al.*, 2017).

## CONCLUSIONS

Extensive grazing has an impact on the synthesis and accumulation of TSS in mesquite trees. Therefore, the consumption of branches, leaves, and fruits decreases TSS concentrations both in the stem and the root. Consequently, regulating the intensity of grazing and pasture rotation is fundamental to favor the accumulation of carbohydrates required for the formation of meristematic tissues.

## ACKNOWLEDGEMENTS

The authors would like to thank the CONACyT, for the support provided to Mrs. Gabriela Armijo Nájera (MSc) for her ScD studies. They would also like to thank the authorities of the ejido Emiliano Zapata, Durango, Mexico and Julia Núñez Contreras, who facilitated the field sampling.

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# Antibacterial activity of the *Calendula officinalis* L. essential oil on *Escherichia coli*

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**ABSTRACT**

In developing countries, the risk of getting sick from eating food contaminated with *Escherichia coli* is very high. As a consequence of the multidrug resistance of this bacterium, a therapeutic alternative has been sought in the plant kingdom. The **Objective** of this research was to evaluate the antibacterial effect of pot marigold (*Calendula officinalis*) essential oil (EO) on the growth of *E. coli*. **Design/Methodology/Approach**: The antibacterial activity was determined using a Kirby-Bauer disk diffusion susceptibility test. A 90 to 60% dilution of EO generated 24 to 22 mm halos. The EO was subjected to a GC/MS analysis. The **results** showed that cadinene (53.8%) was the main constituent, followed by germacrene (22.5%). The minimum inhibitory concentration was 7 $\mu$g mL$^{-1}$. **Findings/Conclusions**: *C. officinalis* EO can be considered as an option in the treatment against this enterobacteria.

**Keywords**: *Calendula officinalis*, pot marigold, antimicrobial resistance, essential oil (EO), antibacterial compound.

**Citation**: Ruíz-Posadas, L. del M., Rodríguez-López, V. A.[†], Cadena-Iñíguez, J., Delgadillo-Martínez, J., San Miguel-Chávez, R., Salazar-Aguilar, S., & Valdez-Carrasco, J. (2023). Antibacterial activity of the *Calendula officinalis* L. essential oil on *Escherichia coli*. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2520

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 19, 2022.
**Accepted**: February 17, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 25-31.



## INTRODUCTION

Infectious diseases are a public health problem whose frequency and lethality are increasing by leaps and bounds and is therefore considered as one of the greatest threats to world health, food security, and development (WHO, 2020). The pathogenic microorganisms' antibiotic resistance is a consequence of the indiscriminate or wrong use of drugs and the presence of natural processes that produce mutations in bacteria and fungi (Aslam *et al.*, 2018). Antibiotic resistance also leads to a great increase in the costs of the health sector, because the number of patients admitted to hospitals and, therefore, the number of medicines supplied have increased (Dadgostar, 2019).

Image by Mareefe at Pixabay

Antibiotics only kill sensitive bacteria. Resistant bacteria survive, reproduce, and thrive by natural selection (Aslam *et al.*, 2018), creating multidrug-resistant microorganisms. *Escherichia coli* is part of the common gut flora found in the intestine of humans and animals. Nonetheless, it is one of the main agents of infections transmitted by water, food, and direct contact with infected people. Approximately 30% of the bacteremia cases in hospitals are caused by this bacterium —for example, a 71% increase in the bloodstream infections reported by the European Antimicrobial Resistance Surveillance Network. This figure is responsible for approximately 280 million cases of diarrhea and bloodstream infections and 400,000 deaths every year, most of them among young children from all over the world (Salame-Khouri *et al.*, 2018; Steffen *et al.*, 2015). In addition, the antibiotic resistance of some *E. coli* strains has been detected in all parts of the world and is one of the major public health challenges. In Mexico City, multidrug-resistant strains of *E. coli* have been reported: 80% are resistant to rifaximin, 30% to ampicillin, and 50 to 90% to fosfomycin, trimethoprim-sulfamethoxazole, neomycin, furazolidone, chloramphenicol, and ciprofloxacin (Novoa-Farias *et al.*, 2016).

This situation, as well as the side effects of certain antibiotics, have encouraged researchers to find new antimicrobial agents in phytotherapy, especially in plants which have been traditionally used in Mexico. *Calendula officinalis* L. is an herbaceous plant of the Asteraceae family that contains phytochemicals with antibacterial activity, including flavonoids, saponins, carotenoids, triterpenoids, and tannins. Therefore, the purpose of this work was to research the effect of pot marigold EO on a multidrug-resistant strain of *E. coli*.

## MATERIALS AND METHODS

### Inflorescence production

Except for the gas chromatography analysis, all phases of this work were carried out at the Colegio de Postgraduados, Campus Montecillo. Pot marigold was sown in a peat moss:agrolite:soil (2:2:1 v/v) substrate, inside 5-cm diameter black polyethylene bags, at 20 to 30 °C, under greenhouse conditions. After 120 days, the seedlings were transplanted into the open field. The rows were established 70 cm apart from each other and there were 30 cm between plants. The agronomic management included irrigation, weed removal, and control of pests and diseases. The flower heads were harvested with a 0.5-cm peduncle cut. The harvested inflorescences were shade dried at room temperature (18 °C) for 7 days. An average yield of 420 kg $ha^{-1}$ of dry heads was obtained.

### Obtaining the EO

The dried plant material was subjected to steam distillation for 2 h in a 60-L distiller. Afterwards, the water-oil emulsion was recovered and three applications of 50 mL of methylene chloride ($CH_2Cl_2$) were added to separate the oil. Anhydrous sodium sulfate ($Na_2SO_4$) was then added to completely remove the water. Subsequently, the result was concentrated in a Buchi B-480 Water Bath at 40 °C (baine-marie). The EO was transferred with a Pasteur pipette to a 1.5-mL vial and stored at 4 °C.

### Gas chromatography analysis of the EO

The samples were analyzed at the Unidad de Servicios de Apoyo a la Investigación y a la Industria, Facultad de Química, UNAM, in order to determine the chemical composition of pot marigold EO, using a Pegasus 4D gas chromatography/mass spectrometry (GC/MS) (LECO), with a Time of Flight analyzer. The GC/MS system used a stationary phase HP-5MS (DB5) / 10m × 0.18mm × 0.18mm thick column and Ultra High Purity Grade 5.0 helium (Praxair) was used as carrier gas at a flow of 1 mL $min^{-1}$. The following standards were used: naphthalene, 1, 2, 3, 5, 6, 8a-hexahydro-4, 7-dimethyl-1-(1-methylethyl)-,1S-cis) and 1,6-Cyclodecadiene, 1-methyl- 5-methylene-8-(1-methylethyl)-,[s-(E,E)].

### Bioassays

The Instituto de Ciencias Básicas e Ingeniería de la Universidad Autónoma del Estado de Hidalgo provided a strain of the multidrug-resistant enterohemorrhagic *Escherichia coli* (EHEC), which can resist the following antibiotics: amoxicillin-clavulanic acid, colistin, erythromycin, gentamicin, and neomycin (Gómez -Aldapa *et al.*, 2016). It was cultivated in a Petri-dish with an Eosin-Methylene Blue (EMB) Agar (BD®) solid culture medium. Antibacterial activity was assessed using the Kirby-Bauer disk diffusion susceptibility test. The bacterial inoculum was 100 $\mu$L at $1\times10^8$ cells $mL^{-1}$ and had a radiant streaking method. We evaluated 13 treatments: pure EO, 10-90% dilutions of EO, and controls (water and amikacin). A 5-mm diameter Whatman® No. 1 filter paper disc was placed in the center of each Petri-dish, with 10 $\mu$L per treatment and four repetitions. Petri-dishes were incubated at 37 °C for 24 h.

### Inhibition diameter

After incubation, all Petri dishes were photographed and scanned with an HP Deskjet multifunctional. The GIMP (GNU Image Manipulation Program) free software was used to edit the threshold of the files, converting them into black and white images. Inhibition zone diameters were measured using the IMAGEJ digital image processing program. An analysis of variance and a Tukey's mean comparison ($\alpha$, 0.05) were performed using the SAS® v. 9 statistical package.

### Determination of minimum inhibitory concentration (MIC)

MIC values were determined considering the lowest concentration of the crude extract than can inhibit the visible growth of *E. coli* after the incubation period.

## RESULTS AND DISCUSSION

### Phytochemical analysis

Some climate components, crop management, and the phenological stage of the plant at the time of harvest affect its content of secondary metabolites (Russo *et al.*, 2015).

The analysis showed that the EO is composed of 95.85% sesquiterpenes and 4.15% other hydrocarbons. The most abundant components (germacrene and cadinene) are non-oxygenated sesquiterpenes that account for 76.4% of the total oil. These results match the findings of Salome (2015), who determined that cadinene was the most abundant compound in all the floral morphotypes of pot marigold that they studied.

As seen in Figure 1, there are several important peaks with retention times of 606.816 s (cadinene) and 604.066 s (germacrene).

## Antibacterial activity

The essential oil has great potential for antibacterial activity against *E. coli*, according to both the interpretation of the inhibition zones (in mm) in the Kirby-Bauer disk diffusion susceptibility tests and the significant differences in the various concentrations of the essential oil.

Figure 2 shows the susceptibility groups found with the statistical analysis. The bacterium turned out to be susceptible to treatments with a 100 to 70% dilution of EO, with average diameters of 30, 28, 27, and 26 mm. However, 60-40% concentrations had an intermediate effect (23, 20, and 16 mm). Finally, levels of 30-10% allowed the bacteria to grow relatively undisturbed. All dilution levels showed a visible effect and had a metallic green sheen resulting from the rapid lactose fermentation and strong acid production (Figure 3).

Likewise, the results showed that, in the control treatments with methylene chloride ($CH_2Cl_2$), bacterial growth was 100%, ruling out any toxic effect of the solvent. The inhibition of bacterial growth reached MIC values of 7 $\mu$g/mL.

Radioza *et al.* (2007) found that pot marigold EO inhibited the in vitro growth of *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*. Its

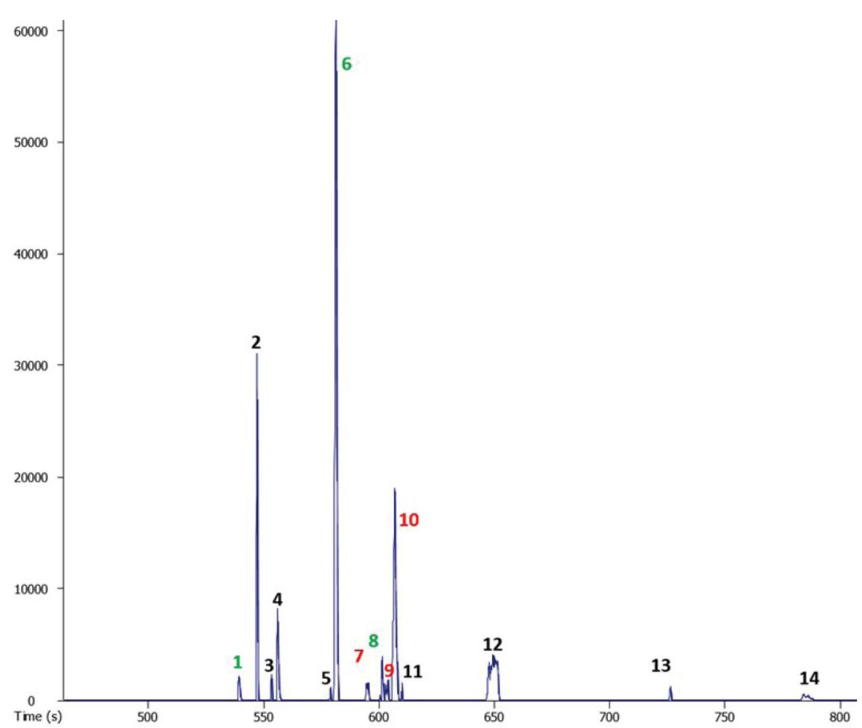


**Figure 1**. Phytochemical profile of pot marigold EO obtained with a gas chromatography/mass spectrometry: 1, 6, and 8 Germacrene; 2 Cubebene; 3 Copaene; 4 -caryophyllene; 5 Isoledene, 7, 9 and 10 Cadinene; 11 Seychellene; 12 Muurolene; 13 Eicosane; 14 Heptadecane.

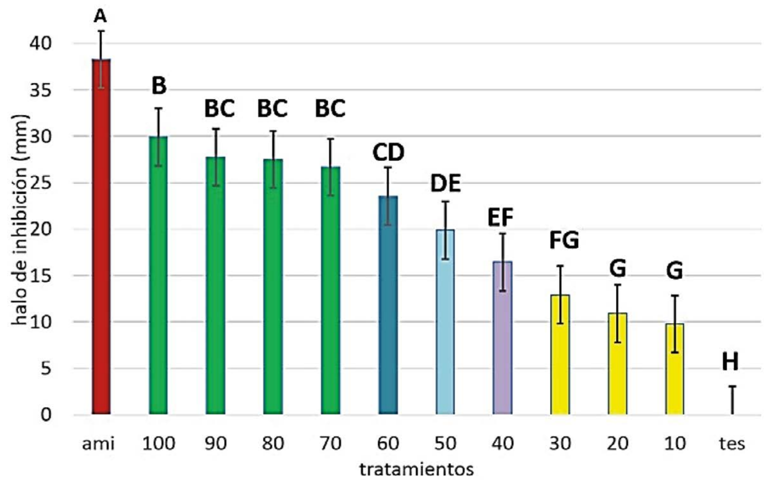


**Figure 2**. Inhibition halos of the *in vitro* growth of *E. coli* produced by different concentrations (10-100%) of *Calendula officinalis* EO, with amikacin (ami) and water (tes) as positive and negative controls. Each value is the mean of 4 replicates ± SE. Different letters are treatments with statistical significance ($p>0.0001$, $\alpha=0.05$, Tukey).

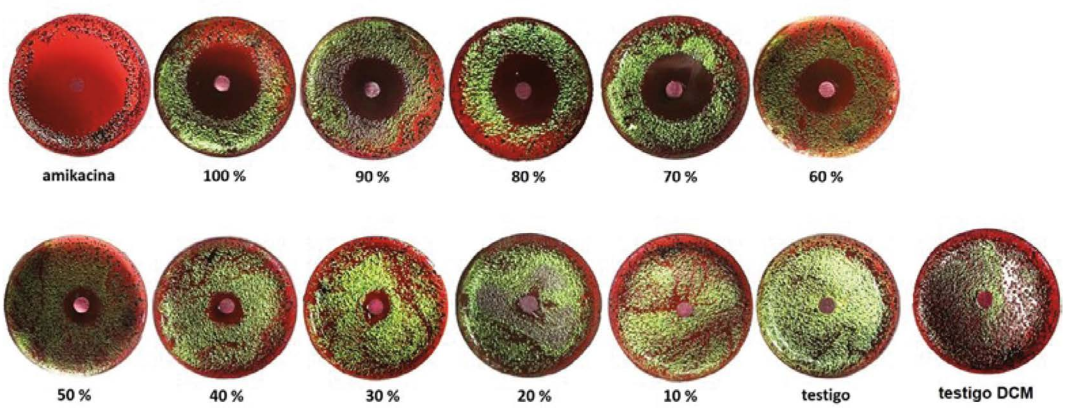


**Figure 3**. Inhibition halos caused by different dilutions (expressed as a percentage) of pot marigold EO on the growth of *E. coli in vitro*. The 100-10% dilutions are compared with a commercial antibiotic (amikacin), the control (distilled water), and the DCM control (methylene chloride).

abundant content of sesquiterpenes —which act as phytoalexins, antibiotic compounds that are capable of intercalating in the lipids of the bacterial cell membrane— distorted the structure of their membranes and make them more permeable (Devi *et al.*, 2010). Salazar-Gómez *et al.* (2020) have likewise reported that this group of compounds has anticancer, anti-inflammatory, and antimicrobial activity. Although the antibacterial effect of an essential oil depends on its mixture of metabolites, its activity can be attributed to its major components. Pot marigold EO is rich in compounds such as: cadinene (Salome-Abarca *et al.*, 2015), which has anti-inflammatory and antiseptic properties (Kaufman *et al.*, 1999); germacrene, which has insecticidal activity against mosquitoes, in addition to its well-known antimicrobial (De Lima *et al.*, 2010), cytotoxic (Palazzo *et al.*, 2009), and antibacterial effect against *Pseudomonas syringae*; caryophyllene, which has proven to be active against *S. aureus* (De Lima *et al*., 2010); and cubeben, which inhibits the growth of *S. aureus*, *B. subtilis*, *Klebsiella pneumoniae*, and *P. aeruginosa* (Sharifi-Rad, 2014).

Based on the evidence found in the scientific literature and the results of this work, sesquiterpenes could be the most important active principle in the antibacterial action of this plant, given both their pharmacological actions and their abundance in pot marigold. Nevertheless, the action of other active principles, such as flavonoids —to which antibacterial and antiallergic actions are attributed—, has not been ruled out. The antihemorrhagic, anti-inflammatory, antipyretic, antiseptic, and phlebotonic properties of this species, reported by Melgarejo *et al*. (2008), could also support the patient's full recovery.

## CONCLUSION

Due to the abovementioned results, the EO of *C. officinalis* could be used to support the treatment of the disease caused by the antibiotics-resistant *E. coli*.

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# Morphology and biochemistry of achacha (*Garcinia humilis* (Clusiaceae) (Vahl) C.D. Adam) fruits harvested at three consumption ripeness stages

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**ABSTRACT**

**Objective**: To identify the morphological and biochemical variations of achacha (*Garcinia humilis* (Clusiaceae) (Vahl) C.D. Adam) in three ripeness stages of consumption.

**Design/Methodology/Approach**: The morphological and biochemical variables of fruits in three ripeness stages were evaluated to identify the preferred stage for consumption. We collected 15 fruits from 10 productive-stage trees (n=150) for each ripeness stage for their subsequent evaluation in the laboratory. The morphological and biochemical variables were evaluated in each one. Each fruit was considered an experimental unit. The morphological and biochemical variables were evaluated for each unit.

**Results**: Delaying the harvest for a few days increases the weight, length, diameter, and pulp:seed ratio of the fruits and decreases the firmness of the epicarp and the acidity. The °Brix increases and the fruits lose a considerable weight during storage. The pulp of the ripest fruits had light and unsaturated colors.

**Study Limitations/Implications**: Changes in the conditions of the production systems and the variation in the availability of rainwater throughout the year and from one year to another can cause variation in fruit quality.

**Findings/Conclusions**: The ripest fruits had outstanding biochemical parameters for its consumption and better morphological characteristics (length, total weight, diameter, and pulp:seed ratio). With adequate training, the results enable the color of the epicarp and the pulp variables to be used as a harvest index.

**Keywords**: Post-harvest, Harvest Index, consumption ripeness, Total Soluble Solids (TSS).

## INTRODUCTION

The genus Garcinia comprises more than 1,300 species, including achacha (*Garcinia humilis* (Clusiaceae) (Vahl) C.D. Adam), also known as Bolivian bacupari (Lorenzi *et*

**Citation**: Villalobos-Villalobos, V., López-Martínez, V., Aguirre-Medina, J. F., Guzmán Camposeco, F., Domínguez-Liévano, A., Espinosa Zaragoza, S., & Pérez de la O, N. B. (2023). Morphology and biochemistry of achacha (*Garcinia humilis* (Clusiaceae) (Vahl) C.D. Adam) fruits harvested at three consumption ripeness stages. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2521

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 14, 2022.
**Accepted**: February 18, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 33-39.

*al.*, 2006). During the last decade, the "Select" achacha, also known as achachairú or mangosteen, has shown its potential as a commercial species in various regions of Brazil (Melo *et al.*, 2017). Cury *et al.* (2016) report that this species has various uses: it has healing, laxative, anti-inflammatory, and digestive properties; likewise, it prevents rheumatism and gastric ulcers. The same authors mention that bioflavonoids, benzophenones, and other substances can be extracted from its leaves and fruits. These substances increase its anticancer, antioxidant, and anti-inflammatory potential. The fruit can be found in the tropical rainforests of Bolivia, Guyana, Panama, and much of the Caribbean. It was also introduced to Australia where it has become a commercial crop (Lim, 2012).

The achacha has been studied and propagated in other parts of the world where commercial plantations have been established for various purposes (medicinal studies and trade). However, despite the recent boom of this fruit tree in in the various places where its plantation are located, the optimal maturity stage for ripeness and consumption —which can affect its organoleptic and biochemical properties— has not been determined. The objective of this work was to identify the morphological and biochemical variation of achacha in three consumption ripeness stages and consequently to guarantee acceptable organoleptic properties that facilitate its commercialization, generating alternatives for fruit diversification in the Soconusco region, in Chiapas, Mexico.

## MATERIALS AND METHODS

Productive "Select" achacha trees were identified in an orchard with eight-year-old trees. They had been planted in a 10×10 m square system, in the Santa Cecilia community, Huehuetán, Chiapas, Mexico (15° 03' 12.4" N, 92° 20 ' 60" W, and 311 m.a.s.l.). Ten productive-stage trees were selected and 15 fruits in different ripeness stages (E1, E2, and E3) were collected from each tree to evaluate the corresponding variables. The soil of the orchard has a vegetal cover mainly composed of pampano (*Calathea lutea* (Aubl.) E. Mey. ex Schult.) and kudzu (*Pueraria phaseoloides* (Roxb.) Benth.). It also has *Theobroma cacao* L., *Ananas comosus* (L.) Merr., and *Cedrela odorata* L. intercrops. The dominant soil group is Acrisol, with a clay loam texture, 6.12 pH (1:2 water), 3.4% OM, 3.16% $NO_3^-$ (mg kg$^{-1}$), 1.72% P (mg kg$^{-1}$ Bray), 22.9% $Fe_2^+$ (mg kg$^{-1}$), 13.4% $Mn_2^+$ (mg kg$^{-1}$), 0.1% $Zn_2^+$ (mg kg$^{-1}$), 0.1% B (mg kg$^{-1}$), 58.7% $K^+$ int. (mg kg$^{-1}$), 39.7 $Ca_2^+$ (mg kg$^{-1}$), and 98.3% $Mg_2^+$ (mg kg$^{-1}$), 0.07% EC (Dsm$^{-1}$ at 25 °C), and a ≥18% slope. The following data were also recorded: a 63% saturation point, a 33.8% field capacity, a 20.1% permanent wilting point, a 0.90 cm h$^{-1}$ hydraulic conductivity, and a 1.03 apparent density. The climate belongs to the Am(w")ig type, with 3,000 to 3,500 mm of rainfall and minimum, average, and maximum temperatures of 19, 27, and 35 °C, respectively, according to García (1973).

### Variables

A digital scale (OHAUS® USA) was used to determine the total weight (g) and weight loss (g) of the fruits. Fifteen fruits were selected and the weight loss was recorded every 72 h. To determine the pulp:seed ratio, each part of the fruit was weighed separately using a digital scale. A FOY analog vernier (USA) was used to determine the polar and equatorial diameter of the fruits. The CIE (International Commission on Illumination) system was used to evaluate the color of the epicarp and the pulp of the fruits in the L*C*h° colour space where L* is lightness, C* is chroma, and h° is hue angle, which are measured with an X-Rite® brand colorimeter. Once the color of the epicarp was measured, it was removed to measure the color of the pulp.

The firmness of the epicarp was determined with an FDV-30 manual texture analyzer (Brand Chatillon, USA), measuring the force (N) required to penetrate it. The Total Soluble Solids (TSS, expressed as °Brix), the pH, and the titratable acidity were calculated through destructive samplings, weighing 10 g of pulp, adjusted to 50 mL with distilled water. The °Brix were determined with a digital refractometer (SPER SCIENTIFIC, model 300034), following the AOAC methodology (1990), while the pH was determined with a 230[a] potentiometer (Thermo Orion, USA). The titratable acidity was calculated following the volumetric method of the AOAC (1990): 10 g of liquefied pulp were poured into 50 mL of distilled water; subsequently, a 20-mL aliquot was titrated with NaOH (0.1 N) and four phenolphthalein drops were added as indicator. The following formula was used to make the conversion to citric acid: (Volume spent*N*Meq*Total volume/Sample weight*mL used)*100. °Brix were divided by acidity (°Brix/acidity) to calculate the flavor index. The resulting data was processed with the SAS software ver. 9.0 (SAS Institute, 2009). An analysis of variance (ANOVA) was performed to establish the statistical difference between the three ripeness. Any differences were subjected to a Tukey's mean comparison ($\rho \leq 0.05$).

## RESULTS AND DISCUSSION

Three different stages were determined depending on the color of the fruit at harvest time (according to the producer's experience): ripeness stage 1 (E1), fruits with green epicarp; ripeness stage 2 (E2), yellow fruits; and ripeness stage 3 (E3), reddish-orange fruits (Figure 2). The weight of the achacha fruits differed depending on the degree of ripeness at which they were harvested; the average weight per ripeness stage was 28.9 g (E1), 32.5 g (E2), and 40.6 g (E3). Since the fruits harvested in E3 are on average larger, E3 recorded the highest weight and was higher than E1 (28.6%) and E2 (19.90%). Weight is lost over

**Figure 1**. Achacha (*Garcinia humilis* Vahl) fruits: oval-shaped berries with a yellow to reddish-orange color at the stage of physiological maturity. They almost always have one normal seed and one rudimentary seed.


**Figure 2**. Ripeness stages of achacha fruits at harvest time.

storage time, because of transpiration; the E3 fruits lost less weight. The values obtained in this work are like the results of Cotty-More *et al.* (2019), who reported a daily weight loss of 0.71 to 1.1 g, as well as a reduction in diameter and in pulp weight, as a result of dehydration (Table 1). Water loss is the main cause of fruit deterioration, since it has a quantitative impact on weight loss and, consequently, on appearance (Kader, 1992).

The amount of pulp increased significantly in the riper fruits (E3) (Table 1, Figure 3). Melo *et al.* (2017) recorded averages ranging from 7.21 to 8.50 g of pulp, in achacha fruits collected from three parts of the crowns in Brazilian plantations. These results are higher than those obtained in the present work. The pulp:seed ratio of the "Select" type achacha is three times higher and reaches a ≈3 proportion (CIAT, 2004). The E3 value was close to 2.5 (Table 1).

The E1 has heavier (11.56%) and its peel was thicker (12.51%) than E2 and E3. The epicarp of the same fruits also was firmer, showing a statistical difference —this phenomenon can be attributed to the lower ripeness degree, which directly impacts the morphological characteristics of the fruit (Castedo, 1999). Regarding their length and diameter, the E3 fruits recorded the highest average with 48.34 and 35.87 mm, respectively; they were

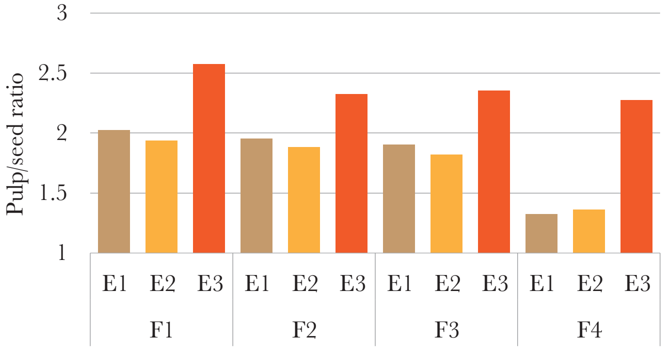


**Figure 3**. Achacha pulp weight:seed weight ratio.

**Table 1**. Characterization of achacha fruits in three ripeness stages.

| Variable | Maturity 1 | Maturity 2 | Maturity 3 | VC % |
|---|---|---|---|---|
| **Fruit morphology** | | | | |
| Total weight (g) | 28.99 c | 32.55 b | 40.64 a | 9.05 |
| Pulp/seed ratio | 2.02 b | 1.93 b | 2.57 a | 19.03 |
| Firmness (N) | 23.18 a | 20.28 b | 20.53 b | 8.14 |
| Longitude (mm) | 45.89 b | 45.89 b | 48.34 a | 4.51 |
| Diameter (mm) | 34.69 b | 35.28 ab | 35.87 a | 2.84 |
| Weight loss | 28.01 a | 25.69 b | 28.47 a | 5.44 |
| Shape index | | | | |
| **Peel colour** | | | | |
| Brightness (L*) | 61.95 b | 66.07 a | 57.86 c | 3.91 |
| Chroma (C*) | 51.80 b | 57.25 a | 46.80 c | 3.96 |
| Hue (°h) | 81.69 a | 83.43 a | 67.62 b | 4.11 |
| **Pulp Colour** | | | | |
| Brightness (L*) | 22.75 c | 37.97 b | 55.25 a | 13.34 |
| Chroma (C*) | 3.05 c | 5.92 b | 10.00 a | 23.78 |
| Hue (°h) | 84.14 a | 116.37 a | 76.37 b | 12.78 |
| **Biochemistry fruit** | | | | |
| Acidity | 2.39 a | 3.08 a | 2.15 a | 42.32 |
| pH | 2.86 ab | 2.64 b | 2.93 a | 8.64 |
| °Brix | 13.19 a | 12.18 a | 10.71 b | 10.58 |
| Ratio °Brix/Acidity | 5.78 ab | 3.79 b | 7.48 a | 57.4 |

* Values with the same letter within each factor and row are equal, according to Tukey's test ($P \leq 0.05$).
** CV: Coefficient of Variation.

statistically superior to the fruits with E1 and E2 ripeness. Lim (2012) reported achacha fruits with a length of 55 to 60 mm and a diameter of 44 to 55 mm in an orchard with commercial agronomic management, while the achacha trees in this study are planted with an intercropping system.

Along with the fruits of E2 (°Hue 83.43), the color of the fruits of E1 was closer to yellow (°Hue 91.69), while the fruits in E3 showed a tendency towards red and were statistically different from the fruits in E1 and E2 (Figure 2 and Table 1). Regarding the lightness (L*) and chroma (C*) results, the values of E3 stand out: the fruits had less lightness and less shade, along with a slight tendency towards red and their tone was firmly closer to orange. With respect to pulp color, the results indicate that E3 had similar (L*) values (*i.e.*, a light-coloured pulp). For their part, Santos and Pereira (2012) determined that the color of the epicarp in mango cultivars changed in a green-yellow-orange-red-purple gradation during the ripening process; likewise, they mention that the hue angle (h°) adequately describes the coloring change of the epicarp and it is a sign of the optimum fruit ripeness.

Regarding the total soluble solids, E3 had higher results than E1 and E2 (Table 1). They have a 10.71-13.19 range and are slightly lower than the results reported by Melo *et al.* (2017), who found a 14.08-16.36% °Brix range in an achacha plantation in Brazil. The pH value of the fruits does not show a trend towards the ripeness degree, although it has statistical differences. Unlike the case of °Brix, the pH results obtained with E1 and E2 were similar to those reported by Melo *et al.* (2017), who recorded 2.66-2.80 pH values; however, the averages of the E3 fruits were higher than the results reported by those authors (Table 1). Melo *et al.* (2017) also mention that the values indicate that the fruit is very acid and it is therefore recommended for the manufacture of juices and other industrialized products. The percentage of citric acid ranged from 2.1 to 3.0 in the three ripeness stages, without significant differences (Table 1). These results are lower than the values reported by Chávez *et al.* (2012), who recorded an average of 13.2% citric acid in achacha; however, they are higher than those reported by Melo *et al.* (2017) and CIAT (2004), both of whom recorded <1.90% citric acid values.

## CONCLUSIONS

We recommend E3 for harvest, given its outstanding biochemical parameters for consumption purposes and because it has better morphological characteristics. The color of the epicarp and pulp are likely a determining factor to decide the optimal ripeness stage (when the harvest should be carried out); therefore, the reddish-orange color can be considered a harvest index. The fruits in E3 should be evaluated during post-harvest to calculate the potential commercialization of the fruit in other markets.

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Colegio de Postgraduados

# Increase of the motility of buck semen with Andromed® extender and the addition of HTF

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**Citation**: Quintero-Elisea, J. A., Clemente-Sánchez, F., Cortez-Romero, C., Velázquez-Morales, J. V., Olguín-Arredondo, H. A., & Orozco-Lucero, E. (2023). Increase of the motility of buck semen with Andromed® extender and the addition of HTF. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i4.2532

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 02, 2022.
**Accepted**: February 27, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 41-47.



## ABSTRACT

**Objective**: To determine if HTF (Human Tubal Fluid) can protect buck sperm cells, increasing semen motility when used by itself or combined with the Andromed® commercial extender.

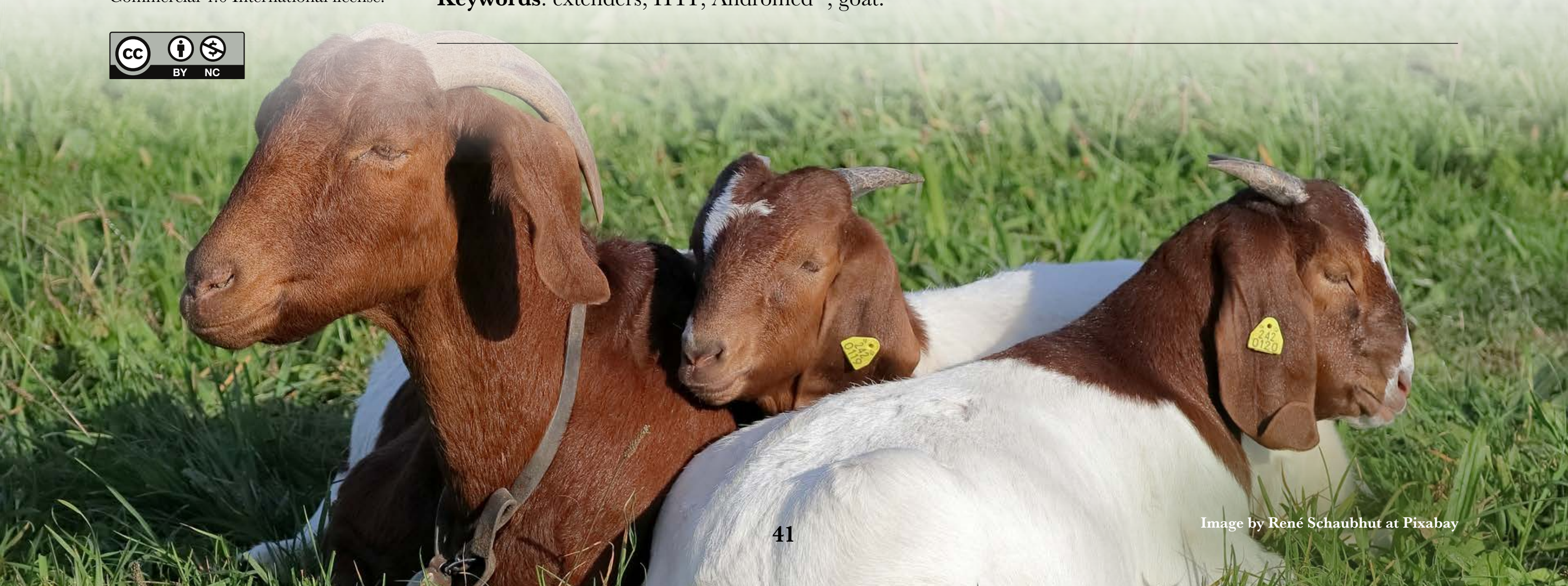
**Design/Methodology/Approach**: The semen of eight bucks of the Boer breed was used (10 ejaculates per male). Each ejaculate was diluted in three treatments (5 repetitions per treatment): 1) only Andromed®; 2) only HTF; and 3) Andromed® + HTF. The following variables were evaluated: mass motility (MM) and progressive motility (MP) in fresh (37 °C) and refrigerated (5 °C) semen. The differences between treatments were detected, based on the MM and MP in fresh and refrigerated semen variables, using the GLM procedure (SAS V9). In the case of the MM evaluated in three different periods, the measures repeated over time were analyzed using the Proc Mixed procedure (SAS V9).

**Results**: Regarding the quality of fresh spermatozoa, MM and MP recorded similar values ($p>0.05$) for the three treatments. On the one hand, MM registered 97.1% for Andromed®, 95.8% for HTF, and 97.5% for Andromed® + HTF; its highest value was achieved with the Andromed® + HTF combination, followed by Andromed® and HTF. On the other hand, the highest MP percentage was observed with the combination of Andromed® + HTF (91.0%), followed by Andromed® (90.4%), and HTF (89.0%). Regarding the quality of the sperm refrigerated at 5 °C, the combination of Andromed® + HTF had a higher MP value ($p<0.05$) than other treatments.

**Study Limitations/Implications**: In all the evaluated treatments, the motility of sperm decreased over time (0 to 24 hours after cooling); however, the treatments with Andromed®, and Andromed® + HTF maintained a high motility.

**Findings/Conclusions**: The combination of Andromed® and HTF can be used to dilute both fresh and cooled semen.

**Keywords**: extenders, HTF, Andromed®, goat.



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## INTRODUCTION

Regarding artificial insemination (AI), highlighting the importance of several factors that affect the desired level of fertility is imperative. In this sense, Allai *et al.* (2018) emphasize the importance of preserving ovine semen as one of the determining factors to be considered during the implementation of an AI program. Consequently, extenders play a fundamental role in the preservation and prolongation of sperm viability before and during AI. Currently, there is no ideal extender that provides the desired sperm quality, because each species needs different conditions.

HTF (Human Tubal Fluid) is an alternative that contains commercial extenders that contribute to the embryonic maturation process. It is a highly nutrient-rich medium, as a result of the compounds (*e.g.*, sodium chloride, potassium chloride, dehydrated calcium chloride, and hepta-hydrated magnesium sulfate) that it contains. The composition of the HTF is based on the compounds of the human oviduct and it has been widely used in the manipulation of gametes and embryos in humans, rats, mice (Aoto *et al.*, 2011; Cossiello *et al.*, 2012; Kleijkers *et al.*, 2016), and more recently in horses (Arroyo-Salvo *et al.*, 2019). The effect of HTF on sperm cells is directly based on sperm capacitation and changes in the levels of reactive oxygen species (ROS), which have been studied in humans but not yet in domestic species (Shih *et al.*, 2016; Hernandez *et al.*, 2021). Therefore, it may be acceptable as a reliable medium in the processing of domestic animal semen. Meanwhile, the development of new *in vitro* technologies —such as the CASA system (Computer Assisted Sperm Analysis System)— enables the study of multiple functional and morphological characteristics of spermatozoa, which are used to predict their fertilizing capacity (Quintero-Moreno *et al.*, 2011). Likewise, this reliable method is the most frequently used tool for the study of fresh or frozen diluted semen (Vicente-Fiel *et al.*, 2013) and it provides the bases for the hypothesis of the present work. There is at least one increase in MM and/or MP between treatments. Therefore, the objective of this study was to test if HTF can protect buck sperm cells by itself or combined in a certain ratio with the Andromed® commercial extender, in order to achieve better fertility in the AI.

## MATERIALS AND METHODS

A total of eight Boer bucks (2-4 years old in average) were used to obtain 10 ejaculates per male during the reproductive season. The semen was collected twice a week until the total number of ejaculates per male was completed. For this purpose, the males were induced to ejaculate by means of the presence of a doe in heat and an artificial vagina. Prior to the application of the treatments, the ejaculates were qualitatively evaluated following the procedure described by Clemente-Sánchez *et al.* (2017) and those that did not show favorable qualitative characteristics were discarded and no longer considered in the treatments. Each ejaculate obtained was subjected to a particular treatment (Andromed®, HTF, and Andromed® + HTF); each treatment included five repetitions per treatment and their dilution rate was 1:5. The statistical analysis of the treatments was based on a total of 400 observations. The treatments (T) were made up as follows: Andromed® (T1); HTF medium (T2); 2/3 of Andromed® + 1/3 of HTF (T3).

After the dilution, the sperm characteristics (Mass Motility (MM) and Progressive Motility (MP)) were first evaluated in diluted fresh semen (37 °C) and, subsequently, in refrigerated semen (5 °C). Additionally, the spermatozoa MM was evaluated three times (at 0, 12, and 24 h) after they had been refrigerated at 5 °C. The evaluations were carried out using the Sperm Vision CASA (Computer Assisted Sperm Analysis System) from MOFA Global Company. Finally, the differences between treatments (along with the behavior of each treatment) were determined according to the variables evaluated. The MM and MP variables were analyzed using the Proc GLM procedure (SAS V9), followed by Tukey's multiple range test ($p<0.05$). The MM evaluated at three different times was subjected to a repeated measures analysis over time using the Proc Mixed procedure (SAS V9).

## RESULTS AND DISCUSSION

In terms of the MM and MP variables, the fresh sperm quality results obtained from the comparison of treatments did not show any differences between treatments ($p>0.05$): Andromed® + HTF (97.5% and 91.0%), Andromed® (97.1% and 90.4%), and HTF (95.8% and 89.0%) (Table 1). Mortimer (1997) mentions that the use of computerized systems such as CASA leads to changes in the sperm flagellum, causing a decrease in motility; these changes were not observed in the fresh semen values, because the numbers obtained provided appropriate motility values for use in AI. However, the CASA System has the advantage that all the readings are standardized, avoiding the sampling or evaluator effects.

Most of researches about ruminant semen consider MM as the main variable to determine semen quality. However, the evaluation by MM as the only semen quality parameter is not enough to predict the fertilizing capacity of semen (Grasa *et al.*, 2005). Clemente-Sanchez *et al.* (2012) developed a linear model to determine semen quality in white-tailed deer, known as the Deer Semen Quality Index (SQID), which in addition to MM considers individual motility, sperm concentration, percentage of live spermatozoa, and percentage of normal spermatozoa. The result of this model provides a criterion of semen quality that is closer to reality and its use is recommended as a standardization tool in the deer semen evaluation process. Likewise, it is a useful tool in conducting researches where evaluating the semen is necessary or for treatments such as those developed in this study. However, although this study only considered the mass and progressive motility variables, all the sperm cell variables that the CASA system includes must be taken into consideration, including curvilinear velocity, progressive velocity, linear velocity, linearity

**Table 1**. Evaluation of mass motility (MM) and progressive motility (MP) in the spermatozoa of diluted fresh semen of buck.

| Tratamientos | Motilidad | |
|---|---|---|
| | MM (%)±EE | MP (%)±EE |
| Andromed® | $97.1^{a}\pm2.3$ | $90.4^{a}\pm2.4$ |
| HTF | $95.8^{a}\pm1.7$ | $89.0^{a}\pm2.6$ |
| Andromed® + HTF | $97.5^{a}\pm1.9$ | $91.0^{a}\pm2.0$ |

[a] Values with the same letter within columns are not different ($p<0.05$).
±ES (±EE)=Standard Error.

index, straightness index, oscillation index, and mean amplitude of lateral head displacement (Hernández-Corredor *et al.*, 2013). These variables provide objective parameters for the evaluation of semen diluted in different extenders to facilitate the standardization of the morphometric characteristics of spermatozoa (Vicente-Fiel *et al.*, 2013). Other reports describe the evaluation of buck semen, including a study by Evans and Mawell (1990), who describe the procedure for accepting or rejecting the ejaculate to be used in AI, based on a series of parameters, such as MM, MP, ejaculate volume, sperm vigor, sperm concentration, vital coloration, sperm morphology, and endosmosis test.

Regarding the MM and MP of sperm refrigerated at 5 °C (Table 2), the combination of Andromed® + HTF showed a significant difference ($p<0.05$) in the MM variable with respect to the other treatments. This may be the result of the combination of Andromed® and HTF: once the semen is subjected to cold temperatures, it maintains a high motility, as a consequence of the large amount of nutrients provided jointly by these two substances. However, spermatozoa tend to suffer damage and deterioration when they are diluted and preserved at low temperatures. Therefore, it is necessary to use extenders —especially those based on soybean lecithin, and which are free of egg yolk, given their great cryopreservation capacity to preserve the viability, membrane integrity, motility, and progressivity of spermatozoa when buck semen is frozen (Hernández-Corredor *et al.*, 2017). In this regard, Singh *et al.* (2012) found that using the Andromed® extender increases the spermatic motility of semen stored at 5 °C. MM and MP values above 80% for buck semen refrigerated at 5 °C are both considered excellent for later use in assisted reproduction programs. Therefore, the results of this study indicate that the evaluated treatments —particularly the Andromed® + HTF combination— can be used as extenders. This result is similar to that reported by Roof *et al.* (2012), who corroborated that the composition of the extender is crucial for the protection of spermatozoa against cryopreservation (Jiménez-Rabadan *et al.*, 2012), because the structural changes that take place at cellular level during the cryopreservation process cause irreversible damage to spermatozoa and affect motility (Dorado *et al.*, 2009).

The MM results of the spermatozoa evaluated over time are shown in Figure 1. There were no differences ($p>0.05$) at zero (0) h; however, the highest percentage of motile spermatozoa was observed in the Andromed® + HTF (92.4%). At 12 h, MM decreased in all treatments, with differences between them ($p<0.05$). The HTF treatment showed 15% reduction in mass motility. Just like the HTF, it greatly reduced mass motility

**Table 2.** Evaluation of mass motility (MM) and progressive motility (MP) in the spermatozoa of diluted and refrigerated (5 °C) semen of buck.

| Tratamientos | Motilidad | |
|---|---|---|
| | **MM (%) ±EE** | **MP (%) ±EE** |
| Andromed® | $86.0^{a}\pm3.2$ | $84.9^{a}\pm2.3$ |
| HTF | $84.1^{a}\pm2.6$ | $84.6^{a}\pm2.0$ |
| Andromed® + HTF | $90.1^{b}\pm2.1$ | $86.3^{a}\pm1.6$ |

[a, b] Values with different letter within columns are different ($p<0.05$).
±SE (±EE)=Standard Error.

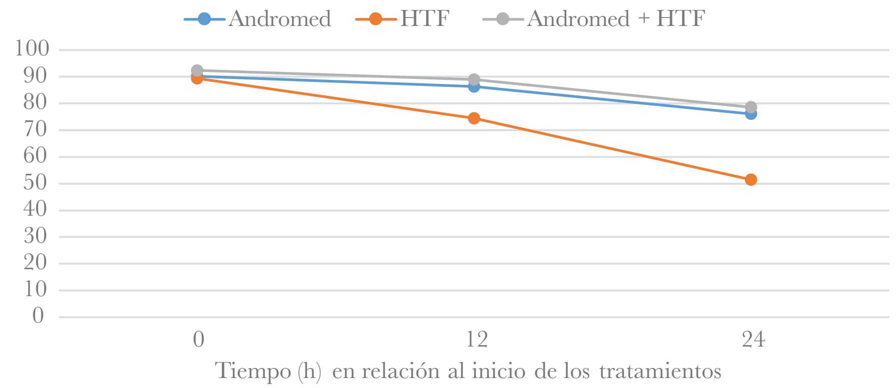


**Figure 1**. Mass motility (MM) of spermatozoa in semen diluted in three mediums (treatments), evaluated at 0, 12, and 24 h after refrigeration at 5 °C.

(22.9%), twenty-four hours after refrigeration. Likewise, it was different than the other two treatments ($p<0.05$).

Both Andromed® and the Andromed® + HTF combination recorded 50 to 75% motility values, which are desirable in the refrigerated semen stored for up to 24 h that will be used in AI. According to Hernandez *et al.* (2014), the use of animal protein-free extenders (*e.g.*, Andromed®) provides a better response, because its phospholipid content is obtained from soybean extract and spermatozoa are not affected by the phospholipase used in other extenders. Paulenz *et al.* (2002) evaluated an extender over time (0, 6, 12, and 24 h), and found that motility remains acceptable (70-80%) for a long period. Diaz *et al.* (2015) also evaluated sperm quality over time (0, 2, 4, 6, and 24 h) with different extenders and found that the best MM is recorded 4 to 24 h after the semen has been refrigerated at 5 °C. Because there is scarce information about the parametric evaluation of buck sperm using the CASA system (Hernández-Corredor *et al.*, 2013), it would be advisable to highlight that this system represents an innovative technology that determines the values of the behavior of spermatozoa stored under fresh, refrigerated, or frozen conditions (Quintero-Moreno *et al.*, 2011). Coupled with an adequate selection of an extender for the conservation of buck semen (Hernández-Corredor *et al.*, 2013), this system is a practical alternative for the conservation of the germplasm that is used in species improvement programs.

## CONCLUSIONS

With respect to the MM and MP of spermatozoa from fresh and refrigerated semen, the highest percentage was found in the combination of extenders (Andromed® + HTF). Consequently, Andromed®, in combination with other and by itself serves as an appropriate medium to maintain and improve the characteristics of fresh and refrigerated semen. Regarding sperm quality over time, the Andromed® + HTF combination recorded the highest percentage in mass motility among the three compared extenders. Therefore, we conclude that Andromed® can be combined with HTF to dilute both fresh and refrigerated semen, maintaining an acceptable quality for up to 24 h. However, when using only HTF as an extender, we advise using it only in fresh semen; if it is used to dilute refrigerated semen, the sperm quality will record a considerable fall.

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Colegio de Postgraduados

# Structure and phenology characterization of four tree species of tropical deciduous forests

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**ABSTRACT**

**Objective**: To determine the structural and phenological characteristics of four major tree species in the tropical deciduous forests.

**Design/Methodology/Approach**: To determine their structure, ≥10 cm wide trees were registered at a height of 1.30 m in 30 sites of 500 $m^2$. For phenological purposes, seven sites were selected. The following phenological stages were evaluated: dormancy, vegetative, flowering, fruiting, and seeding.

**Results**: Four hundred and five individuals belonging to the four species under study were found: copal ancho (*Bursera copallifera* (Sessé & Moc.) ex DC. Bullock), Mexican kidneywood (*Eysenhardtia polystachya* (Ortega) Sarg.), tecolhuixtle (*Mimosa benthamii* (J.F. Macbr. var. bentha-mii)), and tepemezquite (*Lysiloma divaricatum* (Jacq.) Macbride). Tepemezquite ranked first according to the Relative Importance Value Index. Based on the phenological characteristics observed, three species lost foliage at some time of the year and only tecolhuixtle was evergreen.

**Study Limitations/Implications**: The results and conclusions are limited only to individuals with ≥10 cm in diameter. Further results can be extrapolated from this data.

**Findings/Conclusions**: The structural and phenological features of the four species evaluated in ejido El Limón have similar characteristics to those that have been described for this type of vegetation in other locations in Mexico.

**Keywords**: copal ancho, Mexican kidneywood, tecolhuixtle, tepemezquite.

**Citation**: Fierros-González, A. M., Sánchez-Hernández, M. A., Cortés-Díaz, E. & Velázquez-Martínez, A. (2023). Structure and phenology characterization of four tree species of tropical deciduous forests. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i5.2535

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 19, 2022.
**Accepted**: March 01, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 49-56.



## INTRODUCTION

Tropical deciduous forests (TDF) cover 35% of the total area of Mexico and 70% of the State of Morelos, in which the Reserva de la Biosfera Sierra de Huautla (REBIOSH) is located. However, they have not been given enough attention (Osorio-Beristaín *et al.*, 2012).

Image by Nika Akin at Pixabay

The analysis of the structural and phenological characteristics of the tree species of the TDF helps to understand the functioning of this ecosystem and to make informed decisions about its proper management (Graciano-Ávila *et al.*, 2017). Additionally, it is fundamental for conservation programs, forest management, and wilderness management planning (Delgado-Demera *et al.*, 2018). Therefore, the objective of this study was to determine the structural and phenological characteristics of four major tree species in the tropical deciduous forest of the *ejido* El Limón in Tepalcingo, Morelos.

## MATERIALS AND METHODS

The study was carried out in the *ejido* El Limón de Cuauchichinola, municipality of Tepalcingo, Morelos. The following species were selected: copal ancho, Mexican kidneywood, tecolhuixtle, and tepemezquite. These species were selected based on their high ecological and social and cultural values.

### Structural characteristics

To determine the structural characteristics of the four species, 30 circular sites of 500 $m^2$ were established, in which $\geq 10$ cm wide trees were registered at 1.30 m from the ground. To obtain the phenological data, the seven sites with the highest concentration were chosen and the phenological stages of the four species were monitored for 12 months. Height was established with a 5-meter GEO-SURV® telescopic levelling rod, model FGTS-5 (individuals ≤ 7 m) and a Suunto® clinometer, model PM5/360PC (trees > 7 m). The horizontal structure was described using the distribution of the number of trees per diameter category, density by species (Zarco-Espinosa *et al.*, 2010), and basal area (BA), which was obtained with the following formula:

$$BA = \frac{\pi}{4000} * D^2$$

Where: $BA$ = basal area; $D$ = Diameter of the tree at 1.30 m above the ground (in centimeters).

The Relative Importance Value Index (RIVI) was calculated through the sum of the relative density (RD), relative dominance (RDom), and relative frequency (RF) (Mostacedo and Fredericksen, 2000).

### Phenology

The following phenological stages were observed every two months: dormancy (with foliage), vegetative (without foliage), flowering (number of flowers), fruiting, and seeding-The Fournier (1974) methodology —which consists of applying the scale described in Table 1— was applied.

## RESULTS AND DISCUSSION

Four-hundred and five individuals from the four species under study were recorded. The largest number of individuals belonged to tepemezquite (164), followed by tecolhuixtle,

**Table 1**. Evaluation scale according to the Fournier methodology (1974).

| Scale | Description | Range |
|---|---|---|
| 0 | absence of characteristic | 0 |
| 1 | presence of the characteristic, with a magnitude between | 1-25% |
| 2 | presence of the characteristic, with a magnitude between | 26-50% |
| 3 | presence of the characteristic, with a magnitude between | 51-75% |
| 4 | presence of the characteristic, with a magnitude between | 76-100% |

A figure indicating the percentage of trees observed in each phenological stage was developed to describe the phenology of each species.

copal ancho, and Mexican kidneywood. Table 2 shows the number of individuals per hectare.

None of the four selected species was present in all the sites. The most frequent was tepemezquite, which was found in 83% of the sites. Sixty-three-point-one percent of the total BA of the four species (7.08 $m^2$ $ha^{-1}$) was covered with tepemezquite (Table 2). The average height of the four species was 8.7±3.2 m (Table 2).

Three strata categories were differentiated: lower, from 1 to 5.9 m (17% of the individuals); intermediate, 6 to 10.9 m (46%); and upper, 11 to 15.9 m (37%).

In average, the trees in *ejido* El Limón were slightly taller in this study than the trees measured by Guillermo-Sandoval *et al.* (2021) in the same area (average height: 8 m). A similar height (6-8 m) was recorded in different locations in Mexico and the Caribbean islands (Álvarez-Yépiz *et al.*, 2008; Guillermo-Sandoval *et al.*, 2021).

The average diameter per species is shown in Table 2. In the diameter distribution, most of the individuals (78%) measured from 10 to 20 cm, which matches the findings of Gutiérrez-Báez *et al.* (2012), who link this behavior with the age of the community and considers that, as the vegetation of a place recovers, the number of individuals with small diameter decreases; therefore, the community shows a good state of recovery. The average general diameter is 17.3±5.9 cm with an interval of 10 to 45 cm.

Regarding the distribution of individuals by species based on height, copal ancho (75%) and Mexican kidneywood (83%) are distributed mostly in the intermediate stratum, while tecolhuixtle is mainly distributed in the lower stratum (56%), and tepemezquite (59%), mostly in the upper stratum.

**Table 2**. Number of individuals per hectare, frequency, basal area, height, and average diameter of the four species of the tropical deciduous forests.

| Specie | No. Ind. $ha^{-1}$ | Frequency (%) | Basal Area ($m^2$ $ha^{-1}$) | Average Height (m) | Average Diameter (cm) |
|---|---|---|---|---|---|
| Copal ancho | 39 | 53% | 1.07 | 6.7 | 17.9 |
| Palo dulce | 8 | 17% | 0.19 | 6.5 | 16.8 |
| Tecolhuixtle | 59 | 40% | 1.35 | 5.02 | 16.2 |
| Tepemezquite | 164 | 83% | 4.47 | 10.6 | 17.6 |
| Average | | | | 8.7 | 17.3 |
| Total | 270 | | 7.08 | | |

Tepemezquite recorded the highest RIVI and *Eysenhardtia polystachya* had the lowest value (Table 3).

The results match the findings of Hernández-Silva *et al.* (2011) in three *ejidos* of the Sierra de Huautla, Morelos, in which tepemezquite and tecolhuixtle registered a high RIVI, which places them within the first 10 species in this category. However, it is important to point out that they carried out censuses of species without taking the diameter into account.

Vallejo (2009) also carried out a study in the Sierra de Huautla, evaluating the RIVI in different areas: near the foothills, near the smooth hills, far from the foothills, and far from the smooth hills. Vallejo's results showed that tepemezquite is the dominant and most important species in the entire study area, which matches several reports for the TDFs that are distributed along the Pacific coast of Mexico (Pineda-García *et al.*, 2007).

**Phenology**

In total, 80 trees were monitored. The species with the highest number of individuals was tecolhuixtle (25), followed by copal ancho (22), tepemezquite (21), and Mexican kidneywood (12).

**Copal ancho**

Flowers were observed in 95.5% of the copal ancho individuals in the first data collection (June 2018) (Figure 1a). Out of this total, 4.5% were found in rank 0 (without flowers), 13.6% in rank 1, 45.5% in rank 2, 27.3% in rank 3, and only 9.1% in range 4, according to the Fournier scale (Figure 1b).

The results of the phenological observations of the second and third survey (August and October 2018) showed that the highest percentage were in the fruiting stage. In December, most of them were already in the seeding stage (81.8%) and some had begun to lose their leaves (9.1%).

In February 2019, 91% of the individuals were dormant; in April, 91% were already in a vegetative stage; and in June all the individuals had some percentage of flowering. Medina-Lemos (2008) determined that the flowering stage lasts from May to June and the fruiting stage goes from June to November, which matches the results of this study.

**Mexican kidneywood**

In June 2018, 66.7% of the individuals of this species were in a vegetative stage and 33.3% had begun to flower. In August, 75% were in a flowering stage and 25% had begun

**Table 3**. Relative Importance Value Index (RIVI/IVIR) of the four species.

| Specie | Relative Dominance | Relative Density | Relative Frequency | RIVI |
|---|---|---|---|---|
| Tepemezquite | 63.23 | 60.49 | 44.0678 | 167.80 |
| Tecolhuixtle | 19.04 | 21.98 | 20.33898 | 61.36 |
| Copal ancho | 15.11 | 14.57 | 27.11864 | 56.79 |
| Palo dulce | 2.62 | 2.96 | 8.474576 | 14.06 |

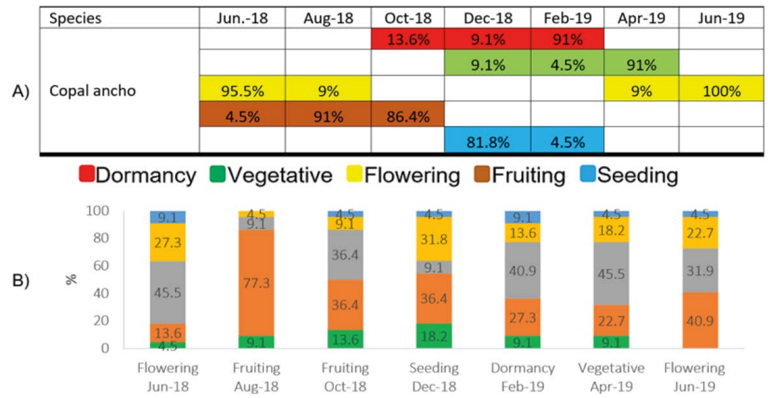


**Figure 1**. Bimonthly phenological stages from June 2018 to July 2019.

to bear fruits. In October, most of the individuals (83.4%) were at some point of the fruiting range and, in December, 83.3% were in the seeding stage. In February and April 2019, they were in the dormancy stage and, in June, 83.3% were in a vegetative stage (Figure 2a). Figure 2b shows the measurement of phenological characteristics, according to the Fournier scale.

The results of Cervantes-Sánchez and Sotelo-Boyas (2002) are similar to those reported in this study. However, differences can be observed with the datasheets for this species published by Vázquez-Yanes *et al.* (1999), who reported that fruiting occurs from November to December.

## Tecolhuixtle

This species did not fully lose its foliage at any time and therefore never entered the vegetative stage. It was not until December 2018 that flowers were observed in 96% of the individuals. In February 2019, 76% of the individuals were already in some point of the fruiting stage; in April, 96% were already in the seeding stage and, in June, the same percentage were dormant (Figure 3).

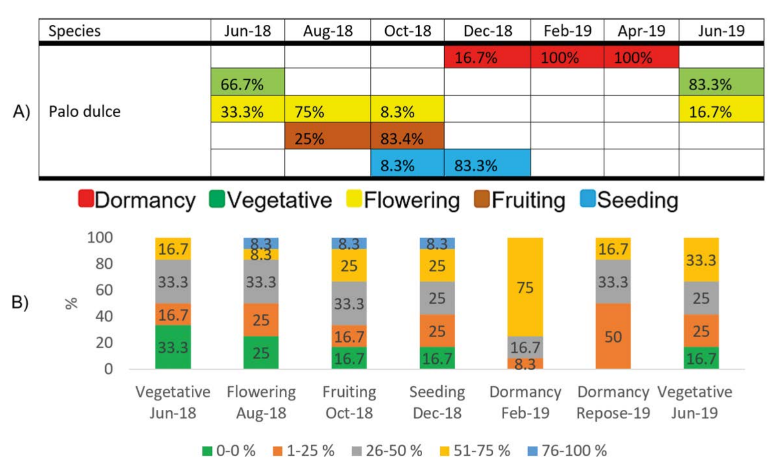


**Figure 2**. Bimonthly phenological stages from June 2018 to July 2019.

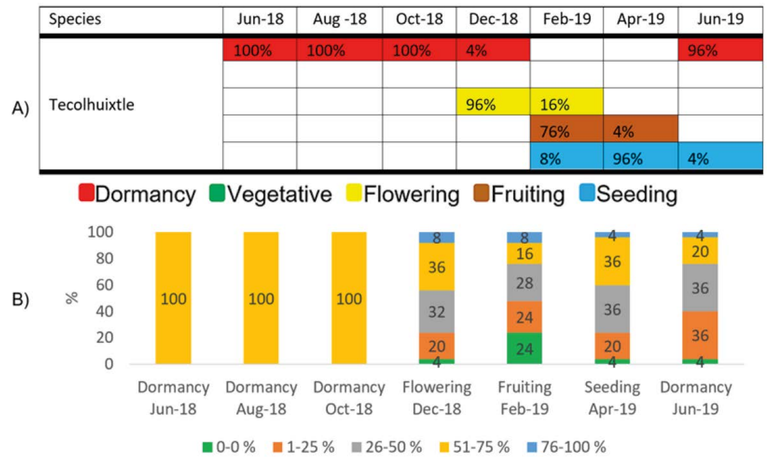


**Figure 3**. Bimonthly phenological stages from June 2018 to July 2019.

Rico-Arce (2001) reports that this species flowers from September to March and bears fruit from February to June, which matches the results of this study. The reproductive periods of plants are generally associated with water and nutrient availability cycles, as well as climatic factors.

**Tepemezquite**

Regarding phenology, in June 2018, 90.5% of the individuals were in a vegetative stage and the remaining 9.5% began to flower. In August, 85.7% were already flowering. In October, 90.5% were at some point of the fruiting range, while in December, 95.2% were within the seeding stage. In February and April 2019, most of them were already dormant and, in June, 85.7% of the individuals were in a vegetative stage (Figure 4). The results match the findings of Cervantes-Sánchez and Sotelo-Boyas (2002), who reported that this species usually remains dormant from November to April and enters a vegetative stage from May to October, before it flowers from June to September.

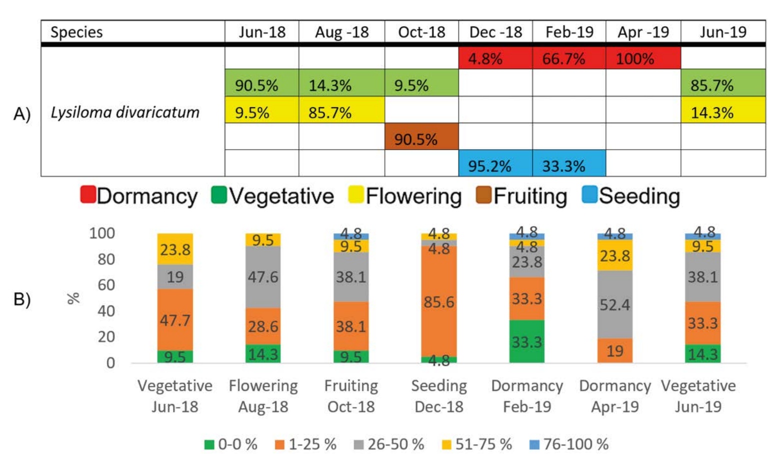


**Figure 4**. Bimonthly phenological stages from June 2018 to July 2019.

## CONCLUSIONS

The structural characteristics of the four woody species analyzed from *ejido* El Limón are similar to what has been described for this type of vegetation in other locations in Mexico.

The most important —due to its structural contribution to the community— and most widely distributed species was Lysiloma divaricatum. This tree has multiple uses (firewood, living fence, or posts), which may favor its use. The quantification of the phenology allows to determine the availability of the fruits and seeds of each site, tree, and species.

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Colegio de Postgraduados

# Postharvest characteristics of Ataulfo mango grown in Soconusco, Chiapas

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**ABSTRACT**

**Objective**: To determine the main evolution indexes of the postharvest ripening of the Ataulfo mango variety grown in Soconusco, Chiapas, Mexico.

**Design/Methodology/Approach**: The variables determined were, mango skin color (B*, a*, and b*), starch index, total soluble solids (TSS) (°Brix), pH, fruit firmness, and color/skin firmness correlation.

**Results**: As the fruit ripeness evolution advances from stage 1 to stage 5, the values of the B*, a*, and b* (color) variables, the TSS, and the pH also increase; however, starch concentration and skin firmness decrease. The negative correlation between the B*, a*, and b* (color) and the fruit firmness,suggests the use of the color variable to measure the ripeness of the Ataulfo variety.

**Study Limitations/Implications**: The ripeness evolution of mango has been the subject of several studies, focused on different varieties (*e.g.*, Tommy Atkins, Keitt, Alphonso, etc.), particularly in Ataulfo mangoes grown in different regions of Mexico. Understanding the physicochemical evolution of the Ataulfo mango grown in Soconusco, Chiapas is fundamental for the producers.

**Finding/Conclusions**: The results obtained could help to determine the ripeness state of the Ataulfo mango, under the environmental conditions of Soconusco, Chiapas.

**Keywords**: Color, °Brix, skin firmness, pH, starch.

**Citation**: Salvador-Figueroa, M., Rosas-Quijano, R., Martínez-Luzuriaga, J. D., Gómez-Estrada, J. A., Vázquez-Ovando, A., & Gálvez-López, D. (2023). Postharvest characteristics of Ataulfo mango grown in Soconusco, Chiapas. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2544

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: February 11, 2023.
**Accepted**: May 15, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 57-71.



## INTRODUCTION

Ataulfo mango (*Mangifera indica* L.) is native from Tapachula, municipality of Tapachula, in the southern Mexican state of Chiapas, Mexico. As a result of its organoleptic characteristics, the export quotas of this variety have increased. Additionally, mango is a tropical fruit that is mainly harvested at the beginning of its physiological ripeness, in order to minimize the quality loss resulting from the post-harvest handling and transportation (Padda *et al.*, 2011). During the beginning of its physiological ripeness, mango has a hard or firm texture, a low soluble solids content, and a high acidity. Consequently, it requires a ripeness period to achieve organoleptic maturity —*i.e.*, the desired flavor and texture at the moment of consumption (Cortés *et al.*, 2016; Ibarra-Garza *et al.*, 2015).

Mexico has a protected appellation of origin for some of its products. This "protected appellation origin" label belongs to the geographical location where a specific good is

Image by u_e5c33shc37 at Pixabay

grown or produced. The label establishes a certain production and sowing process and specific characteristics. The goods are considered as a unique product that must be legally protected. The Ataulfo mango was awarded the "Ataulfo mango of Soconusco, Chiapas" protected appellation origin in 2012 (Mendoza-Hernández *et al.*, 2020; DOF, 2012).

The ripening process of mango includes a series of significant molecular, physiological, and biochemical changes. These changes cause ethylene production, an increase in the respiration rate, a remodeling of structural polysaccharides through a coordinated action of the enzymes that cause fruit softening —the result of the progressive depolymerization of pectic and hemicellulosic polysaccharides, which causes a significant galactose, arabinose, and mannose residues loss—, chlorophyll degradation, and carotenoid biosynthesis (Gill *et al.*, 2017; Lawson *et al.*, 2019; Yashoda *et al.*, 2005).

Among the thousand mango varieties grown worldwide, the commercial brands that dominate the market include Tommy Atkins, Haden, Kent, Keitt, Alphonso, and Ataulfo (Lawson *et al.*, 2019). The Mexican states with the largest sowing areas are Chiapas, Sinaloa, and Guerrero. Meanwhile, the most productive state is Sinaloa (>423,517 t), followed by Guerrero (404,561 t), Nayarit (334,914 t), and Chiapas (272,384 t) (SIAP, 2021). Mango exports from Mexico to USA reached 312,390.42 and 346,250.42 tonnes in 2021 and 2022, respectively (Agtools, 2023). Sixty-five percent of the mangoes produced in Mexico is consumed in the USA. Ataulfo is the most produced mango variety in Mexico and it has the highest volume of exportation (Sánchez *et al.*, 2017). Ataulfo mango is grown all along the Pacific coast, from Chiapas to southern Sinaloa.

The protected appellation origin of the Ataulfo mango of Soconusco, Chiapas (Mendoza-Hernández *et al.*, 2020; Villa-Corrales *et al.*, 2010) establishes all the quality parameters for its commercialization (NOM-188-SCFI-2012). Additionally, the post-harvest quality criteria of various mango varieties have been widely described. The main physicochemical attributes of mango related to fruit ripeness quality are pulp firmness, total soluble solids content, and the aroma volatile compounds (Ellong *et al.*, 2015; Liguori *et al.*, 2020). The National Mango Board (NMB) —an agency that defines fruit quality for the American market— has established specific reception and ripeness protocols for Ataulfo mango. These protocols are based on a fruit ripeness guideline, which includes such parameters as the visual index of the pulp color on mangoes cut by half, range of the soluble solids content, and pulp firmness (NMB, 2015). Although these physicochemical criteria are available for Ataulfo mango, developing *in situ* criteria to determine the ripeness evolution of this variety could be useful for local producers. This process would establish the ripeness degree of a recently harvested fruit under the same weather conditions of the region, before it is included in the commercialization process.

Fruit ripeness evolution has been studied in other fruit species such as papaya, tomato, apple, banana, and pear (Basulto *et al.*, 2009; Okiror *et al.*, 2017; Zhang *et al.*, 2021; Li *et al.*, 2011; Saquet 2019) and their findings are used today as criteria to define the commercial quality of those fruits. Currently, most of the researches about mango ripeness have focused on the Tommy Atkins, Keitt, and Alphonso varieties, among others. However, post-harvest quality can differ depending on the variety (Lawson *et al.*, 2019); consequently, specific studies about every local mango variety are required. In this regard, producers must be aware of the evolution process of the Ataulfo's physicochemical criteria. Therefore, the objective of this study was to determine the main post-harvest ripeness evolution indexes for Ataulfo mangoes grown in Soconusco, Chiapas, establishing alternative measures at harvesting time.

## MATERIALS AND METHODS

### Biological material

Ataulfo mangoes were collected from four certified orchards, located in the municipalities of Acapetahua (15.27985 N, 92.94173 W), Frontera Hidalgo (14.79168 N, 92.21614 W), Huehuetán (15.02958 N, 92.34744 W), and Tapachula (14.85765 N, 92.34577 W). These municipalities constitute the protected appellation origin area of the Ataulfo mango in Soconusco, Chiapas (DOF, 2012). The fruits were sampled from January to June, 2022, and their physiological ripeness was determined according to the ripeness guide of the NMB. In order to avoid variability, 15 fruits (caliber 6) were collected exclusively from one tree per municipality.

The biological material was transported to the Genética de Poblaciones laboratory of the Instituto de Biociencias-UNACH. Afterwards, the samples were washed with water and detergent and were kept at room temperature in order to carry out the analysis. Subsequently, the following stages were established: stage 1 (day 0 or cutting day); stage 2 (day 3 after the harvest (DAH)); stage 3 (5 DAH); stage 4 (7 DAH); and stage 5 (9 DAH). The fruits were kept constantly at room temperature (27 °C average) until the analysis was completed.

### Determination of the skin color

The color variable was analyzed following the methodology proposed by Lawson *et al.* (2019), using a MiniScan EZ Hunter Lab colorimeter and quantifying the B*, a*, and b* indexes (*i.e.*, the CIELAB color space). The measurements were carried out in three areas of the fruit, along its longitudinal axis: apical, central, and basal. These indexes are located within the color chromaticity, where B* indicates luminosity-brightness (from 0 = black to 100 = white); a* indicates color directions (+a* = red, −a* = green); and b* indicates color directions (+b* = yellow, -b* = blue). Additionally, the exterior of the fruits was monitored using a Nikon D3300 professional camera. The pictures were taken at a distance of 30 cm from the fruits.

### Starch analysis

The starch analysis was carried out using the iodine-starch test (Pitts and Cavalieri, 1988). This test consists of the reaction created by the iodine in contact with the starch granules; the latter are transformed into sugar, thus resulting in a dark purple (almost black) coloration of the pulp. The lower the amount of starch, the lower the coloration. In order to carry out this test, the fruit was cut lengthways, very close to the seed, and, using tweezers, the fruit was placed face down in a stray with a 0.5% I2-KI solution. The internal face was observed after 5 minutes. The results were photographed with a Nikon D3300 professional camera. The pictures were taken 30 cm apart from the fruits.

**Determining the physicochemical variables**

The physicochemical variables analyzed included total soluble solids (TSS) (°Brix), pH, and firmness (N). Each evaluation was carried out using 15 fruits per tree as replicates and each sample was analyzed in triplicate for all the measurements.

**Total soluble solids (°Brix)**

The TSS (°Brix) were evaluated according to the NOM-188-SCFI-2012, using a digital refractometer (ATAGO). The TSS was determined cutting the pulp in small portions and then pressing and squeezing the portions in order to obtain a concentrated juice, which was finally filtered. The reading of the concentrated juice of each fruit was performed in triplicate.

**Determining the pH**

The pH was measured using a HANNA digital potentiometer, following the method proposed by Maldonado-Astudillo *et al.* (2016). The pulp was homogenized using a common blender; a 25 g solution was prepared from the pulp for each of the five ripeness stages and distilled water was poured into a volumetric flask until the mixture reached 100 mL. Afterwards, the suspension was filtered, and the result was used to determine the pH.

**Firmness**

Firmness is one of the quality measures for fruits and vegetables. This variable was expressed in Newtons (N) and was evaluated using a LLOYD Instruments Plus® texture analyzer with a 2-mm wide plunger. The measurements were carried out in the equatorial sector of the unpeeled fruit. The parameters were: preload/tension (5.6000 N); preload/tension/speed (30.000 mm/min); speed (30.000 mm/min); limit (30.000 mm); and length of the test (30.000 s).

**Statistical analysis**

All the measured variables were subjected to an analysis of variance (ANOVA) and an analysis of mean differences (Tukey's Test; $\alpha<0.05$), using the Infostat v. 2016 software (Di Rienzo *et al.*, 2016). Additionally, the skin firmness with B*, a*, and b* color and the skin during the different ripeness stages of the fruit were subjected to a correlation analysis. The Infostat v. 2016 software was used for this purpose, taking into account a significance value of $p<0.05$.

**RESULTS AND DISCUSSION**

**Determining skin color**

During the ripening process, skin brightness (B*) increased from stage 1 to stage 4, with mean values of 59.77and 67.65, respectively. Subsequently, a reduction of the mean value (65.5) was recorded during stage 5 (Figure 1A). There were significant differences between the ripeness stages ($p<0.005$), but not between municipalities.

Meanwhile, the a* values of the skin recorded significative differences between the ripeness stages ($p<0.005$), but there were no differences between municipalities. In average, an increase was recorded from stage 1 (−3.98; unripe fruit) to stage 5 (20.88; yellow fruits). These values showed clear changes in every ripeness stage. The values were negative when the fruit was green (stages 1 and 2); however, the values became positive in stages 3, 4, and 5. These results indicate that the a* skin value can be a useful indicator during the monitoring stages of fruit ripening (Figure 1B).

Meanwhile, the b* values of the skin increased from stage 1 to 4, with mean values of 59.77 and 67.65, respectively. A reduction was recorded during stage 5 (Figure 1C). The skin color of the fruit changed from green to yellow during the ripeness stage. Significative differences were recorded between ripeness stages ($p<0.005$), but not between municipalities.

**Starch analysis**

As the ripeness physiology of the Ataulfo mango advanced, the fruit changed its color, recording the highest starch degradation during stages 1, 2, and 3 (Figure 2). Meanwhile, starch degradation was lower or null during stages 4 and 5. On the one hand, when Ataulfo mango is unripe —*i.e.*, it has not reached the cutting point—, the pulp is white and, therefore, the starch test turns the fruit completely black. On the other hand, during the cutting point, the amount of starch accumulated in the mesocarp is high and, consequently, the color is dark with some yellow lines. At this point, the °Brix are low (5 °Brix in average); however, this is a sign that the degradation of the accumulated starch has started, transforming it

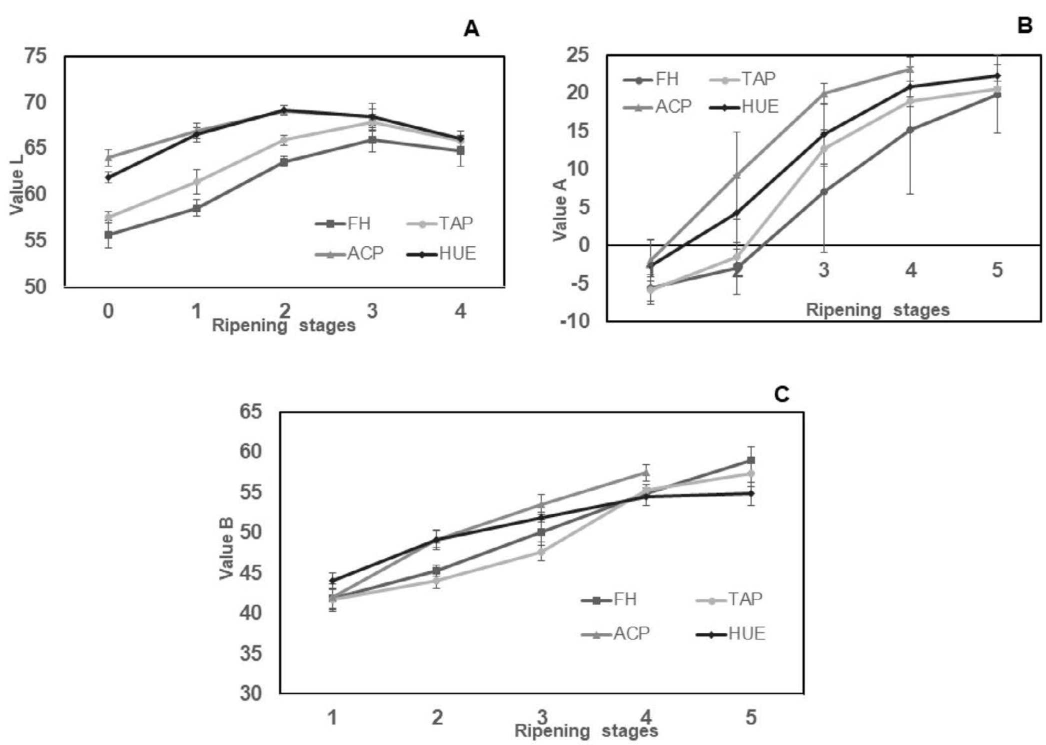


**Figure 1**. Skin color of the Ataulfo mango during different ripeness stages, in Soconusco, Chiapas: Frontera Hidalgo (FH), Tapachula (TAP), Acapetahua (ACP), and Huehuetán (HUE). A) B* skin brightness. B) a* skin color components. C) b* skin color components.

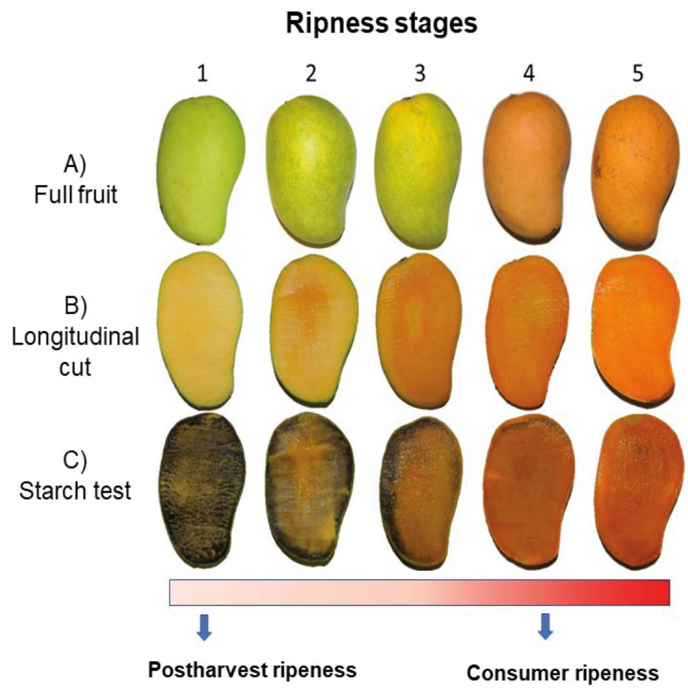


**Figure 2**. A) Visual appearance of uncut Ataulfo mangoes during each ripeness stage. B) Visual appearance of the insides of Ataulfo mangoes during each ripeness stage. C) Color of the fruit during the starch test.

into soluble sugars. In contrast, during the last ripening phase, the coloration of the starch is almost non-existent, because the fruits have reached their maximum ripeness and all the starch has become soluble sugars. This is the point where the desirable degree of sweetness is reached.

## Determining the physicochemical variables

### Total soluble solids

Significative differences were found ($p<0.005$) regarding the sugar content (°Brix) during the ripeness evolution of the Ataulfo fruit. Meanwhile, °Brix increased from stage 1 (average 8.14) to stage 4 (20.53). However, sugar concentration decreased in stage 5 (19.10 °Brix) (Figure 3).

### Determining the pH

The pH variable recorded significant differences ($p<0.005$) between the different ripeness stages. The pH increased from stage 1 to stage 5, reaching 3.93 and 5.67, respectively (Figure 4).

### Firmness

There were significant differences ($p<0.05$) regarding the firmness values of the stages evaluated in the different municipalities. Skin firmness values reached their peak during stage 1 (a 286.34 N average value). However, firmness values drastically decreased (50.24 N) during stage 5. As the ripeness days advanced, ripeness values decreased (Figure 5).

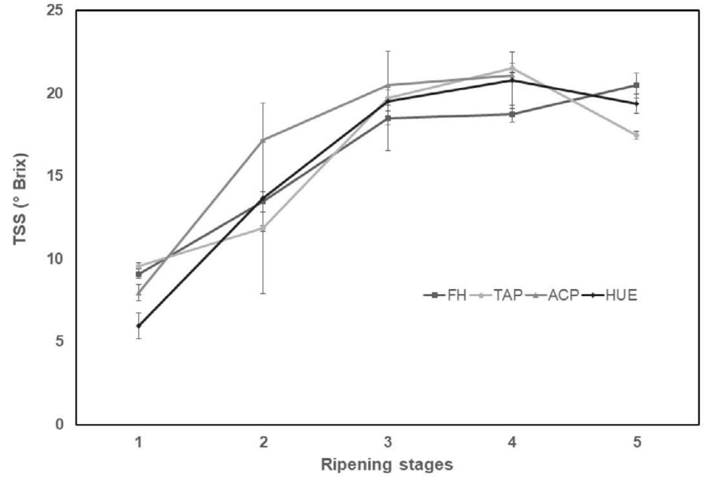


**Figure 3**. Total soluble solids (°Brix) during the different ripeness stages in the municipalities of Frontera Hidalgo (FH), Tapachula (TAP), Acapetahua (ACP), and Huehuetán (HUE).

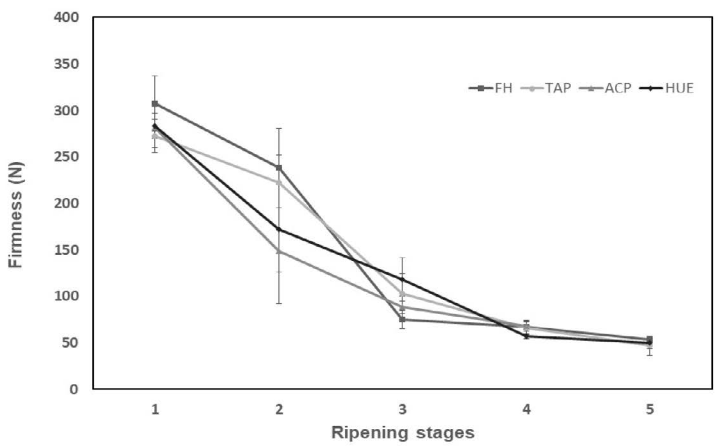


**Figure 4**. pH of the pulp of the Ataulfo mango during different ripeness stages in the municipalities of Frontera Hidalgo (FH), Tapachula (TAP), Acapetahua (ACP), and Huehuetán (HUE).

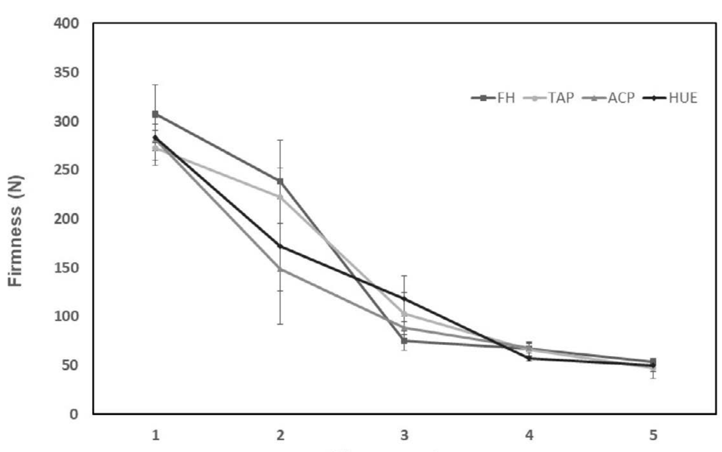


**Figure 5**. Skin firmness of Ataulfo mangoes during different ripeness stages in the municipalities of Frontera Hidalgo (FH), Tapachula (TAP), Acapetahua (ACP), and Huehuetán (HUE).

**Skin firmness/skin color correlation**

There is a highly significative negative correlation in all the municipalities between skin firmness and fruit color (B*, a*, and b*) (Table 1). As the ripeness evolves, the fruit loses its firmness and the color variable increases. Therefore, a lower firmness entails a higher brightness (B*), reduces the direction of green (a*); and increases yellow (b*).

Several authors have described the post-harvest quality criteria of different varieties of mangoes. The National Mango Board (NMB) has defined specific reception and ripeness protocols for the Ataulfo mango and its approval for the American market. These protocols are based on fruit ripeness guidelines, which include parameters such as: visual index of the color of the pulp (fruit cut in half), ranges of the soluble solids content, and pulp firmness. However, the color index only works if the guidelines are used in an appropriate manner (National Mango Board, 2015). These parameters are used by both exporters and marketers in Mexico and by the NMB in USA. Nevertheless, there are no guidelines for the ripeness evolution of the Ataulfo mango in Soconusco, Chiapas. Such guidelines could help to establish the ripeness degree of a freshly cut fruit, before it becomes part of the commercialization process.

Studies about the ripeness evolution of other fruits have also been carried out with other species. These fruits have included papaya (Santamaría-Basulto *et al.*, 2009), tomato (Okiror *et al.*, 2017), apple (Zhang *et al.*, 2021), banana (Li *et al.*, 2011), and pear (Saquet, 2019). This study shows that the evaluated parameters prove the ripeness evolution of the Ataulfo mango. Our results were able to determine the ripeness degree of the fruit with greater accuracy.

**Table 1**. Correlation between skin firmness and the B*, a*, and b* color variables during the fruit ripening.

| Municipality | | Firmness (N) | Value A | Value L | Value B |
|---|---|---|---|---|---|
| Frontera Hidalgo | Firmness (N) | 1 | −0.876** | −0.962** | −0.884** |
| | Value A | | 1 | 0.853** | 0.941** |
| | Value L | | | 1 | 0.872** |
| | Value B | | | | 1 |
| Tapachula | Firmness (N) | 1 | −0.964** | −0.926** | −0.914** |
| | Value A | | 1 | 0.853** | 0.942** |
| | Value L | | | 1 | 0.814** |
| | Value B | | | | 1 |
| Acapetahua | Firmness (N) | 1 | −0.963** | −0.949** | −0.923** |
| | Value A | | 1 | .945** | .905** |
| | Value L | | | 1 | .857** |
| | Valor B | | | | 1 |
| Huehuetán | Firmness (N) | 1 | −.773** | −.620* | −.891** |
| | Value A | | 1 | .660** | .923** |
| | Value L | | | 1 | .698** |
| | Value B | | | | 1 |

** The correlation is significant at the 0.01 level (bilateral).

The skin color of Ataulfo mangoes is an empirical parameter used by producers in the production areas. Producers base their decisions on the color or pigmentation of the fruits or in the degree to which the peduncle is split at the shoulder of the fruit. However, sometimes fruits do not reach the ripeness degree desired by the market and, consequently, they are rejected. Although other models have also used a colorimeter to measure skin color, marketers do not trust that this test can define the post-harvest ripeness of the fruits. If the Ataulfo mangoes are not harvested at the appropriate post-harvest ripeness time, they will never reach the desirable color values. Additionally, the visual attribute of fruit skin color defines the preference of the consumers in certain countries (Kayesh *et al.*, 2013), particularly regarding Ataulfo mango: if the fruits do not reach the market with the desired color (yellow), the costumer will not buy them.

Color development is an important phenomenon that the fruit undergoes in the various stages of its physiological ripeness (Hernández-Estrada *et al.*, 2022; Kapoor *et al.*, 2022). There were significant differences in this study regarding the B* variable; nevertheless, they were similar to those recorded by Rivera-Castro *et al.* (2022), who registered 50.23 to 68.90 values for Ataulfo mangoes grown in the state of Guerrero. Our results are also similar to the findings of Karanjalker *et al.* (2018), who reported that brightness was significatively higher (62.86) for the yellow Arka Anmol variety than for the green Amrapali variety. Meanwhile, the lowest value was recorded for the red Janardhan Pasand variety. Regarding the a* variable, the results of the Ataulfo mango of Chiapas match the Amrapali varieties, whose negative values for stages 1 and 2 indicate an unripen fruit (−8.66); however, these results differ from the findings of Liu *et al.* (2013), who reported values of 10.5, 10.87, 10.31, and 9.80 for the Keitt, Irwin, Tainong No. 1, and JinHwang varieties, respectively. These differences can be attributed to the different shades of red that characterize the skin of these varieties, while the skin of the Ataulfo mango is yellow. Meanwhile, the b* values reported in this study are supported by the work of Rivera-Castro *et al.* (2022), who reported 40.34-52.30 values for Ataulfo mango during stages 1 and 5. These values are also similar to the findings of Karanjalker *et al.* (2018), who recorded that the highest value (48.86) was obtained by the Arka Anmol variety. They also reported a significatively lower value for the Amrapali and Janardhan Pasand varieties. These results also match the findings of Liu *et al.* (2013), who recorded similar results, obtaining values of 49.67, 52.39, 48.01, and 52.27 for the Taining No. 1, Irwin, JinHwang, and Keitt varieties, respectively.

Meanwhile, the starch metabolism of fleshy fruits is a complex and dynamic process, characterized by its accumulation and degradation (Thammawong and Arakawa, 2007). On the one hand, starch accumulation takes place during fruit development and until fruits have completed their cellular division —*i.e.*, when they stop growing (Nardozza *et al.* 2010, 2013). On the other hand, starch degradation starts when fruits begin their ripeness process. Consequently, almost all the starch of the fruits has transformed into soluble sugars after the harvest, increasing and providing the sweetness associated with a ripe fruit (Cordenunsi-Lysenko *et al.*, 2019). In the case of climateric fruits such as mango, ethylene production and respiration rate increase after the harvest and until the fruit reaches its complete ripeness. This process is simultaneously associated to the starch content loss. Shafique *et al.* (2006) reported that, as the sugar levels of the fruits slowly increase, the tritatable acidity rate slowly increases until it reaches its the maximum value, before it ultimately decreases.

Doerflinger *et al.* (2015) applied a tincture to Gala, Honeycrisp, McIntosh, and Empire apple varieties in order to observe their starch concentration and found that starch concentrations linearly diminished after the post-harvest. These results match the findings of Zhang *et al.* (2021), who studied Fuji apples and proved that the apple quality indicators are significatively different during different harvest ripeness stages. Additionally, they reported that the starch index in apples gradually diminished during different post-harvest ripeness stages. This test is very frequently used by apple producers and marketers. It is a highly trustable test and it is unexpensive. Consequently, it could be used to complement the studies about the ripeness of Ataulfo mango. This test has also been used for the color evolution analysis in fruits such as tomato (Huang *et al.*, 2017; Steinhauser *et al.*, 2010), melon (Dai *et al.*, 2011), and peach (Lombardo *et al.*, 2011).

Meanwhile, the TSS and the sugar/acid indexes (TSS/AT) are used as primary indexes to determine the fruit quality of the acceptable ripeness state for commercialization. The minimum requirements to accept the fruits specify the appropriate TSS concentrations and the TSS/AT proportions for each crop (Daniels *et al.*, 2019). These concentrations depend on the solubilization of the sugars in the pulp, which will be the key for the final sweetness of the fruit. The NMB defines the °Brix range for the different mango varieties, according to their ripeness degree. For example, the initial indexes for the Tommy Atkins and Keitt varieties have ranges from 5 to 7, while the Kent, Haden, and Francis varieties have an acceptance range of 6 to 8 or 9. These concentrations guarantee the physiological ripeness of the fruit (National Mango Board, 2015).

In the case of the Ataulfo variety, the NMX-FF-058-SCFI-2006 standard indicates that the minimum acceptable value for fruit ripeness is 2.9 °Brix. For its part, the NOM-188-SCFI-2012 standard specifies that the minimum acceptable range for its commercialization is 8 °Brix, while the NMB uses a range of 5-7 °Brix (National Mango Board, 2015). Slightly differences were found in this study between municipalities. These differences can be the result of the genetic nature of the Ataulfo mango (Salvador-Figueroa *et al.*, 2008). However, these data are similar to the results reported by Rivera-Castro *et al.* (2022), who recorded values of 6.02 °Brix for the Ataulfo variety during the cutting stage. Other mango varieties, such as Langra and Khirshapath (Islam *et al.*, 2013), also recorded values similar to those of the Ataulfo mango. Therefore, the NOM-188-SCFI-2012 standard should be updated to include 5 °Brix for the Ataulfo mango.

Regarding the ripeness evolution of the fruit, the TSS values at the cutting stage increased from stage 1 until they reached their maximum values in stage 4 (when it reached its maximum sweetness). Finally, a slight decrease was recorded during stage 5. These results match the findings of Islam *et al.* (2013), who reported that the TSS values (°Brix) of the Langra and Khirshapath varieties evolved with a similar behavior, because the TSS quickly increased from the first day to the sixth day and then significatively decreased. Ranges have already been established for this TSS attribute for several commercial mango varieties; however, it changes depending on the evaluated variety and the region or location of the said variety. Some mango varieties store lower or higher amounts of sugar in the pulp, ranging from 3 to 8 °Brix during physiological ripeness and reaching 23 °Brix when the fruit is in advanced stage of ripeness (Jha *et al.*, 2010). Finally, the NMB establishes a maximum value of 18 °Brix, while the Ataulfo mango of Soconusco reaches up to 20.5 °Brix.

Regarding the pH variable, Ataulfo mango evolved from 3 to 5.67, which matches the findings of Liu *et al.* (2013), who reported pH values of 3.95, 4.56, 4.90, and 5.58, for the Tainong No. 1, Irwing, JinHwang, and Keitt varieties, respectively. The pH values are also similar to those reported by Shafique *et al.* (2006), who recorded 2.5-5.4 pH values for the Fazli, Ashina, Langra, Surjapuri, Khirshapat, Gopalbhog, Kisanbhog, Mohanbhog, Latabombai, and Ranipasand varieties. Cissé *et al.* (2020) recorded 4.8 pH values for the Kent variety, while Islam *et al.* (2013) obtained 3.5-7 pH values for the Langra and Khirshapat varieties. Their results also support the findings of this study. The NMX-FF-058-SCFI-2006 standard and the NOM-188-SCFI-2012 official protected designation origin standard do not mention optimal pH values for the Ataulfo variety; however, understanding the evolution of this variable during the ripeness stage is a useful indicator of fruit quality.

Meanwhile, fruit firmness is an important physical parameter that the mango industry frequently seeks. Additionally, it is widely used to determine the ripeness state of the fruit (Jassi *et al.*, 2019). Depending on the marketer or producer requirements, this variable can be measured using a texture analyzer, which can be fixed (in a lab) or handheld (portable or that can be used on the field). In the case of Ataulfo mango, fruit firmness is not a parameter specified in the NOM-188-SCFI-2012 standard, although, the NMB carries out this test in order to receive the fruits. According to the ripeness guideline of the NMB, fruit firmness must be measured in the pulp of a peeled fruit, using a penetrometer with an 8-mm tip. The values reported by the NMB range from 209 to 97.8 N, during the initial organoleptic maturity, and 13 to 4.4 N when the ripeness state advances. In this study, firmness was measured in an unpeeled fruit. An average of 286 N was recorded for Ataulfo mango in the cutting day. This measure decreased under the environmental conditions of the Soconusco region, reaching 50 N during the last ripeness stage.

The skin firmness of the analyzed fruits of the four municipalities decreased throughout the different ripeness stages. This decrease can be the result of the different metabolic changes that take place during the ripeness process of mango, as a consequence of the ethylene production and the hydrolase enzymes of the polysaccharides. This situation causes several biochemical changes, a respiration rate increase, the remodeling of the structural polysaccharides of the cellular wall, and consequently fruit softening (Gill *et al.*, 2017). The fruits analyzed in this study had similar values to those reported by Cissé *et al.* (2020) for the Kent and Côte d'Ivoire varieties (45-50 N). For their part, Aular and Natale (2013) mentioned that fruit texture has a series of internal and external characteristics that depend on environmental and nutrient factors, as well as on the parameters used to program the equipment used for the analysis (texturometers and tools). Therefore, the skin firmness could be a useful variable in the analysis of fruit quality and should be included in the update of the NOM-188-SCFI-2012 standard for Ataulfo mango.

Finally, in other studies about other climacteric fruits —such as oriental persimmon (Salvador *et al.*, 2006) and papaya (Santamaría-Basulto *et al.*, 2009)—, the correlation between the B*, a*, and b* values and the fruit firmness allowed the use of color as an unequivocal value to measure fruit ripeness. Consequently, according to the high negative correlation found between the firmness of the unpeeled fruit and the color values (B*, a*, and b*), the non-destructive color variable could be used to determine the physiological ripeness of the Ataulfo mango of Soconusco, Chiapas. This variable could also accurately be used in the field, replacing the empirical measurement made by the producers.

## CONCLUSIONS

We established the main ripeness indexes and physicochemical characteristic found during the post-harvest ripeness evolution of the Ataulfo mango variety from Soconusco, Chiapas. According to the obtained results, the generated data are a trustful and easily reproduced guideline that can help producers and marketers to determine the fruit ripeness quality of the Ataulfo mango variety.

## ACKNOWLEDGEMENTS

The CONACYT-APN/2017: 6427 project was funded by the Consejo Nacional de Ciencia y Tecnología. The infrastructure used to carry out this project was acquired through the CONACYT-INFR-2015-01: 254256 project. The authors would like to thank the producers from the certified plantations from the Soconusco region, Chiapas, who provided the fruits for this study.

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Colegio de Postgraduados

# Health and economic crises and the Mexican agricultural sector

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**ABSTRACT**

**Objective**: To determine if the 1994 and 2008 economic crises and the 2009 and 2020 health crises had an impact on the relationship between the price and the production of the main agricultural products in the main producer states of the Mexican agricultural sector.

**Design/Methodology/Approach**: Seventy structural stability tests with dichotomous variables were carried out in order to analyze the effect of these crises on the price-quantity relationship in the main producer states of the five regions in which the Mexican agricultural sector is divided. These states are the main producers of corn, sorghum, and bean grains in Mexico.

**Results**: There were some exceptions but, overall, neither the 1994 and 2008 economic crises, nor the 2009 and 2020 health crises had an impact on the price-production relationship of main products of the main producer states of the five regions of the Mexican agricultural sector.

**Study Limitations/Implications**: We did not evaluate all the products or all the Mexican states.

**Findings/Conclusions**: Overall, economic and health crises did not impact the price-production relationship of the analyzed producer states. The price-production relationship of these states is resilient to economic and health crises —*i.e.*, producers did not significatively alter their production as a consequence of the price changes brought about by the economic and health crises.

**Keywords**: economic crises, health crises, agricultural sector, structural analysis.

**Citation**: Vivanco-Vargas, M., Cruz-Lázaro, L. M. , & Banda-Ortiz, H. (2023). Health and economic crises and the Mexican agricultural sector. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2545

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: March 13, 2023.
**Accepted**: June 15, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 73-82.



## INTRODUCTION

The economic and health crises have an impact on the agricultural sector, because they influence the prices, the demand, and the supply of the agricultural sector, as well as the relationship between these variables. A clear example of this situation is that the economic and health crises have a negative impact on the agricultural workforce and, therefore, a negative impact on the production (supply) of this sector. This situation causes a supply reduction, leading to a price increase (Basurto and Escalante, 2012; Fernández, 2008; Márquez *et al.*, 2006; Rojas, 2009).

Specifically, the 1994 and 2008 economic crises and the 2009 (H1N1) and 2020 (COVID-19) health crises impacted the Mexican agricultural sector. On the one hand, these crises caused a contraction of the demand that affected the economy of the country and had a negative impact on the income of the consumers. On the other hand, the impact

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on the income of the consumers had an impact on the demand. Additionally, producers had less resources to invest and lacked access to credit. Meanwhile, health crises had an impact on the production and demand of agricultural products, as a result of the measures implemented by the governments to contain the epidemic, such closing borders and shutting down the productive sectors (CEPAL, 2020; Ríos, 2020; Ayala and Chapa, 2017; Becerril *et al.*, 2011; Reynoso, 2010; Ortega *et al.*, 2010).

Therefore, the objective of this research was to determine if the 1994 and 2008 economic crises and the 2009 and 2020 health crises had an impact on the price-production relationship of the main agricultural products, in the main producer states of the Mexican agricultural regions.

## THEORETICAL FRAMEWORK

The 1994 and 2008 economic crises impacted the Mexican agricultural sector; the subsequent economic contraction had an impact on the income of the consumers, while the producers did not have resources to invest, because they did not have access to credits. These effects impacted the supply and demand of agricultural products, leading to a negative impact on their prices and production. This situation altered the price-production relationship in the agricultural sector. Additionally, the 2008 crisis caused a contraction of exports (Basurto and Escalante, 2012; Blanke, 2009; Becerril *et al.*, 2011; Gómez, 2008; Tonconi, 2015; Benítez, 2022).

Meanwhile, the 2009 (H1N1) and the 2020 (COVID-19) health crises affected the agricultural sector, as a consequence of the effect that the supply and the demand had on the prices of the products. In this regard, the demand contracted, due to the unemployment and the income contraction resulting from the crisis and the related social restriction measures. It also had a negative impact on the supply, as a result of the measures taken to control the health crisis, which affected the productive structure and lead to a price increase. In order to tackle the crisis, the government closed the borders and shut down the activity of productive sectors. However, the governments tried to minimize the impact of these policies on the agricultural sector and implemented measures aimed to maintain or to increase the agricultural production and to stabilize the prices (CEPAL, 2010, 2020; OCDE-FAO, 2011; Ríos, 2020; Ayala and Chapa, 2017; Obschatko, 2020; Brambila *et al.*, 2014).

Another element to be considered is that the agricultural sector is more resilient to the economic and health crises than other sectors, because the supply and the demand are inelastic. Consequently, the supply and the demand of this sector do not have a significant reaction to changes in the price of products (Basurto and Escalante, 2012; Vilaboa *et al.*, 2021; Cardona *et al.*, 2007; Roitbarg, 2021; Ortega *et al.*, 2010).

## METHODOLOGY

The methodology applied seeks to determine if the 1994 and 2008 economic crises and the 2009 and 2020 health crises had an impact on the price-production relationship of the main agricultural products, in the main producer states of the Mexican agricultural regions. Consequently, seventy structural stability tests with dichotomous variables were carried out. The 1980-2021 price and production annual data bases for the main producer states of the main products of the Mexican agricultural sector was obtained from the Secretaría de Desarrollo y Agricultura (SADER, 2022 a). The price data base was deflated, using the Mexican National Consumer Price (INPC) developed by the Instituto Nacional de Estadística y Geografía (INEGI, 2022 a). The five analyzed regions are the ones used by SADER (2022 b) to oversee the Mexican agricultural sector. The states in which the main products of the agricultural sector (corn, sorghum, and bean grains) are produced were chosen from these regions (SADER, 2022 a b). Table 1 shows the products and the analyzed states in each region.

A structural analysis was carried out to determine if the 1994 and 2008 economic crises and the 2009 and 2020 health crises had an impact on the relationship between price and the production of the main agricultural products in the main producer states of the five regions that make up the Mexican agricultural sector (SADER, 2022 a b). The theoretical basis of this analysis is described in the theoretical framework and the neo-classical economics theory, which has been used to study the Mexican agricultural sector (Cardona *et al*., 2007; Roitbarg, 2021). Table 2 describes the economic and health crises.

**Table 1**. Products and analyzed states by region.

| Region | Corn grain | Bean | Sorghum grain |
|---|---|---|---|
| Northwest | Sinaloa | | |
| Northwest | Chihuahua | Zacatecas | Tamaulipas |
| Center-West | Jalisco | Guanajuato | Guanajuato |
| Center | State of Mexico | Puebla | Morelos |
| South-southeast | Veracruz | Chiapas | Campeche |

Source: table developed by the authors using data from SADER (2022 a b).

**Table 2**. Description of the economic and health crises.

| Year-Crisis | Type | Years before the crisis | Years after the crisis | Description of the crisis |
|---|---|---|---|---|
| 1994 The Tequila Effect | Economic | 1980-1994 | 1994-2021 | In early 1994, the sudden interruption of the flow of foreign capital towards Mexico and the devaluation of the national currency caused an economic contraction. |
| 2008 Subprime Mortage Crisis | Economic | 1980-2007 | 2008-2021 | The international 2008 financial crisis was mainly the consequence of a crisis caused by the low-quality mortgages derivatives in USA. This crisis had global repercussions. |
| 2009 H1N1 Pandemic | Health | 1980-2008 | 2009-2021 | The H1N1 pandemic officially started on March 11, 2009, after the first case was confirmed in Mexico City. From April 2009, this pandemic had an impact on the 32 states of Mexico, leading to an economic crisis. |
| 2020 COVID-19 Pandemic | Health | 1980-2019 | 2020-2021 | To avoid the spread of the COVID 19 disease, many countries closed their borders. Consequently, the exchange of goods and services was reduced in most of the economic sectors, leading to a GDP economic contraction in 2020. |

Source: Table developed by the authors based on data from CEPAL, 2009; Camberos and Bracamontes (2015); De la Luz *et al*. (2015).

## ANALYSIS OF THE STRUCTURAL CHANGE WITH DICHOTOMOUS VARIABLES

Seventy structural analysis tests with dichotomous variables were carried out to determine if there was a structural change in the slope, the ordinate intercept, or in both. Based on the procedure determined by Gujarati and Porter (2010), seventy multiple regressions with dichotomous variables were estimated. The result was Equation 1.

$$Y_t = \alpha_1 + \alpha_2 D_t + B_1 P + B_2 \left(D_t P\right) + B_3 X_1 + B_4 X_2 + u_1 \quad (1)$$

Where: $Y$ = amounted produced variable (one of the three products) in one of the main producer states of the five regions; $\alpha_1$ = is the intercept value; $\alpha_2$ = is the differential intercept value; $D_t$ = is the dichotomous variable, where: 0 is the observation from period 1 to the structural change and 1 is the observation from the structural change to the end of the series; $B_1$ = value of the actual price variable (one of the three products); $P$ = actual price variable (one of the three products); $B_2$ = is the differential slope; $D_tP$ = is the multiplication of the actual price variable times the dichotomous variable; $X_1$ = is the controlled variable of the harvesting area; $X_2$ = is the controlled variable of the economic activity

The cut in the tests will take place according to the dates shown in Table 2. Therefore, the dichotomous variable of the impact analysis before the 2009 (H1N1) health crisis was zero, while it increased to 1 after 2009. Regarding the COVID-19 health crises, the dichotomous variable before 2020 was zero, while after 2020 the dichotomous variable had a value of 1. Meanwhile, the dichotomous variable before the 1994 economic crisis was zero, while after 1994 the dichotomous variable was 1. Finally, before the 2008 economic crises, the dichotomous variable was zero, but it increased to 1 after 2008. Additionally, the following control variables were added to strengthen the analysis: harvested area (SADER, 2022 a) and the Global Indicator of Economy Activity (IGAE, two months average) (INEGI, 2022 b). The periodicity of both variables is the same than the periodicity of the analyzed variables and the deflated IGAE, using the INPC (INEGI, 2022 a).

Once the seventy multiple regressions were estimated (Equation 5), their $R^2$ values were analyzed in order to validate them. For this purpose, >50% values are used as reference. After the validity of the models was established, the *p* values of the differential intercept ($\alpha_2$) and the differential slope ($B_2$) of each model were analyzed. Only <0.05 results were considered statistically significative. If the *p* value of the differential intercept ($\alpha_2$) is <0.05, then the structural change took place in the ordinate intercept. However, if the *p* value of the differential slope ($B_2$) is <0.05, the structural change took place in the slope. Finally, if both are <0.05, the structural change took place in the ordinate intercept and the slope.

## RESULTS AND DISCUSSION

Table 3 shows the results of the structural analysis evaluations used to determine if the 1994 and 2008 economic crises and 2009 and 2020 health crises caused a structural

**Table 3**. Results of the structural analysis evaluations with dichotomous variables.

| **Region** | **Model** | | **1994** | | **2008** | | **2009** | | **2020** | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Beta value** | **P-value** | **Beta value** | **P-value** | **Beta value** | **P-value** | **Beta value** | **P-value** |
| Northwest | Real price of corn kernel in the state of Sinaloa | Differential intercept | -59439.31 | 0.5063 | -283244.7 | 0.0047 | -280387.3 | 0.0056 | 188659.6 | 0.811 |
| | | Differential slope | 29.48359 | 0.0541 | 69.9895 | 0.0071 | 68.23946 | 0.0127 | -75.24794 | 0.7594 |
| | | Coefficient of determination $R^2$ | 0.969521 | | 0.961615 | | 0.96165 | | 0.955297 | |
| | Real price of beans in the state of Sinaloa | Differential intercept | 11413.9 | 0.5944 | 3039.708 | 0.9093 | 8383.427 | 0.7591 | 4014.682 | 0.9717 |
| | | Differential slope | -0.64402 | 0.6272 | 0.130477 | 0.933 | -0.036013 | 0.9818 | -0.38968 | 0.9537 |
| | | Coefficient of determination $R^2$ | 0.862518 | | 0.862643 | | 0.864312 | | 0.861621 | |
| | Real price of sorghum kernel in the state of Sinaloa | Differential intercept | 128415.5 | 0.0222 | -112198.8 | 0.1691 | -94128.72 | 0.2157 | 159530.6 | 0.7838 |
| | | Differential slope | -8.407028 | 0.5329 | 6.482687 | 0.793 | -1.947047 | 0.933 | -64.93095 | 0.7462 |
| | | Coefficient of determination $R^2$ | 0.771865 | | 0.701326 | | 0.740756 | | 0.624008 | |
| Northeast | Real price of corn kernel in the state of Chihuahua | Differential intercept | 259662.1 | 0.1204 | 5805.61 | 0.9652 | 33012.08 | 0.8019 | 189216.9 | 0.515 |
| | | Differential slope | -40.12901 | 0.1014 | -20.65636 | 0.5402 | -31.91615 | 0.3533 | -60.20869 | 0.4398 |
| | | Coefficient of determination $R^2$ | 0.525507 | | 0.520508 | | 0.540048 | | 0.502766 | |
| | Real price of beans in the state of Zacatecas | Differential intercept | -98276.22 | 0.2962 | 78637.39 | 0.3527 | 87558.55 | 0.3045 | -29129.37 | 0.912 |
| | | Differential slope | 7.004287 | 0.1958 | -9.615242 | 0.1623 | -9.356099 | 0.1756 | 5.98185 | 0.7793 |
| | | Coefficient of determination $R^2$ | 0.816137 | | 0.819495 | | 0.817231 | | 0.811483 | |
| | Real price of sorghum kernel in the state of Tamaulipas | Differential intercept | 215661.3 | 0.0758 | -320043 | 0.1218 | -312505.2 | 0.1258 | -511860.3 | 0.4367 |
| | | Differential slope | -5.714089 | 0.8391 | 71.99688 | 0.2578 | 67.20681 | 0.2891 | 174.7614 | 0.4286 |
| | | Coefficient of determination $R^2$ | 0.727336 | | 0.641589 | | 0.646987 | | 0.613503 | |
| Center-West | Real price of corn kernel in the state of Jalisco | Differential intercept | 128601.3 | 0.1328 | -37297.35 | 0.7034 | -31001.35 | 0.7516 | 127376.4 | 0.5568 |
| | | Differential slope | -10.0009 | 0.5057 | 4.787082 | 0.8463 | 5.761058 | 0.8165 | -34.11174 | 0.5572 |
| | | Coefficient of determination $R^2$ | 0.827216 | | 0.79518 | | 0.793549 | | 0.794665 | |
| | Real price of beans in the state of Guanajuato | Differential intercept | -8640.629 | 0.6576 | -5710.094 | 0.7887 | -4447.371 | 0.8348 | 32272.51 | 0.545 |
| | | Differential slope | 0.563108 | 0.5782 | -0.163361 | 0.931 | -0.204689 | 0.915 | -3.138259 | 0.5168 |
| | | Coefficient of determination $R^2$ | 0.830192 | | 0.834509 | | 0.833445 | | 0.830657 | |
| | Real price of sorghum kernel in the state of Guanajuato | Differential intercept | 7062.67 | 0.8378 | 7608.88 | 0.069 | 84079.1 | 0.0496 | 77491.15 | 0.5691 |
| | | Differential slope | -11.56745 | 0.1422 | -18.59756 | 0.1106 | -19.54512 | 0.0912 | -19.57048 | 0.6013 |
| | | Coefficient of determination $R^2$ | 0.796583 | | 0.768321 | | 0.772127 | | 0.748515 | |

Table 3. Continues...

| Region | Model | | 1994 | | 2008 | | 2009 | | 2020 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Beta value | P-value | Beta value | P-value | Beta value | P-value | Beta value | P-value |
| Center | Real price of corn kernel in the state of México | Differential intercept | 190659.6 | 0.3632 | -785667.5 | 0.0208 | -857702.4 | 0.0186 | 184266.4 | 0.9604 |
| | | Differential slope | 190659.6 | 0.3632 | 381.9905 | 0.0496 | 351.6687 | 0.0307 | 14.91217 | 0.9861 |
| | | Coefficient of determination $R^2$ | 0.688453 | | 0.72508 | | 0.7273 | | 0.691173 | |
| | Real price of beans in the state of Puebla | Differential intercept | 855.7167 | 0.8041 | -5742.959 | 0.3419 | -5111.738 | 0.4403 | 35950.25 | 0.87 |
| | | Differential slope | -0.849161 | 0.9357 | 1.310649 | 0.1751 | 1.069406 | 0.2369 | -2.455735 | 0.8768 |
| | | Coefficient of determination $R^2$ | 0.850657 | | 0.858358 | | 0.856923 | | 0.851512 | |
| | Real price of sorghum kernel in the state of Morelos | Differential intercept | 12547.9 | 0.6137 | 43600.43 | 0.2606 | 19713.95 | 0.5936 | -682543.2 | 0.2299 |
| | | Differential slope | 51.27476 | 0.8968 | -60.69458 | 0.0642 | -41.55941 | 0.1077 | 174.6457 | 0.2173 |
| | | Coefficient of determination $R^2$ | 0.772585 | | 0.795232 | | 0.79968 | | 0.781566 | |
| South-southeast | Real price of corn kernel in the state of Veracruz | Differential intercept | 15888.65 | 0.8219 | 146107.9 | 0.1163 | 83421.09 | 0.3889 | 7228.048 | 0.9324 |
| | | Differential slope | -888.0041 | 0.1209 | -42.53137 | 0.4461 | -88.71212 | 0.0539 | -6.855907 | 0.7533 |
| | | Coefficient of determination $R^2$ | 0.924621 | | 0.924701 | | 0.928601 | | 0.918344 | |
| | Real price of beans in the state of Chiapas | Differential intercept | -7227.075 | 0.2606 | 6467.361 | 0.4631 | -2061.854 | 0.8601 | -159226.3 | 0.5614 |
| | | Differential slope | 15.18808 | 0.4531 | -2.754115 | 0.0632 | -1.33232 | 0.3345 | 11.48052 | 0.5597 |
| | | Coefficient of determination $R^2$ | 0.874855 | | 0.886231 | | 0.884342 | | 0.884342 | |
| | Real price of sorghum kernel in the state of Campeche | Differential intercept | -5976.195 | 0.3446 | -24302.11 | 0.0917 | -21414.12 | 0.14 | 42917.9 | 0.2837 |
| | | Differential slope | 0.374026 | 0.8056 | 6.244166 | 0.0715 | 5.881178 | 0.0914 | -14.33184 | 0.2466 |
| | | Coefficient of determination $R^2$ | 0.978857 | | 0.979391 | | 0.979394 | | 0.978594 | |

Source: Table developed by the authors based on data from Eviews.

change in the price-production relationship for the main products, in the main producer states of the five regions of the Mexican agricultural sector.

The results in Table 3 show that the models are valid because the $R^2$ has >0.50 values. Additionally, except for a few cases, the 1994 and 2008 economic crises and the 2009 and 2020 health crises did not cause a structural change in the differential intercept and the differential slope ($p$>0.05), regarding the price-production relationship of the main producer states of the five regions of the Mexican agricultural sector. Table 4 shows the exceptions found in the study.

**Table 4**. Exceptions.

| Crises | Exceptions | Place where the change took place | value |
|---|---|---|---|
| 1994 Economic Crisis | Actual price of sorghum grain in the State of Sinaloa. | In the differential intercept | The value of the differential intercept is <0.05. |
| 2008 Economic Crisis | Actual price of corn grain in the State of Sinaloa. | In the intercept and the slope. | The values of the differential slope and differential intercept are <0.05. |
| | Actual price of corn grain in the State of Mexico. | | |
| 2009 Health Crisis | Actual price of corn grain in the State of Sinaloa. | In the intercept and the slope. | The values of the differential slope and the differential intercept are <0.05. |
| | Actual price of corn grain in the State of Mexico. | | |
| | Actual price of sorghum grain in the State of Guanajuato. | In the intercept. | The values of the differential intercept are >0.05. |
| 2020 Health Crisis | There were no exceptions | | |

Source: Table developed by the authors.

Therefore, the results indicate that, except for a few cases, the economic and the health crises do not have an impact on the price-production relationship of the main producer states of the five regions of the Mexican agricultural sector. These states are the main producers of corn, sorghum, and bean grains. Our results are different from the findings of other researches about this subject (Basurto and Escalante, 2012; OCDE-FAO, 2011; Brambila *et al.*, 2014; Tonconi, 2015; Fernández, 2008; Flores, 2014; Méndez, 2011; Guzmán *et al.*, 2012; García, 2020; Benítez, 2022; Rojas 2009; Ortega *et al.*, 2010). These studies indicate that the economic and health crises have an impact on the price-production relationship of the product of the agricultural sector.

On the one hand, our results differ from the findings of Basurto and Escalante (2012), Becerril *et al.* (2011), Gómez (2008), Reynoso (2010), Ortega *et al.* (2010), and Blanke (2009). These authors indicated that the 1994 and 2008 economic crises —which had an impact on the economy, the supply, and the demand— affected the price-production relationship in the Mexican agricultural sector. Additionally, our results differ from the findings of CEPAL (2020), OECD-FAO (2011), Aparicio and Delgado (2009), Ayala and Chapa (2017), and Ríos (2020). These institutions and authors pointed out that the 2009 (H1N1) and 2020 (COVID-19) health crises had an impact on the price-production relationship of the Mexican agricultural sector.

On the other hand, our results about the 2020 (COVID-19) health crisis match the results of Vilaboa *et al.* (2021), Reynoso (2010), Brambila *et al.* (2014), Sosa and Ruíz (2017), Flores (2014), Orozco *et al.* (2017), and Obschatko (2020). These authors indicated that the agricultural sector was resilient to the crisis. Given its importance, the policies implemented to contain the pandemic were less severe for this sector. Additionally, measures aimed at maintaining or increasing the agricultural production during the health crises were implemented.

Basurto and Escalante (2012), OECD-FAO (2011), Roitbarg (2021) and Vilaboa *et al.* (2021) pointed out that the agricultural sector was more resilient to the economic and health crises than other sectors. Cardona *et al.* (2007), Roitbarg (2021), and Ortega *et al.* (2010) pointed out that the supply and the demand of the agricultural sector are inelastic. Consequently, the supply and the demand of this sector do not have significant reactions to changes in the price of products, because, even in the face of such changes, society keeps demanding the products.

## CONCLUSIONS

The objective of this research was to determine if the 1994 and 2008 economic crises and the 2009 and 2020 health crises had an impact on the relationship between the price and the production of the main agricultural products in the main producer states of the five regions of the Mexican agricultural sector. Seventy structural stability tests with dichotomous variables were carried out. The five analyzed regions are the ones used by SADER (2022 b) to oversee the Mexican agricultural sector. The main producer states of each of the five regions were chosen, because they grow the main products of the agricultural sector (corn, sorghum, and bean grains). The results of the structural analysis evaluations indicate that the 1994 and 2008 economic crises and the 2009 and 2020 health crises did not cause structural changes, except for the following four cases: one during the 2009 health crisis, one case during the 1994 economic crisis, and two during the 2008 economic crisis.

Overall, the economic and health crises did not have an impact on the price-production relationship in the main producer states of the five regions of the Mexican agricultural sector. Therefore, the price-production relationship of the Mexican agricultural sector is resilient to economic and health crises —*i.e.*, the producers do not significatively alter their production in reaction to price changes resulting from disturbances caused by such crises. Therefore, the production of the agricultural sector is inelastic. The results about the non-existent relationship contradict several studies mentioned in the theoretical framework and the discussion section; however, some previous studies are consistent with the results of our study.

Therefore, we consider that the objective of this study was achieved. There were some limitations in this research: we did not evaluate all the products or all the states of the Mexican agricultural sector. Further research should focus on the structural analysis evaluations of other relationships and variables of the agricultural sector.

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# Multifunctionality in maize production systems in the Mixteca Alta region of Oaxaca

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**ABSTRACT**

**Objective**: To infer the role of multifunctionality in the milpa systems of the Mixteca Alta region of Oaxaca, Mexico.

**Design/Methodology/Approach**: The methodological framework of the Multifunctionality Index of Agricultural Production Systems (IMSPA) was used, applying semi-structured interviews to producers.

**Results**: The multifunctionality level of each evaluated systems was determined and the Milpa Intercropped with Fruit Trees (MIAF) showed the greatest multifunctionality and potential attributes for local development.

**Study Limitations/Implications**: The resistance among producers to make changes in their plots usually leads to a refusal to participate and to mistrust this type of research.

**Findings/Conclusions**: It is necessary to follow up on multifunctionality evaluations, since some systems are at a point where their functions can advance or regress.

**Keywords**: Intercropped milpa, MIAF system, multifunctional agriculture.

**Citation**: Marcos-Rivera, U., Ugalde-Lezama, S., Valdés-Velarde, E., Tarango-Arámbula, L. A., & Buendía-Espinoza, J. C. (2023). Multifunctionality in maize production systems in the Mixteca Alta region of Oaxaca. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i4.2548

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: December 15, 2022.
**Accepted**: March 17, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 83-96.



## INTRODUCTION

In recent years, alternative approaches that allow better use of the land have emerged. The multifunctionality of agriculture (aka, multifunctional agriculture) is one those approaches and refers to the ability to generate different types of products and services derived from agricultural practices (Gómez-Limón *et al*., 2008; Silva, 2010). These practices have a direct impact on the economy and society as a whole (Bonnal *et al*., 2003). In addition, multifunctional agriculture directly contributes to well-being and social inclusion, through the increase in the flow of money, ecotourism activities, and the management and appreciation of natural resources (Ikova and Todorova, 2014). From its origin, the concept of multifunctional agriculture (MFA) has drawn the attention of academics, institutions, and countries (Cuevas *et al*., 2015).

Image by Leopictures at Pixabay

MFA has been practiced by small farmers in rural communities as a self-sufficiency and diversification strategy for decades; however, it was not considered in global development, political, and science forums before the Rio Earth Summit of 1992 (Callo-Concha, 2018). In 2001, the Organization for Economic Co-operation and Development (OECD) established the objectives of the MFA, highlighting the preservation of production and consumption, the promotion of agricultural development, and the improvement of the economic benefit of farmers (OECD, 2001).

In Mexico, one of the main multifunctional agricultural systems is the milpa, particularly in rural regions where the main crop is maize, which can be associated with both domesticated and wild species (Cotler-Ávalos and Lazos-Chavero, 2019). This production system has a spatiotemporal dynamic, related to the natural environment (soil, microclimates, nutrients, water), the socio–cultural context (family dynamics, lore, practices, institutional arrangements), and the economic–political aspects (market behavior, labor, development policies). This situation leads to multiple crop management and decisions, making the milpa an exponentially complex and multifunctional agroecosystem (Lazos, 1995; Cotler-Ávalos and Lazos-Chavero, 2019).

Within the Mexican territory, the southeastern region is where maize (*Zea mays* L.) cultivation is most important and it is considered one of the centers of origin of this species (Salinas Moreno *et al.*, 2013). Approximately 90% of the cultivated maize surface of the State of Oaxaca is sown with native maize of different races, colors, textures, and crop cycles (Aragón *et al.*, 2006). There are areas where maize is grown in interaction with other crops and fruit trees (MIAF); this interaction provides more benefits than monocultures (traditional milpa) (Turrent *et al.*, 2017).

The Mixteca Alta region of Oaxaca is a mountain range whose inhabitants cultivate the milpa in a traditional way; nevertheless, some of them are gradually adopting and implementing other multifunctional production systems. The multifunctionality of the milpa in this region has not been evaluated yet, neither with the systems analysis or any other approach. Therefore, the objective of this study was to determine the multifunctionality of milpa systems in the Mixteca Alta region of Oaxaca, Mexico.

## MATERIALS AND METHODS

### Methodological framework of the Multifunctionality Index of Agricultural Production Systems (IMSPA)

The IMSPA is an index made up of two interrelated key concepts: agroecology and sustainable development. It takes up the systemic perspective of agriculture and defines the agricultural production system itself as the level of analysis. It has a local approach, in which the producers manage the resources and, consequently, their agricultural practices have an impact on the surrounding environment. The index allows the evaluation of the multifunctionality degree to which an agricultural production system contributes through four areas: 1) Territorial Scope (TS); 2) Environmental Scope (EnS); 3) Economic Scope (EcS) and; 4) Social Scope (SS). These areas were analyzed using 12 functions (three per scope) (Figure 1).

| | | | |
|---|---|---|---|
| **Function 1: Landscape settings.**<br>Exclusionary variable: Spatial heterogeneity<br>Indicator: Number of crops established in the last year.<br>-One crop, value = 1.75<br>-2 to 3 crops, value = 3.5<br>-4 to 5 crops, value = 5.25<br>-Greater than 6 crops, value = 7 | **Territorial scope** | **Economic scope** | **Function 7: Rural viability.**<br>Excluding variable: Employment.<br>Indicator: Type and duration of employment generated<br>-Temporary family employment, value = 2.5<br>-Constant family employment, value = 5<br>-Constant and temporary external family employment, value = 7.5<br>-Constant family and external employment, value = 7.5 |
| **Function 3: Agricultural history.**<br>Excluding variable: Exchange holding<br>Indicator: System orientation determined by the number of crops developed over time.<br>-Simplification (crop reduction) value = 2.33<br>-Stable (equal number of crops) value= 4.66<br>-Complexity (crop increase) value = 7 | **Function 2: Spatial connectivity.**<br>Additive variable: Source of supply.<br>Indicator: Type of source of water for the development of the system.<br>-Value by type = 1.2<br>-Irrigation unit/district<br>-Rain<br>-Water dam<br>-River<br>-Waterhole | **Function 8: Strengthening local economy.**<br>Excluding variable: Market.<br>Indicator: Production integrated to the local market.<br>-No distribution, value = 1.875<br>-Distribution external to the locality, value = 3.75<br>-Local and external distribution, value = 5.625<br>-Local distribution only, value = 7.5 | **Function 9: Food security.**<br>Excluding variable: Self-consumption.<br>Indicator: Production for direct or indirect self-consumption through a livestock subsystem<br>-Only direct consumption, value = 2.5<br>-Only indirect consumption, value = 5<br>-Direct and indirect consumption, value = 7.5 |
| | **Index of Multifunctionality of Agricultural Production System** | | |
| **Function 4. Provision of shelter and habitat.**<br>Additive variable: Wildlife protection<br>Indicator: Sighting of burrows.<br>Value by type = 1.125<br>-In arboreal stratum<br>-In stone fences or other materials<br>-In subsoil or wall<br>-In cultivation | **Function 5. Soil and water conservation.**<br>Additive variable: Sustainable strategies<br>Indicator: Implementation of enabling practices<br>Value by practice = 1,683<br>-Incorporation of organic matter<br>-Vermiculture practices<br>-Soil rehabilitation<br>-Cover crops<br>-Contour furrow<br>-Crop rotation<br>-Minimum tillage<br>-Drip irrigation | **Function 12: Social cohesion.**<br>Excluding variable: Participation.<br>Indicator: Degree of participation in social platforms-<br>-No integration, value = 0<br>-If you belong to any social group, value = 2.1875<br>-If you attend meetings, value = 4.375<br>-If you participate in agreements, value = 6.5625<br>-Is part of the organizational group, value = 8.75 | **Function 11: Territorial roots.**<br>Excluding variable: Local price.<br>Indicator: sense of belonging<br>-Land lent or rented and external origin, value = 1,875<br>-Own land and external origin, value = 3.75<br>-Land rented or lent and local origin, value = 5,625<br>-Own land and local origin, value = 7.5 |
| **Function 6. Preservation of biodiversity.**<br>Additive variable: Promotion of diversity<br>Indicator: Practices to promote biodiversity.<br>Practical value = 3<br>-Incorporation of more than one variety per crop<br>-Use of two or more different crops<br>-Use of hedges in burrows and/or within the system<br>-Incorporation of livestock activity | **Environmental scope** | **Social scope** | **Function 10: Protection of cultural heritage**<br>Additive variable: Agricultural worldview.<br>Indicator: Availability and/or practice of the producer.<br>Value by option: 1.75<br>-Use various crops in time and space<br>-Produce without chemical inputs or machinery<br>-Use ecological interdependencies to prevent pests and diseases<br>-Produce in adverse environments due to temperature and / or slope of the land<br>-Use wild plants and/or animals medicinally |

**Figure 1**. General composition of the IMSPA.

The sum of all the scopes integrates the value of the IMSPA.

$$IMSPA = TS + EnS + EcS + SS$$

Where:

$$\frac{TS\left(Territorial\ \ Scope\right) = \sum Functions\ 1,2,3}{Maximum\ \ EnS\ \ value = 20}$$

$$\frac{EnS\left(Environmental\ \ Scope\right) = \sum Functions\ 4,5,6}{Maximum\ \ EnS\ \ value = 30}$$

$$\frac{EcS\left(Economic\ \ Scope\right) = \sum Functions\ 7,8,9}{Maximum\ \ EnS\ \ value = 25}$$

$$\frac{SS\left(Social\ \ Scope\right) = \sum Functions\ 10,11,12}{Maximum\ \ SS\ \ value = 25}$$

The IMSPA has a 0-100 scale. When a system is closer to 100, its multifunctionality is greater. It is classified into five categories that define its multifunctionality degree. It also helps to locate the result of the system's status; consequently, an ongoing monitoring leads to an increase in the speed of the changes (Table 1).

**Table 1**. IMSPA categories.

| IMSPA Categories | Definition |
|---|---|
| I (<20)<br>Low multifunctionality | System in critical state due to the minimum contribution of functions both inside and outside it, located at the extreme of the conventionality of its form of production. |
| II (20.1 - 40)<br>Medium-low multifunctionality | System thet in its greatest production is handled in a conventional way, can have a marked contribution in any of the four scopes. |
| III (40.1 - 60) Intermediate multifunctionality | System in a vulnerable state to improvement or setback in terms of the production of functions. |
| IV (60.1 - 80)<br>Medium-high multifunctionality | System in a favorable path to produce functions in the various fields, although not proportionally. It is considered that these systems defined their paths towards diversification and develop practices that benefit the multifunctionality of the system. |
| V (80.1 - 100)<br>High multifunctionality | Category that defines an excellent state in terms of the contribution of functions in the four scopes that the system generates and that have a positive impact on the environment and society. Ideal systems to replicate or augment. |

**Study area**

The Mixteca is one of the eight regions that make up the State of Oaxaca. It is located in the northwest of the state and covers an area of 15,671.08 $km^2$. The Mixteca Alta is an area characterized by high mountains, where flat morphology is scarce and the Nochixtlán valley is the most extensive flat area (Solís-Castillo *et al.*, 2018).

The dominant climate is temperate sub-humid with rainfall in summer (Cw) and a marked midsummer heat period between July and August (García, 2004). The mean annual temperature is 15 °C and the average annual rainfall is 649 mm. The predominant vegetation is secondary pine and oak forest in the upper parts, with some primary relicts (Solís-Castillo *et al.*, 2018).

Asunción Nochixtlán is one of the seven districts that make up the Mixteca region. The municipalities of Santa María Apasco, Santiago Apoala, and San Miguel Huautla are located in this district (Figure 2). Their inhabitants cultivate maize under different Agricultural Production Systems (APS).

## RESULTS AND DISCUSSION

### Application of IMSPA to maize systems

Eight agricultural production systems (PS) —which together cover an area of nine hectares— were identified and evaluated in the three selected communities (Table 2). The APS and the plots were located through key informants who are aware of the local agricultural management. Each APS was considered as a level of analysis and a semi-structured interview was applied to the person responsible for its management, in order to evaluate both the APS and its producers. The surveys were applied from May to July 2022.

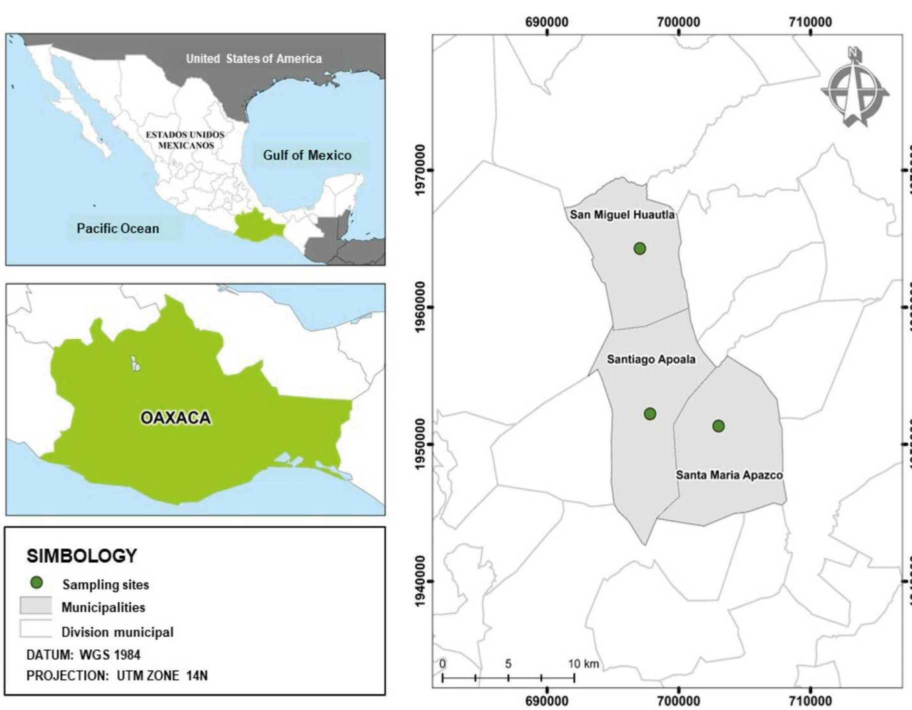


**Figure 2**. Location of the sampling sites in the Mixteca region of Oaxaca, Mexico.

**Table 2**. General characterization of the Agricultural Production Systems evaluated.

| System | Area (ha) | General characteristics |
|---|---|---|
| Apasco corn | 1.0 | Conventional cultivation with application of agrochemicals, production for consumption and sale |
| Apoala corn | 1.5 | |
| Huautla corn | 1.0 | |
| Corn-Bean Apasco | 0.5 | Conventional cultivation with application of agrochemical, small areas, with production destined for corn for consumption, and beans for consumption and sale |
| Corn-Bean Apoala | 0.5 | |
| Corn-Fruit Apoala | 1.5 | Intercropping with milpa and fruit trees such as: peach, avocado, apple. They are not governed by the methodology of the MIAF system. |
| Corn-Fruit Huautla | 1.0 | |
| MIAF Apasco | 1.0 | It is characterized by following the methodologies of sowing, planting, pruning and fertilization of the MIAF system. The fruit trees are peach, apple and avocado. |

## Multifunctionality of agricultural production systems in the Mixteca Alta region of Oaxaca

The analysis of the 12 functions for each of the four evaluated areas (territory, environment, economy, and society) are shown below, along with their graphic representation and their integration into the IMSPA. It is important to mention that the variables and functions included do not limit the broad concept of multifunctionality; however, they certainly contribute to the way in which it can be approached.

## Territorial scope

The landscape configuration function is mainly understood as the result of the management of natural resources by human beings (Ruiz *et al.*, 2016). The components of the agricultural landscape act as jigsaw pieces that can favor or interrupt environmental processes and ecosystem services (Francesconi and Montagnini, 2015; Clemente-Ortega and Álvarez, 2019). Therefore, a production system directly influences the landscape by creating a composition that indicates functionality and continuity: the greater the spatial heterogeneity, the greater the multifunctionality (Ruiz *et al.*, 2016). The indicator used to evaluate the said heterogeneity was the number of crops implemented per year. The results for this function showed that no system had more than six crops established in the last year: 12.5% of the evaluated systems implemented 4 to 5 crops, 37.5% established 2 to 3 crops, and the remaining 50% only had 1 crop.

Agriculture is an economic activity that consumes a high amount of water resources. The spatial connectivity function considers the importance of production systems in the continuity or fragmentation of the agricultural space; this function does not only impact the landscape, but also interrupts ecological interactions (Ruiz *et al.*, 2016). Water was considered as a fundamental requirement for the productivity and continuous production of agricultural systems. On the one hand, having a non-rainfall supply source will be decisive. On the other hand, the source of water was considered for the evaluation of the function. The results showed that rain is the only source of water supply in five systems, while the remaining three have an alternate source that guarantees the continuity of production.

Lastly, the agricultural history function considers that crop changes in the system allow the restructuring of the landscape of a given territory, determining its specific characteristics. Consequently, it was possible to identify the systems' tendency towards complexity (increase in the number of crops), constancy (same number of crops), or even simplification (reduction of crops). Changing from a monoculture to agroecological diversification promotes natural processes that preserve the health, productivity, and self-sustainability of the agroecosystem (Nicholls *et al.*, 2015). The results showed that 87% of the evaluated systems has a stable trend, because the producers have always developed the same number and type of crops, while only 12.5% (one system) has a trend towards complexity and increases on a regular basis (Figure 3).

**Environmental scope**

The refuge and habitat provision function considers that a production system can provide refuge for wildlife, forming microhabitats in which different species can develop (Canavellis and Zaccagnini, 2007; Figueroa-Sandoval *et al.*, 2019). Therefore, the sighting of burrows and nests within the system is a good indicator of multifunctionality (Ruiz *et al.*, 2016). Burrows and nests were only observed in one location within each system (fences, subsoil, and inside the crop). No sightings were recorded in the MIAF - Apoala, while in the MIAF - Apasco they were found in two different locations within the system (fences made of stone or other materials and in the subsoil). More burrows were recorded within this crop than in others.

The soil and water conservation function considers that agricultural practices have a direct impact in the conservation or deterioration of these resources. Adopting and implementing new soil conservation techniques will pose a major challenge for authorities, producers, and academia (Gómez-Calderón *et al.*, 2018). Minimum tillage is the most frequently used of the eight practices evaluated in this research. In addition, in the Maíz - Huautla system only one practice is carried out, in contrast with the MIAF - Apasco

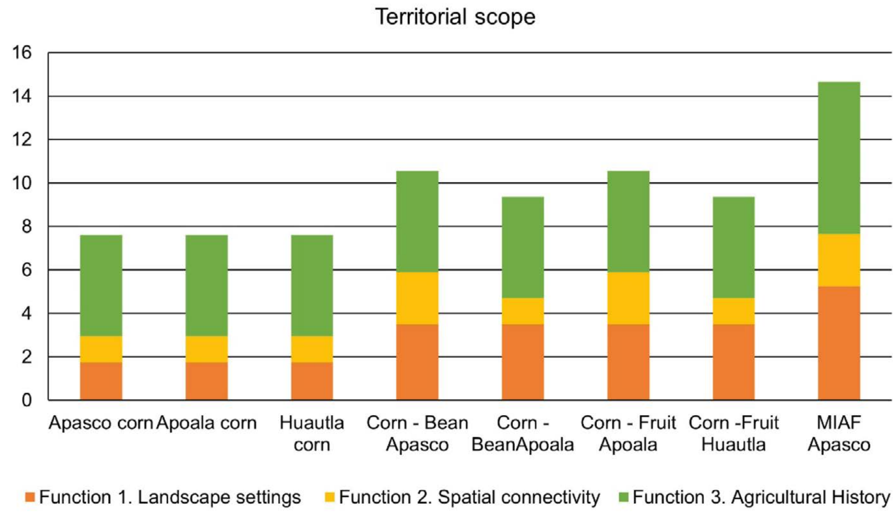


**Figure 3**. Accumulated value of functions for the territorial scope by system.

system, which recorded the highest number of implemented techniques (incorporation of organic matter, soil rehabilitation, crop rotation, minimum tillage, and drip irrigation).

The biodiversity preservation function considers the management that the producer carries out to promote the conservation of biodiversity in the system. The FAO (1999) mentions that the different agricultural practices can have a positive or negative impact on diversity and, that the greater the promotion of diversity, the greater the multifunctionality displayed by the system. Aiming agricultural practices towards a diverse agroforestry system can unite two factors in the dynamics of society: sustainable production and biodiversity protection (Saborío, 2016). The results showed that the use of two or more crops and the incorporation of livestock activity are the most frequent practices. None of these practices were used in the Maíz – Apasco, while the MIAF - Apasco system reported three of the four practices (Figure 4).

## Economic scope

The rural viability function considers that a production system is viable when it offers attractive options that motivate the producer to persist in that area; part of this attraction involves a guaranteed employment and income (Ruiz *et al.*, 2016). The results showed that 75% of the systems generate temporary employment for the family. The Maiz - Frutal Huautla system generates a constant family employment. Finally, the MIAF - Apasco system generates constant employment for both the family and external employees, resulting in a greater multifunctionality in the system and a positive impact on society.

The function of strengthening the local economy takes into account the integration of products in the local market where the system is developed. The greater the placement of products in the local market, the greater the multifunctionality (Ruiz *et al.*, 2016). In this regard, the whole production of 50% of the systems is only used for self-consumption; in 12.5% of the systems, the totality of the production is distributed outside the locality of origin; in 12.5%, the totality of the production is distributed within the locality of origin;

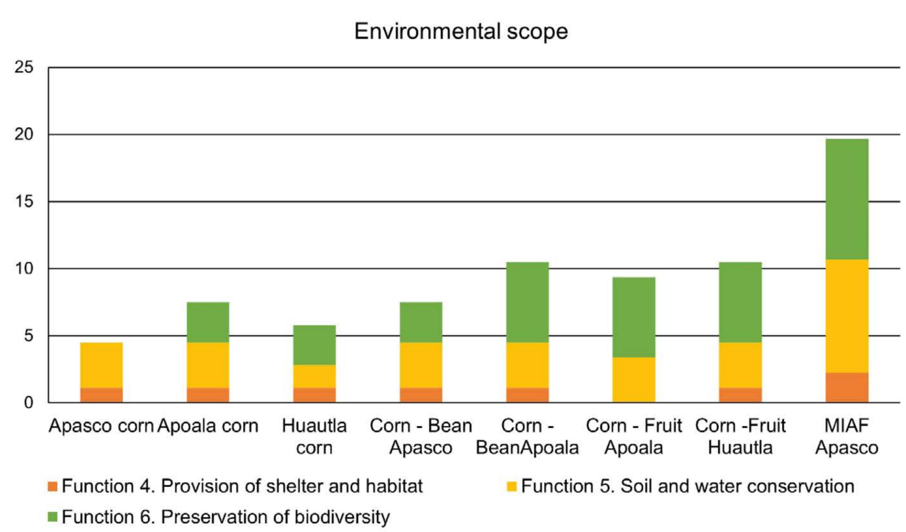


**Figure 4**. Accumulated value of functions for the environmental scope by system.

and, finally, 25% of the systems have a shared distribution of production (within and outside the locality of origin).

Regarding the food security function, diversified production systems that favor self-consumption provide greater food security, either through the direct consumption of products provided by the system or indirect consumption of other products in livestock subsystems (Urquía-Fernández, 2014; Ruiz *et al.*, 2016). The results showed that, in 87.5% of the systems, direct self-consumption is favored by the system manager, while both types of self-consumption were registered in only 12.5% of the systems (Figure 5).

**Social scope**

The protection of cultural heritage function considers the preservation of the lore regarding the cultivation of the land; therefore, it is directly related to the knowledge of the person responsible for the management of each system (Ruiz *et al.*, 2016). Farmer lore is linked to the reality of rural communities and has enabled the production of staple food for subsistence under different conditions. The results reflected that 62.5% of the systems usually produce in adverse environments (temperature and/or land slope), while 50% use different crops at the same time and in the same space. Two systems still make use of wild plants and/or animals for medicinal purposes. Only one system does not use chemical inputs or machinery and relies on ecological interdependencies to prevent pests and diseases.

The deep-rootedness function refers to the sense of belonging that the producers have towards the system they manage and the appreciation will be greater if they own the land where the system is developed and if they were born in the locality. All of the systems are managed by local land-owners.

Finally, the social cohesion function recognizes that there is a network of social actors (ejidos, associations, etc.) who seek to develop the countryside. This function considers the level of integration of the system manager. The results show that, although 100% of the

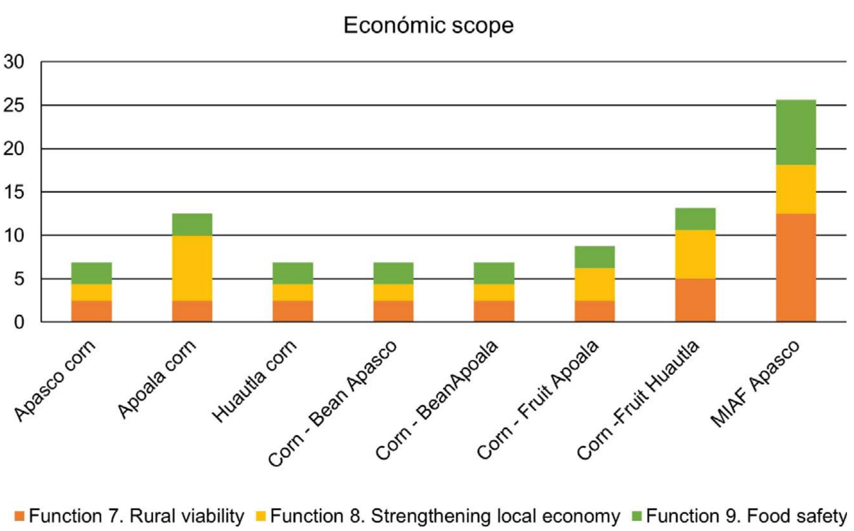


**Figure 5**. Accumulated value of functions for the economic scope by system.

systems are developed by producers who belong to a social group, only 50% attend the meetings and only 25% have a say in local matters (Figure 6).

**IMSPA Integration**

Once all the functions were analyzed, the value for each scope was determined. Subsequently, those values were added and the Multifunctionality Index of Agricultural Production Systems (IMPSA) was integrated (Table 3). The results showed that two of the five categories managed by the IMSPA were not represented in any system: category I (low-multifunctionality system) and category IV (with medium-high multifunctionality system).

Category II (medium-low multifunctionality systems) included the Maíz - Apasco, Maíz - Apoala, Maíz - Huautla, Maíz - Frijol Apasco, and Maíz - Frijol Apoala, which accounted for more than 50% of the systems. Figure 7 shows that the territorial and social scopes were the best represented functions, in contrast with the environmental and economic scope functions. These systems are developed in places where the producers own the land and constantly practice conventional agriculture, always growing the same number of crops per harvest.

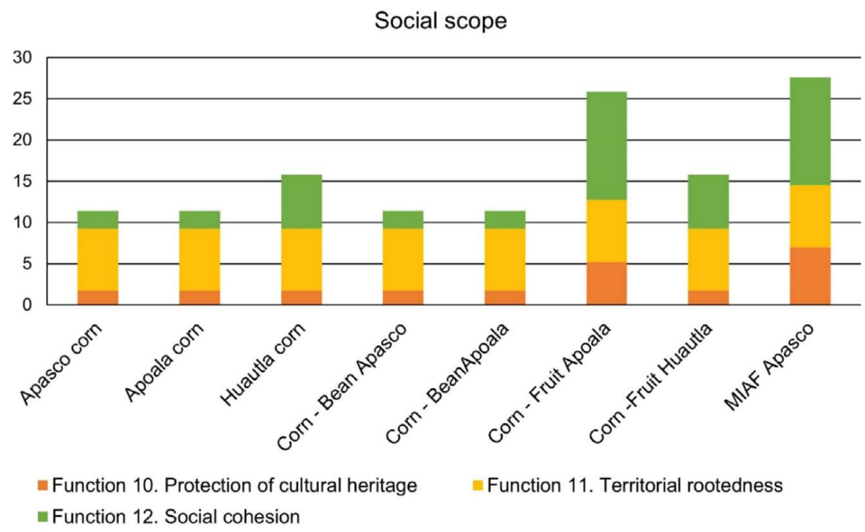


**Figure 6**. Accumulated value of functions for the social scope by system.

**Table 3**. IMSPA integration.

| System | Territorial (20) | Environmental | Economic | Social (25) | IMSPA (0-100) | Category |
|---|---|---|---|---|---|---|
| Apasco corn | 7.61 | 4.491 | 6.875 | 11.4375 | 30.4135 | II |
| Apoala corn | 7.61 | 7.491 | 12.5 | 11.4375 | 39.0385 | II |
| Huautla corn | 7.61 | 5.808 | 6.875 | 15.8125 | 36.1055 | II |
| Corn-Bean Apasco | 10.56 | 7.491 | 6.875 | 11.4375 | 36.3635 | II |
| Corn-Bean Apoala | 9.36 | 10.491 | 6.875 | 11.4375 | 38.1635 | II |
| Corn-Fruit Apoala | 10.56 | 9.366 | 8.75 | 25.875 | 54.551 | III |
| Corn-Fruit Huautla | 9.36 | 10.491 | 13.125 | 15.8125 | 48.7885 | III |
| MIAF Apasco | 14.65 | 19.665 | 25.625 | 27.625 | 87.565 | V |

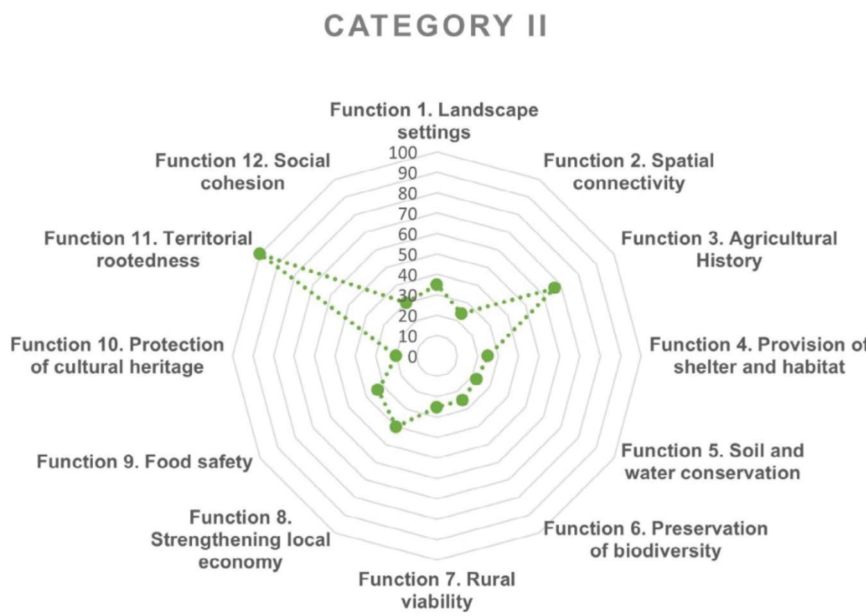


**Figure 7**. Representation of the average system with medium-low multifunctionality.

Category III (intermediate multifunctionality systems) was only represented by the Maíz - Frutal Apoala and Maíz - Frutal Huautla systems, which obtained an IMSPA of 54.551 and 48.7885, respectively. The production of these systems' functions is vulnerable to improvements or setbacks; therefore, it is worth highlighting the need to tip the scales in favor of an increased multifunctionality. The systems in this category contributed to all the evaluated functions. However, the functions of the territorial, economic, and social scopes make a bigger contribution than other functions (Figure 8).

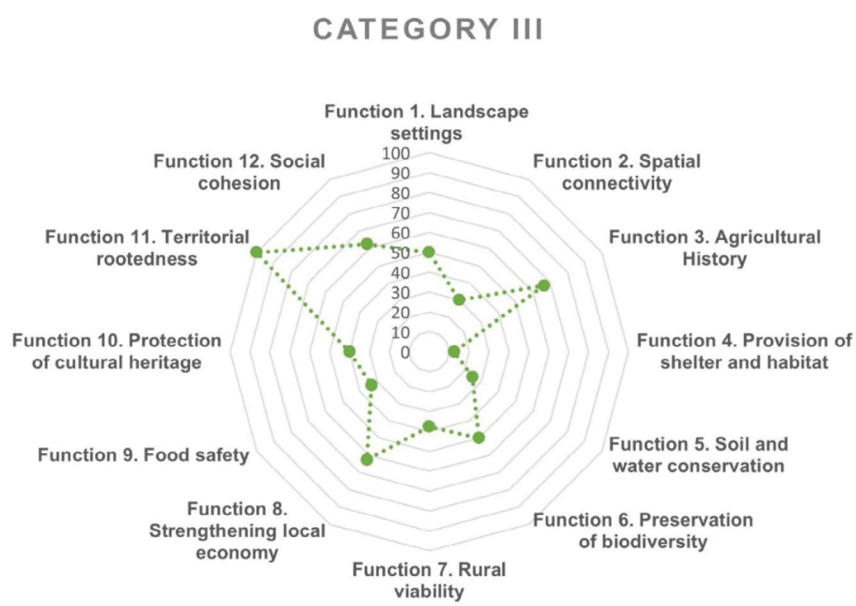


**Figure 8**. Representation of the average system with intermediate multifunctionality.

Finally, only the MIAF Apasco system was classified in category V (high multifunctionality systems), which shows that it is the only system that is effectively performing various functions. The multifunctionality in this system is not fully equitable in all scopes; nevertheless, an increase in the functions of the environmental scope was indeed observed. None of the other systems evaluated included this scope (Figure 9).

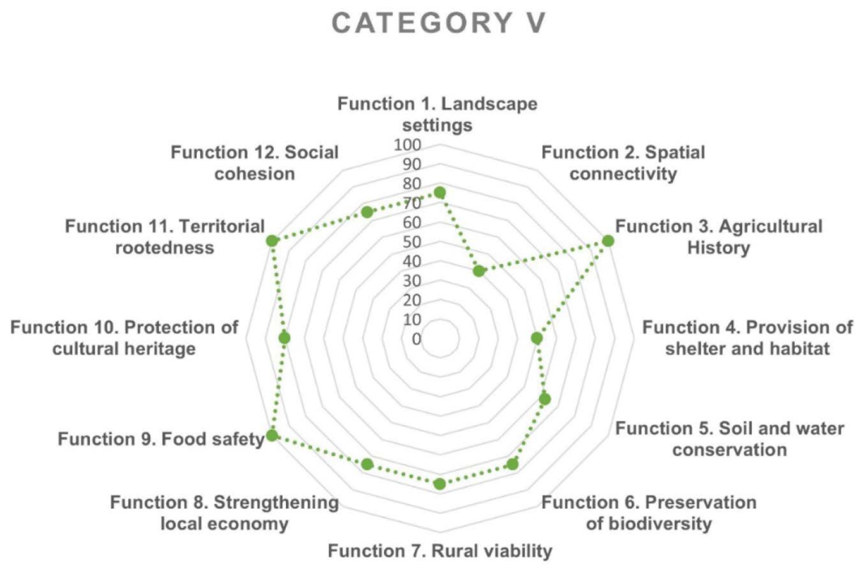


**Figure 9**. Representation of the average system with high multifunctionality.

## CONCLUSIONS

The multifunctionality in maize productive systems can be extremely interesting for the inhabitants and producers of these communities. Their role and performance in their plots are fundamental. The MIAF-Apasco system is a clear example of how committed producers can transform a conventional agricultural practice into something more diversified and multifunctional. Nevertheless, a real change requires time, dedication, effort, and a lot of motivation. Hence the importance of understanding agriculture as a multifunctional activity that can contribute to the progress or to strengthen the arrangements that enable the maintenance or improvement of rural development. The implementation of a multifunctional agriculture makes a positive contribution to the partial achievement of the global sustainability objective. However, we must highlight the difference between both terms: while sustainability guides the objectives, multifunctionality is a characteristic of the agricultural production process. The IMSPA enters the theoretical and practical debate on the multifunctionality of agriculture. This article exposes the capacity of the IMSPA as a tool to analyze productive systems, evaluating the degree of multifunctionality that they generate. The index can be used to monitor the multifunctionality of the systems over time, favoring decision-making that encourages a management that promotes sustainability in the agricultural field.

## ACKNOWLEDGEMENTS

The authors would like to thank the maize producers of the Santa María Apasco, Santiago Apoal, and San Miguel Huautla municipalities, who kindly agreed to participate in this study, as well as Consejo Nacional de Ciencia y Tecnología (Conacyt) for the support received.

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# Relationships between technological and nutritional meat quality traits in native Mexican *Meleagris gallopavo gallopavo* L.

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**ABSTRACT**

**Objective**: To evaluate the relationships between some technological and nutritional meat quality traits in native Mexican Guajolote (*M. g. gallopavo*).

**Design/methodology/approach**: In the study, a total of 35 carcasses from male native guajolotes (32-40 weeks old; approximately 2.9 kg carcass weight) were used. Some technological [pH, colour (*L**, *a**, *b**), water-holding capacity (WHC), drip loss (DP), and cooking loss (CL)] and nutritional [dry matter (DM), crude protein (CP), crude fat (CF), crude ash (CA) and energy content (EC)] properties of breast and leg meat were evaluated. Pearson correlation of SAS software was used for data analysis.

**Results**: In breast meat, moderate to high positive correlations ($P<0.01$; 0.35 [$pH_{45min}$ *vs* $pH_{24h}$]$\leq r<0.82$ [DM *vs* EC]) were observed, but highly and negatively correlations ($P<0.01$; $-0.36$ [CF *vs* CA]$\leq r<-0.77$ [*b** *vs* DL]) also were found. Similarly, technological and nutritional quality traits in leg meat also showed moderate to high positive correlations ($P<0.01$; 0.38 [$pH_{24h}$ *vs* *L**]$\leq r<0.74$ [DM *vs* CF]); however, high negative correlations ($P<0.01$; $-0.42$ [$pH_{24h}$ *vs* CL]$\leq r<-0.69$ [*a** *vs* *b**]) were observed.

**Limitations on study/implications**: Studies on the factors that affect the technological and nutritional characteristics of meat quality in this poultry species should be carried out

**Findings/conclusions**: The results could be used as an important benchmark of the current state of Guajolote meat quality to develop selection and breeding programs in its genetic improvement.

**Keywords**: *M. g. gallopavo*, Meat quality, Poultry genetic resource, Technological properties.

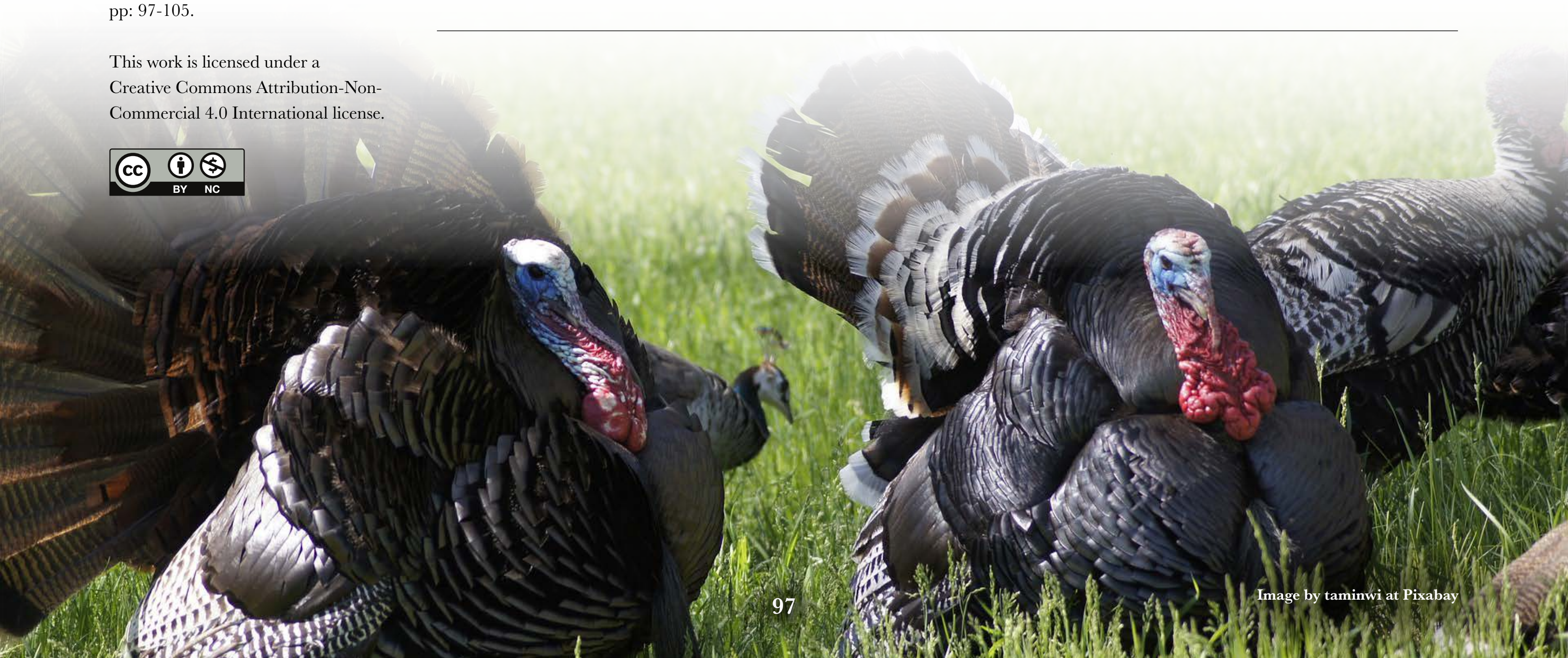
**Citation**: Portillo-Salgado, R., Herrera-Haro, J. G., Bautista-Ortega, J., & Cigarroa-Vázquez, F. A. (2023). Relationships between technological and nutritional meat quality traits in native Mexican *Meleagris gallopavo gallopavo* L. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2395

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: October 08, 2022.
**Accepted**: May 16, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 97-105.





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## INTRODUCTION

In recent years, consumers are more and more interested in the sustainability, health and nutritional aspects of meat poultry products. They are willing to pay higher price for meat that they perceived as naturally produced and with high standard of animal welfare (Cygan-Szczegielniak *et al.*, 2019). In this way, the interest exhibited by the consumers for poultry products deriving from free-range or organic systems encourages the use of native or local genotypes (Cassandro *et al.*, 2015; Dalle-Zotte *et al.*, 2019). Several studies demonstrated differences in meat quality between native and commercial poultry breeds, particularly in terms of physical traits and chemical composition (Cassandro *et al.*, 2015; Uhlířová *et al.*, 2018; Katemala *et al.*, 2022). Additionally, native poultry meat has a unique taste and texture that draws the attention of consumers (Ali *et al.*, 2021).

Meat quality can be characterized through the technological, chemical and nutritional attributes presented (Hiscock *et al.*, 2022). However, meat quality characteristics in poultry may be influenced by many factors such as species and breed, environment, feeding, and care conditions (Uhlířová *et al.*, 2018; Onk *et al.*, 2019). Meat can be classified with traits such as meat colour, pH, and water-holding capacity and therefore these parameters represent important indicators for meat quality. Due to the relationships between these traits, it is possible that directional selection for some of these traits will improve meat quality (Hiscock *et al.*, 2022).

The native Guajolote (*M. g. gallopavo*) is the second poultry species more abundant and important in backyard poultry in rural and suburban communities of Mexico (Romero-López, 2021). Constitutes a poultry genetic resource of great biological and productive value. Also, has a good capacity for adaptation and high rusticity that allows it to reproduce under different environmental and management conditions (Portillo-Salgado *et al.*, 2022a). Recently, Portillo-Salgado *et al.* (2022b) reported that native Guajolote raised traditionally under extensive conditions can achieve relatively high carcase weights and yields, particularly in males, as well as meat of good nutritional quality, making them preferable for meat production. However, no studies are currently available investigating the possible relationships between meat quality traits. Therefore, the aim of this study was to evaluate the relationships between some technological and nutritional meat quality traits in native Mexican Guajolote. A good understanding of the relationship between these meat quality traits should aid in future breeding strategies to improve overall meat quality of this poultry genetic resource.

## MATERIALS AND METHODS

### Ethics statement

The experimental protocol was approved by the Animal Welfare Committee of the Colegio de Postgraduados and they complied with the standards for the care and use of animals used for research (Approval folio: COBIAN 002/21).

### Sample preparation

For the study, carcases of 35 males native guajolotes (32-40 weeks old; approximately 2.9 kg carcase weight) which were raised traditionally under extensive conditions and fed a mixed diet (Portillo-Salgado *et al.*, 2022b), were used. The birds were humanely slaughtered by exsanguination following the Official Mexican Standards (NOM-008-ZOO-1994, NOM-009-ZOO-1994, and NOM-033-ZOO-1995) established for the humane slaughter of animals intended for meat production. The carcases were scalded in hot water to facilitate manual plucking. Later, carcases were eviscerated and dissected as described by Hahn and Spindler (2002). The right breast (*Pectoralis major*) and leg (thigh and drumstick) meat without skin of each bird were ground for evaluate technological properties and proximate composition.

**Evaluation of meat technological properties**

The meat pH was measured at 45 min ($pH_{45min}$) and 24 h ($pH_{24h}$) *post-mortem* using a portable digital pH-meter (Model HI 99161, Hanna Instruments®, USA). Before measurement, the pH-meter was calibrated using buffers of pH 4.0 and 7.0 at room temperature according to the manufacturer's instructions. The pH was evaluated at three different points, adopting the average value of these three readings. The colour parameters were measured using a colorimeter (Model CR-400, Konica Minolta®, Tokyo, Japan), and were expressed in terms of CIELab colour coordinates reporting values for lightness ($L^*$), redness ($a^*$) and yellowness ($b^*$).

Water-holding capacity (WHC; %) was evaluated by the filter paper press method (Biesek *et al.*, 2021). For this, ground meat samples (3 g) were placed between two sheets of filter paper (Whatman® No. 1) and were pressed with standard weight of 2 kg for 5 min. WHC was calculated as the difference between the initial sample weight and the final weight. Cooking loss (CL; %) was determined by placing ground meat samples (20 g) on a absorbent gauze inside sealed plastic bags, and they cooked in a water bath at 85 °C for 10 min (Kokoszyński *et al.*, 2020). CL was expressed as the ratio between the weight before and after cooking. Drip loss (DL; %) was determined by placing ground meat samples (20 g) in two sealable bags (one of the bags was perforated to allow dripping) and storing them at +4 °C for 24 h (Kokoszyński *et al.*, 2020). DL was expressed as the percentage of weight loss of the sample concerning its weight recorded before the refrigeration period.

**Proximate composition of meat**

Proximate analysis was performed to determine dry matter (DM; %), crude protein (CP; %), crude fat (CF; %), crude ash (CA; %) and energy content (EC; cal/g) according to the methods approved by the AOAC (1990).

**Statistical analysis**

Data were analysed using the SAS software, version 9.4 (SAS Institute Inc., Cary, NC). Normal distribution of the variables was analyzed according to the Shapiro-Wilk test. The results are presented as least square means ± standard deviation (SD). Pearson correlation coefficients (r) were evaluated using the PROC CORR procedure to determine relationships between technological and nutritional meat quality traits. Significance was determined when $P \leq 0.05$. Each bird was considered as the experimental unit.

## RESULTS AND DISCUSSION

Table 1 shows the technological properties and nutritional meat quality traits of breast and leg meat from native Mexican Guajolote. The pH values obtained in this study (5.71-6.05) varied within the pH range accepted for commercial poultry meat (5.7-6.4) (Gálvez *et al.*, 2018). The decrease in $pH_{24h}$ compared to $pH_{45min}$ *postmortem* indicates normal biochemical changes in muscles (Biesek *et al.*, 2021). Sarica *et al.* (2011) found pH values that varied from 5.9 to 6.1 and 6.1 to 6.4 for breast and thigh meat in turkeys of different genotypes. According to Chumngoen and Tan (2015), the lower pH levels observed in native poultry meat resulted from the effects of more aggressive behavior in native poultry. The authors evidenced that the greater stresses experienced by native chicken caused more glycogens to be metabolized, consequently affected post-mortem glycolysis, lead to lactic acid accumulation, and thus resulted in lower pH values in meat.

Meat colour is the first trait used by the consumer when buying meat, this due used to assess the freshness and quality of meat and is closely related to the ultimate pH (Sujiwo *et al.*, 2018; Uhlířová *et al.*, 2018). Our results showed that leg meat is darker (lower $L^*$ value) and redder (high $a^*$ value) than breast meat. The difference in redness among muscles could be due to a difference in muscle fiber type and histological muscle structure (Sarica *et al.*, 2011). Similar results were observed by Gálvez *et al.* (2018) in commercial turkeys. Myoglobin content is a major factor contributing to meat colour and is dependent on the species, muscle, and age of the bird (Sarica *et al.*, 2011).

WHC is the ability of meat to retain its juice during application of external forces (cutting, heating, grinding, or pressing), and if it is poor, meat will lack juiciness (Sarica *et al.*, 2011; Cygan-Szczegielniak *et al.*, 2019). In this study, the breast and leg meat had WHC values of 59 and 41%, respectively, indicating that breast meat was more suitable for potential further processing. Biesek *et al.* (2021) argued that the mechanism of WHC in the muscles of the breast and legs may differ, as the levels of individual chemical components (protein) are different in both types of muscles. Also, fiber types differ in their ability shrinkage, so the more muscle fibers shrinks, the larger the leakage of water from the muscle tissue would be obtained. Our results regarding WHC are comparable with those reported by Sarica *et al.* (2011) in the breast (46-47%) and thigh (41-46%) meat of turkeys of different genotypes. The CL values observed in the present study were high compared to those reported in commercial turkeys (Damaziak *et al.*, 2016). Differences in cooking loss with respect to genotype might be attributed to different proteins solubility (collagen) and to different fat content. Cooking temperature and ultimate pH could also play a role (Uhlířová *et al.*, 2018).

The proximate composition of breast and leg meat of native Mexican Guajolote is reported in Table 1. Few data were found in the literature about the nutritional composition of native Guajolote. The dry matter, protein, and ash contents were consistent to those reported in breast and thigh meat of native mexican guajolotes (López *et al.*, 2011) and commercial turkeys genotypes (Sarica *et al.*, 2011). However, the latter presented higher fat contents compared to those observed in this study. Therefore, the Guajolote meat may be a better choice for a lower fat diet because of their low fat contents. According to Dalle-Zotte *et al.* (2019), the discrepancies in terms of meat proximate composition in poultry might be

**Table 1**. Mean ± standard deviation (SD) for technological and nutritional quality traits in breast and leg meat of native Mexican Guajolote.

| Traits | Breast meat | Leg meat |
|---|---|---|
| | Mean±SD | Mean±SD |
| $pH_{45min}$ | 5.78±0.20 | 6.05±0.23 |
| $pH_{24h}$ | 5.71±0.18 | 5.75±0.15 |
| *L** | 45.60±5.10 | 42.13±6.43 |
| *a** | 1.85±1.00 | 6.07±2.71 |
| *b** | 1.80±1.27 | 2.79±1.90 |
| Water-holding capacity; % (WHC) | 59.09±19.00 | 40.90±12.69 |
| Drip loss; % (DP) | 2.92±1.51 | 2.97±1.19 |
| Cooking loss; % (CL) | 24.82±5.49 | 25.93±5.88 |
| Dry matter; % (DM) | 26.16±0.86 | 24.66±0.84 |
| Crude protein; % (CP) | 22.34±1.00 | 20.14±0.79 |
| Crude fat; % (CF) | 1.47±1.03 | 1.93±0.71 |
| Crude ash; % (CA) | 1.05±0.07 | 1.02±0.07 |
| Energy content; cal/g (EC) | 1291.10±79.09 | 1199.56±62.00 |

explained by the lack of standardisation in the productive performances and meat quality traits of these animals.

## Analysis of correlations

Tables 2 and 3 show the Pearson correlation matrices between technological and nutritional quality traits in breast and leg meat of native Mexican Guajolote. For breast meat, the pH values were positively correlated with *b** (r = 0.66), and negatively correlated

**Table 2**. Pearson correlations between technological and nutritional quality traits in breast meat of native Mexican Guajolote.

| | $pH_{45min}$ | $pH_{24h}$ | *L** | *a** | *b** | WHC | DL | CL | DM | CP | CF | CA | EC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $pH_{45min}$ | 1.00 | | | | | | | | | | | | |
| $pH_{24h}$ | $0.35^{*}$ | 1.00 | | | | | | | | | | | |
| *L** | $-0.20^{ns}$ | $0.04^{ns}$ | 1.00 | | | | | | | | | | |
| *a** | $-0.29^{ns}$ | $-0.21^{ns}$ | $0.04^{ns}$ | 1.00 | | | | | | | | | |
| *b** | $0.27^{ns}$ | $0.66^{***}$ | $0.07^{ns}$ | $-0.21^{ns}$ | 1.00 | | | | | | | | |
| WHC | $-0.26^{ns}$ | $-0.68^{**}$ | $0.14^{ns}$ | $0.20^{ns}$ | $-0.70^{**}$ | 1.00 | | | | | | | |
| DL | $-0.28^{ns}$ | $-0.74^{***}$ | $0.22^{ns}$ | $0.32^{ns}$ | $-0.77^{***}$ | $0.65^{**}$ | 1.00 | | | | | | |
| CL | $-0.40^{*}$ | $-0.56^{**}$ | $0.47^{*}$ | $0.35^{ns}$ | $-0.61^{**}$ | $0.52^{**}$ | $0.59^{**}$ | 1.00 | | | | | |
| DM | $-0.19^{ns}$ | $-0.22^{ns}$ | $0.05^{ns}$ | $-0.04^{ns}$ | $-0.11^{ns}$ | $0.05^{ns}$ | $-0.03^{ns}$ | $-0.18^{ns}$ | 1.00 | | | | |
| CP | $0.01^{ns}$ | $-0.15^{ns}$ | $-0.00^{ns}$ | $0.24^{ns}$ | $-0.25^{ns}$ | $-0.17^{ns}$ | $0.25^{ns}$ | $-0.01^{ns}$ | $0.14^{ns}$ | 1.00 | | | |
| CF | $-0.18^{ns}$ | $-0.10^{ns}$ | $0.03^{ns}$ | $-0.13^{ns}$ | $0.08^{ns}$ | $0.09^{ns}$ | $-0.29^{ns}$ | $-0.02^{ns}$ | $0.63^{**}$ | $-0.53^{*}$ | 1.00 | | |
| CA | $-0.03^{ns}$ | $-0.17^{ns}$ | $0.50^{*}$ | $-0.16^{ns}$ | $-0.27^{ns}$ | $0.00^{ns}$ | $0.35^{ns}$ | $0.17^{ns}$ | $0.21^{ns}$ | $0.18^{ns}$ | $-0.36^{*}$ | 1.00 | |
| EC | $-0.09^{ns}$ | $-0.25^{ns}$ | $0.04^{ns}$ | $0.15^{ns}$ | $-0.17^{ns}$ | $0.26^{ns}$ | $0.03^{ns}$ | $0.08^{ns}$ | $0.82^{***}$ | $-0.03^{ns}$ | $0.78^{***}$ | $-0.08^{ns}$ | 1.00 |

$^{ns}$ Non Significant; $^{*}$ P<0.05; $^{**}$ P<0.01; $^{***}$ P<0.001.

with WHC (r=−0.68), DL (r=−0.74) and CL (r=−0.40 and −0.56) (P<0.01). This result indicated that an increase in pH led to significant increase meat yellowness, but to significant decrease the ability of meat to retain water (Biesek *et al.*, 2021). It is well known that the ultimate pH is of importance when considering meat preservation and stability; high muscle pH affects meat moistness, while a low pH is associated with poor WHC and meat colour (Cygan-Szczegielniak *et al.*, 2019).

The *L** value was found to be positively correlated with CL (r=0.47) and CA (r=0.50) (P<0.05), while *b** was negatively correlated with WHC (r=−0.70), DL (r=−0.77) and CL (r=−0.61) (P<0.01). Similarly, Hiscock *et al.* (2022) found that *L** value was also significantly positively correlated with drip loss (r=0.127-0.166) and cooking loss (r=0.130-0.164) of turkey meat. The authors suggested that lightness (*L**) could be an appropriate indicator trait for overall poultry meat quality that could be included as a phenotype in a breeding program.

In this study, the WHC was significantly positively correlated with DL (r=0.65) and CL (r=0.52), and DL was significantly positively correlated with CL (r=0.59) (P<0.01). These findings confirm the close relationship between these meat technological traits, since the WHC also can be evaluated by drip or cook losses (Sarica *et al.*, 2022). The DM was positively correlated with CF and EC with correlation coefficients of 0.63 and 0.82, respectively (P<0.01). Likewise, CF showed a positive correlation with EC (r=0.78) (P<0.001). Instead, CP was negatively correlated with CF (r=−0.53) (P<0.05). In Holli chickens, dry matter content was positively correlated with protein and ash contents, and fat content was positively correlated with the meat texture, pH values and *a**, but negatively correlated with the protein content (Tougan *et al.*, 2013).

Regarding the leg meat (Table 3), $pH_{45min}$ value was positively correlated with *a** (r=0.59), but negatively correlated with *L** (r=−0.65) and b* (r=−0.48) (P<0.01). For

**Table 3**. Pearson correlations between technological and nutritional quality traits in leg meat of native Mexican Guajolote.

| | $pH_{45min}$ | $pH_{24h}$ | L* | a* | b* | WHC | DL | CL | DM | CP | CF | CA | EC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $pH_{45min}$ | 1.00 | | | | | | | | | | | | |
| $pH_{24h}$ | $-0.22^{ns}$ | 1.00 | | | | | | | | | | | |
| L* | $-0.65^{***}$ | $0.38^{*}$ | 1.00 | | | | | | | | | | |
| a* | $0.59^{**}$ | $-0.46^{**}$ | $-0.75^{***}$ | 1.00 | | | | | | | | | |
| b* | $-0.48^{**}$ | $0.49^{**}$ | $0.44^{*}$ | $-0.69^{***}$ | 1.00 | | | | | | | | |
| WHC | $0.23^{ns}$ | $-0.57^{**}$ | $-0.15^{ns}$ | $0.45^{*}$ | $-0.56^{**}$ | 1.00 | | | | | | | |
| DL | $0.37^{ns}$ | $-0.22^{ns}$ | $-0.57^{**}$ | $0.66^{**}$ | $-0.46^{*}$ | 0.42* | 1.00 | | | | | | |
| CL | $-0.12^{ns}$ | $-0.42^{*}$ | $0.06^{ns}$ | $0.22^{ns}$ | $-0.36^{ns}$ | $0.39^{ns}$ | $0.17^{ns}$ | 1.00 | | | | | |
| DM | $0.04^{ns}$ | $-0.08^{ns}$ | $-0.11^{ns}$ | $0.25^{ns}$ | $-0.31^{ns}$ | $0.17^{ns}$ | $-0.16^{ns}$ | $-0.05^{ns}$ | 1.00 | | | | |
| CP | $0.15^{ns}$ | $0.08^{ns}$ | $-0.29^{ns}$ | $0.14^{ns}$ | $-0.27^{ns}$ | $0.26^{ns}$ | $0.09^{ns}$ | $0.07^{ns}$ | $0.08^{ns}$ | 1.00 | | | |
| CF | $-0.00^{ns}$ | $-0.07^{ns}$ | $0.06^{ns}$ | $0.18^{ns}$ | $-0.15^{ns}$ | $-0.05^{ns}$ | $-0.00^{ns}$ | $-0.12^{ns}$ | $0.74^{**}$ | $-0.50^{*}$ | 1.00 | | |
| CA | $-0.00^{ns}$ | $0.16^{ns}$ | $0.10^{ns}$ | $-0.38^{ns}$ | $0.53^{*}$ | $-0.14^{ns}$ | $-0.38^{ns}$ | $-0.24^{ns}$ | $0.13^{ns}$ | $-0.25^{ns}$ | $0.07^{ns}$ | 1.00 | |
| EC | $0.04^{ns}$ | $-0.07^{ns}$ | $0.07^{ns}$ | $0.24^{ns}$ | $-0.34^{ns}$ | $0.13^{ns}$ | $0.22^{ns}$ | $0.08^{ns}$ | $0.72^{**}$ | $0.06^{ns}$ | $0.71^{**}$ | $-0.11^{ns}$ | 1.00 |

$^{ns}$ Non Significant; $^{*}$ P<0.05; $^{**}$ P<0.01; $^{***}$ P<0.001.

$pH_{24h}$, positive correlations with *L** (r=0.38) and *b** (r=0.49) were observed; however, it was negatively correlated with *a** (r=−0.46), WHC (r=−0.57) and CL (r=−0.42) (P<0.05, P<0.01). The above results can be explained by fast acidification of meat and degradation of muscle protein and pigments, this refers to the colour fading (higher *L**, higher *b**, lower *a**), and decreased WHC (increased drip and cooking loss) (Hiscock *et al.*, 2022). Similarly, Barbut (1993) reported high negatives correlations between pH with lightness (r=−0.36) and redness (r=0.38) values.

Meat lightness (*L**) was negatively correlated with *a** (r=−0.75) and DL (r=−0.57) (P<0.01); while positively correlated with *b** (r=0.44) (P<0.05). The results indicate that poultry meat with higher redness tends to present higher levels of lightness and yellowness (Tougan *et al.*, 2013). Positively correlations between reddnes (*a**) and WHC (r=0.45) and DL (r=0.66) (P<0.05, P<0.01) were observed. Yellowness (*b**) was also negatively correlated with WHC (r=−0.56) and DL (r=−0.46) (P<0.05). Interestingly, *b** was significantly correlated with CA (r=0.53) (P<0.05). Finally, DM was positively correlated with CF (r=0.74) and EC (r=0.72) (P<0.01). Instead, a negative correlation (P<0.05) was found between CP and CF (r=−0.50). The latter had a positive correlation with EC (r=0.71) (P<0.01). It is important to examine the relationships between fat content and other nutritional traits of poultry meat closely because low-fat meat is beneficial in a health-driven market (Sarica *et al.*, 2011).

## CONCLUSIONS

This study provides an preliminary description of phenotypic correlations between important quality traits of breast and leg meat from native Mexican Guajolotes. The results could be used as an im-portant benchmark of the current state of Guajolote meat quality to develop selection and breeding programs in its genetic improvement. Future work might address the main factors affecting these meat quality traits (*e.g.*, genetics, management, environmental, ante-mortem behavior, among others).

## ACKNOWLEDGEMENTS

This study was supported by Colegio de Postgraduados through the project "Valorización de la canal y calidad nutricional de la carne del Guajolote Nativo Méxicano en Campeche" [Folio No: CONV_RGAA_2022-30]. Likewise, it is part of the research line: LGAC-11 "Innovación Tecnológica y Calidad Alimentaria en Ganadería ", Colegio de Postgraduados.

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# Yield of Popcorn varieties in different population densities in the Valles Altos region of Mexico

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**Citation**: Tadeo-Robledo, M., Espinosa-Calderón, A., Monter-Santillán, A., Ambrosio-Gil, E., Martínez-Díaz, F. S., Gómez-Montiel, N.O., Sierra-Macías, M., Andrés-Meza, P., & Hernández-Galeno, C. D. A. (2023). Yield of Popcorn varieties in different population densities in the Valles Altos region of Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2595

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: January 14, 2023.
**Accepted**: May 05, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 107-114.



## ABSTRACT

**Objective**: the objective of this study was to determine the grain productivity of four Mexican varieties of popcorn maize (Palomero UNAM, Puma Palomero, Palomero Oro and Palomero Unamita) under three population densities.

**Design/Methodology/Approach**: during the Spring-Summer 2020 agricultural cycle, these four varieties were evaluated in two different environments and three population densities were used: 50, 65 and 80 thousand plants per hectare. The experimental design used was completely randomized blocks, with a factorial arrangement of treatments and four replications.

**Results**: the results showed that the population densities of 65 and 80 plants ha$^{-1}$ were statistically equal with yields of 6340 and 6357 kg ha$^{-1}$, but significantly different to the density of 50 thousand plants per hectare. As for the varieties, Palomero Unamita and Palomero Oro showed yields of 6809 and 6625 kg ha$^{-1}$.

**Study limitations/Implications**: it is important to note that is necessary to continue the evaluation of these varieties in different years and environments to obtain more solid and generalizable results.

**Findings/Conclusions**: based on the results obtained in terms of yield, yield components and outstanding vegetative traits, it can be concluded that there are favorable perspectives for the commercial use of the popcorn varieties evaluated in this study.

**Keywords**: *Zea mays* L., productivity, improved varieties of popcorn, seeds, Valles Altos.

## INTRODUCTION

The popping seed varieties of maize (*Zea mays* L.), or popcorn is one of the many types of corn grown in Mexico, under the management of native people. This maize has cultural, socioeconomic and gastronomic relevance, especially in gastronomy, due to multiple

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nutritional properties of popcorn which then has a high nutritional value. Popcorn contains high amounts of fiber, vitamins, minerals, proteins, and antioxidants. This type of corn in Mexico is in risk of extinction nowadays (De la O-Olán *et al.*, 2018).

Popcorn is one of the most appreciated snacks, mainly in the United States, where more than 60 000 hectares (ha) of improved varieties are planted annually. These varieties have exceptional burst quality in one of the largest areas planted with popcorn in the world, which provides an average yield of 7 Mg ha$^{-1}$. In Mexico, the consumption of popcorn is almost totally dependent on imports. About 400 tons (Mg) are produced in the country, but 80 000 Mg of popcorn kernels are imported.

Those improved varieties had their origin in Mexican germplasm of some of the seven breeds whose kernels can burst and form the popcorn, which are Palomero Toluqueño, Chapalote, Nal-Tel, Arrocillo Amarillo (Bautista *et al.*, 2020), Palomero de Jalisco, Palomero de Chihuahua and Reventador (Bautista *et al.*, 2018; Bautista *et al.*, 2020).

It is considered necessary to implement an ethnobotanical exploration of Palomero Toluqueño in the areas estimated as potential (Romero *et al.*, 2005). In order to generate conservation schemes and favor genetic improvement and use, within their distribution areas (CONABIO, 2012; Bautista *et al.*, 2018; Granados, 2019; Bautista *et al.*, 2020; CONABIO, 2020).

A relevant contribution in the country was the popcorn variety V 460 P, registered in the Mexico's National Catalog of Plant Varieties (CNVV) in 2012, with adaptation to southern Tamaulipas by Dr. Juan Valadez Gutiérrez of INIFAP (Valadez *et al.*, 2012). On the other hand, the Faculty of Higher Studies Cuautitlan under the National Autonomous University of Mexico (FESC UNAM), and Campo Experimental Valle de México (CEVAMEX INIFAP) have worked on genetic improvement to develop varieties of popcorn since 27 years ago. In 1986, the first evaluation of a group of popcorn maize varieties was done, as a product of the studies developed there in collaboration with INIFAP. Improved and native materials were evaluated, protein quality sources (QPM) were combined with native popcorn varieties and inbred lines to offer popcorn with higher amounts of lysine and tryptophan. Then varieties were selected, lines were derived, compounds were integrated in a broad genetic base, crosses were made among better lines, recombination cycles, backcrosses, new self-fertilizations, fraternal, and dialelic lines were integrated, until obtaining quality populations and varieties of popcorn.

As a result of those investigations, there are elite varieties in their last stage of evaluation, seed production, varietal characterization, prior to their registration in the National Catalog of Plant Varieties (CNVV) and their commercial release. The varieties Palomero Oro, Palomero Unamita, Palomero UNAM, Puma Palomero have been defined as outstanding. This study was established based on these varieties, with the aim of determining productivity under three population densities.

## MATERIALS AND METHODS

### Geographic location of study sites

During the Spring-Summer 2020 cycle, two uniform trials were established. The first locality at A) Huexotla, Texcoco, State of Mexico (19° 28' 54.5" N, 98° 52' 34.4" W) at

2240 m altitude. The area has a climate C(Wo)(w)b(i')g that corresponds to temperate climate with rains in summer, the driest of the subhumid climates, with cool and prolonged summers, with temperature annual averages between 12 and 18 °C; the annual oscillation of average monthly temperatures is 5 to 7 °C (García, 2004).

The second locality is B) the Rancho Almaraz of the Facultad de Estudios Superiores Cuautitlán (FESC UNAM), Campo 4 of the National Autonomous University of Mexico (UNAM), located at 19° 41' 35" N, 99° 11' 42" W at an altitude of 2274 m; the climate at Cuautitlan is classified as C $(w_0)$ $(w)$ b (i"). The historical average of annual rainfall is 609.2 mm (García, 2004).

**Genetic material**

Four improved varieties of maize from FESC UNAM, Palomero UNAM, Puma Palomero, Palomero Oro, and Palomero Unamita were evaluated. These varieties were planted in 50, 65 and 80 plants ha$^{-1}$; an experimental design of complete randomized blocks was used under a factorial arrangement with four repetitions (Espinosa-Calderón *et al*., 2018).

**Statistical design and experimental units**

The experimental plot consisted of a row 5 m long by 0.80 m wide. The experiments were established following the complete randomized blocks design, with four repetitions and under rainfed conditions. The plantings were made on foot in the month of June with three seeds deposited in each hole every 0.50 m. Then thinning was done to obtain the population densities of 50, 65 and 80 thousand plants ha$^{-1}$.

In the field, variables evaluated were days to male flowering, days to female flowering, plant height, and plant height to the ear expressed in cm. The harvest was carried out manually in December 2020. In each plot, all the ears were pinched and in a representative sample of five ears the percentage of moisture of the grain was determined, with a Dickey-John Gac 2100® moisture determiner, to obtain the percentage of dry matter (% DM); the percentage of grain/cob by means of ratio between the grain weight and the grain weight plus cob weight (Mexican name, olotes) (% G), volumetric weight, weight of 200 grains, ear length, number of rows in the ear, and grains per row. To obtain the grain yield this formula was applied:

$$Yield = (PC \times \%\, MS \times \%\, G \times FC)/8600.$$

**Statistical analyses**

SAS/STAT® 9.4 was used for all statistical analyses of the data (SAS Institute Inc., 2018). Likewise, a comparison of means was performed (Tukey, $p \leq 0.05$).

## RESULTS AND DISCUSSION

The combined analysis of variance showed significant differences ($p<0.05$) among genotypes for all variables except FF and LM. Significant differences ($p<0.05$) were also detected between environments in all variables except GH; whereas, for plant density as the source of variation, there were significant differences ($p<0.05$) only in the variables RG, PV and LM. In the interaction environments×genotypes, there were significant differences ($p<0.05$) in the variables RG, FM, PV, P200G and LM.

In contrast, in the interaction environment×plant density, significant differences ($p<0.05$) were detected in the variables grain yield, FM, P200G, LM and %GRAIN (Table 1). The coefficients of variation ranged from 0.5 to 13% considered as low, suggesting reliability in the results (Castillo, 2007). Finding statistical differences among genotypes and between localities, as well as high levels of variation in traits, indicates the existence of genetic diversity in the varieties evaluated, which respond differently across environments.

Regarding the productive and agronomic characteristics of the varieties evaluated, the overall average grain yield was 6017 kg $ha^{-1}$. The Palomero Unamita and Palomero Oro varieties yielded 6809 kg $ha^{-1}$ and 6625 kg $ha^{-1}$, statistically different from the other two varieties (Table 2). When comparing these results with a recent study in native popcorn populations in southern Brazil, yields are considered appropriate (Zulkadir and Idikut, 2021).

When comparing the days to flowering among varieties, it was observed that the Palomero Oro variety was of late cycle with 78 and 80 days to male and female flowering. Whereas the Palomero Unamita variety presented an early cycle with 73 and 69 days to male and female flowering (Table 2, Figure 1). According to Gil *et al.* (2004) early varieties generally manage to evade the periods of water deficiencies that manifest when there is low rainfall.

**Table 1**. Mean squares and statistical significance in the combined analysis of four free-pollinated varieties of popcorn; under three population densities in Valles Altos (Mexico), Spring-Summer 2020 cycle.

| Source of variation | Environment | Block | Population density | Varieties | Environment × varieties | Environment × population density | Coefficient of variation (%) |
|---|---|---|---|---|---|---|---|
| GY (kg $ha^{-1}$) | 79305462** | 480871* | 5265535** | 10715819** | 1515295** | 316035** | 4.9 |
| MF (días) | 204.2** | 5.5 | 0.64 | 49.3** | 9.6** | 5.9** | 1.8 |
| FF (días) | 617* | 113.8 | 95 | 263.4 | 54.7 | 99.9 | 13.0 |
| PH (m) | 0.068* | 0.108* | 0.011 | 0.048** | 0.01 | 0.014 | 4.4 |
| PHE (m) | 1.08** | 0.048** | 0.013 | 0.054** | 0.01 | 0.013 | 7.6 |
| VW (kg $hL^{-1}$) | 33.2** | 0.43 | 6.13** | 14** | 4.2* | 1.75 | 1.3 |
| W200G (g) | 335** | 5.0 | 4.1 | 138.9** | 12.9** | 7.5* | 4.2 |
| EL (cm) | 21.3** | 1.33 | 2.02* | 1.05 | 1.38* | 1.56** | 4.1 |
| NRE | 14** | 0.08 | 0.58 | 6.14** | 0.027 | 0.87 | 5.9 |
| GPE | 420 | 324 | 1748 | 5915** | 248 | 1334 | 7.0 |
| DM (%) | 68.6** | 0.08 | 0.09 | 0.77* | 0.2 | 0.17 | 0.5 |
| Grain (%) | 31** | 2.57 | 6.15 | 10.11** | 3.4 | 5.6** | 1.6 |

*: $p<0.05$, **: $p<0.01$; GY: grain yield, MF: male flowering, FF: female flowering, PH: plant height, PHE: plant height to the ear, VW: volumetric weight, W200G: weight 200 grains, EL: ear length, NRE: number of rows in the ear, GPE: grains per ear, %DM: percentage of dry matter.

**Table 2**. Comparison of means among varieties of popcorn maize, considering the mean of the evaluation environments.

| Varieties | Palomero Unamita | Palomero Oro | Palomero UNAM | Puma Palomero | DHS |
|---|---|---|---|---|---|
| GY (kg $ha^{-1}$) | 6809 a | 6625 a | 5910 b | 4728 c | 334 |
| MF (días) | 73 c | 78 a | 76 b | 76 b | 1.6 |
| FF (días) | 69 a | 80 a | 78 a | 78 a | 11.3 |
| PH (m) | 2.2 ab | 2.3 a | 2.2 ab | 2.1 b | 0.11 |
| PHE (m) | 1.0 bc | 1.1 a | 1.07 ab | 0.96 c | 0.09 |
| VW (kg $hL^{-1}$) | 78.4 b | 79.1 b | 80.5 a | 78.1 b | 1.13 |
| W200G (g) | 40.6 a | 36.1 b | 34.4 c | 32.7 d | 1.7 |
| EL (cm) | 16 a | 16 a | 15 b | 16 a | 0.74 |
| NRE | 16 b | 17 a | 16 b | 17 a | 1 |
| GPE | 460 b | 480 ab | 467 b | 510 a | 37.9 |
| DM (%) | 87 b | 88 a | 87 b | 88 a | 0.52 |
| Grain (%) | 83.2 a | 81.9 ab | 81.2 b | 81.5 b | 1.52 |

GY: grain yield, MF: male flowering, FF: female flowering, PH: plant height, PHE: plant height to the ear, VW: volumetric weight, W200G: weight 200 grains, EL: ear length, NRE: number of rows in the ear, GPE: grains per ear, %DM: dry matter.


**Figure 1**. Varieties of popcorn maize. A: palomero Puma; B: palomero oro; C: Palomero UNAM; D: palomero Unamita.

As for the variables AP and AM, the Palomero Oro variety presents high values with 2.3 and 1.1 m; on the contrary, Puma Palomero presented a low size with 2.1 and 0.96 m (Table 2). According to Bernal *et al.* (2021) they are related to the architecture of the plant and, mainly, to the stalk lodging. For the PV variable, the Palomero UNAM variety showed the highest mean (80.5 kg $hL^{-1}$). This indicates that it has a higher specific weight compared to the other varieties (Table 2). This result coincides with previous research conducted by Martínez (2022), who found that the PB6×PB1 crosses turned out to have higher PV than other crossed evaluated.

As for the P200g variable, the variety Palomero Unamita showed the highest mean (40.6 g), followed by the other varieties in descending order. This could indicate that the variety Palomero Unamita has heavier grains compared to the others. Likewise, it was observed that there was a relationship for the variable %GRAIN of 83.2%; on the contrary, Puma Palomero presented the best values for the variable GM. These findings are supported by similar and current research that has found differences in grain yield, volumetric weight, and other characteristics among popcorn varieties.

On the other hand, the best environment was CEVAMEX with 7303 kg $ha^{-1}$, slightly higher than the overall average (Table 3). The yields indicated are competitive with those reported by Miranda (1977) and Valadez *et al.* (2012), although studies were done in different regions, they could be used as references. These results are consistent with previous studies that have shown that popcorn productivity can vary according to differences in nutrient availability, soil management, and climatic conditions.

In regard to the yield components PV, P200G, LM, HM, GH, GM and %GRAIN in this same environment the best values for these variables were recorded (Table 3). According to Valadez *et al.* (2012) these differences may be related to genetic traits in grain size and grain development. Similarly, Reyes *et al.* (2009) have reported variations in the number of grains per ear in popcorn related to grain composition and its endosperm and pericarp ratio. In addition, this type of study requires defining the quality and volume of expansion of the popcorn produced with the varieties, in a given planting density and evaluation

**Table 3**. Comparison of means between evaluation environments, considering the mean of four varieties of popcorn and three population densities.

| Main factor | GY | VW | W200G | EL | NRE | GPR | GPE | Grain |
|---|---|---|---|---|---|---|---|---|
| Environment | (kg $ha^{-1}$) | (kg $hL^{-1}$) | (g) | (cm) | | | | (%) |
| FESC 2020 | 4732 b | 78.2 b | 33.3 b | 15 b | 17 a | 30 a | 482 a | 82.8 a |
| CEVAMEX 2020 | 7303 a | 79.9 a | 38.6 a | 16.3 a | 16 b | 28 b | 476 a | 81.1 b |
| average | 6017.5 | 79.05 | 35.95 | 15.65 | 16.5 | 29 | 479 | 81.95 |
| Density | | | | | | | | |
| 50 000 | 5355 b | 78.5 b | 35.5 a | 16 a | 16 a | 29 a | 469 a | 81 a |
| 65 000 | 6340 a | 78.9 a | 35.9 a | 15 b | 16 a | 30 a | 490 a | 82 a |
| 80 000 | 6357 a | 79.7 a | 36.5 a | 16 a | 16 a | 29 a | 479 | 82 a |
| average | 6017.3 | 79.0 | 36.0 | 15.7 | 16.0 | 29.3 | 479.3 | 81.7 |

GY: grain yield, VW: volumetric weight, W200G: weight 200 grains, EL: ear length, NRE: number of rows in the ear, GPR: grains per row, GPE: grains per ear environment, which is part of other studies within the research group (Soylu and Tekkanat, 2007; Sweley *et al.*, 2012).

Similarly, planting density influenced yield and other characteristics evaluated between planting densities of 50 000, 65 000 and 80 000 ha$^{-1}$ plants. Overall, it was found that the planting density of 65 000 and 80 000 ha$^{-1}$ plants tended to outperform 6340 kg ha$^{-1}$ and 6357 kg ha$^{-1}$, different from 50 thousand plants ha$^{-1}$. These results are consistent with previous studies that have shown that planting density can influence the yield and quality of popcorn (Canales *et al.*, 2017). These results support the importance of considering the growing environment when selecting popcorn varieties, and highlight the need for additional research to better understand the factors influencing the production and quality of popcorn in different environments.

Following the same premise, the yields of the Palomero Unamita and Palomero Oro varieties expressed to some extent the product of the studies in genetic improvement for popcorn varieties (Figure 1), implemented by FESC UNAM and CEVAMEX INIFAP since 1996 (Espinosa-Calderón *et al.*, 2018). These varieties have in their germplasm, source for varieties of popcorn; which is a good argument in favor of the preservation of this type of maize to maintain its permanence in the region where the Palomero Toluqueño breed thrives (Gámez *et al.*, 2014).

## CONCLUSIONS

The Palomero Unamita and Palomero Oro varieties presented high grain yield and outstanding vegetative characteristics through the two evaluation environments. The yields in population densities of 65 and 80 thousand plants per ha-1 were good and indicate the convenience of continuing with this type of studies to confirm the commercial prospects to the use of popcorn maize varieties. Nonetheless, the four evaluated varieties could be options to support the production of popcorn in Mexico; thus, it is advisable to continue in parallel with the development of promotional schemes.

## ACKNOWLEDGMENTS

To the National Autonomous University of Mexico for the financial Support Program for Projects on Research and Technological Innovation (PAPIIT), through the project Num: IT200122, DGAPA-UNAM: "Mejoramiento Genético, Productividad y Calidad de expansión de maíz palomero (*Zea mays everta*) en México" (Genetic Improvement, Productivity and Quality of Expansion of Popcorn maize [*Zea mays everta*] in Mexico).

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Colegio de Postgraduados

# Effects of an increase in Mexican strawberry exports to Canada on the profitability of producers in Mexico

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**Citation**: Hernández-Soto, D., Vivanco-Vargas, M., Banda-Ortiz, H., Cruz-Lázaro, L. M., Gómez-Hernández, D., & Velázquez-Alquicira, D. M. (2023). Effects of an increase in Mexican strawberry exports to Canada on the profitability of producers in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2601

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: March 13, 2023.
**Accepted**: June 19, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 115-124.



## ABSTRACT

**Objective**: determine the viability of increasing the exported quantity of Mexican strawberries to the Canadian market.

**Design/methodology/approach**: Likewise, a simulated scenario was developed with the purpose of carrying out a forecast on the conditions that may occur to have a more accurate knowledge of the operation of the international strawberry trade between Mexico and Canada. To perform this analysis, the international market was represented in a partial equilibrium model.

**Results**: According to the calculated price flexibility, an increase in the exported quantity of Mexican strawberries to Canada of 50% in one year would cause a positive final effect. With this estimate, it can be established that an increase in the exported quantity of Mexican strawberries to Canada of 50% in one year would be viable in the economic sense. In this simulated scenario, the Benefit/Cost Ratio (B/C R) calculated for the producers of Michoacan, Baja California and Guanajuato would be 1.0865, 1.196 and 0.6856 respectively.

**Limitations on study/implications**: not all products and all states of Mexico are examined.

**Findings/conclusions**: The results showed that an increase in strawberry production to export to Canada in Michoacan and Baja California would be profitable for the producer, while an increase in strawberry production for export to the Canadian market in Guanajuato would further decrease profitability.

**Keywords**: agricultural production; econometric analysis; Agricultural sector.

## INTRODUCTION

To carry out this research work, a descriptive study was developed to show the effects of an increase in Mexican strawberry exports to the Canadian market. In order to do it, the Mexican strawberry market between Mexico and Canada was represented in an

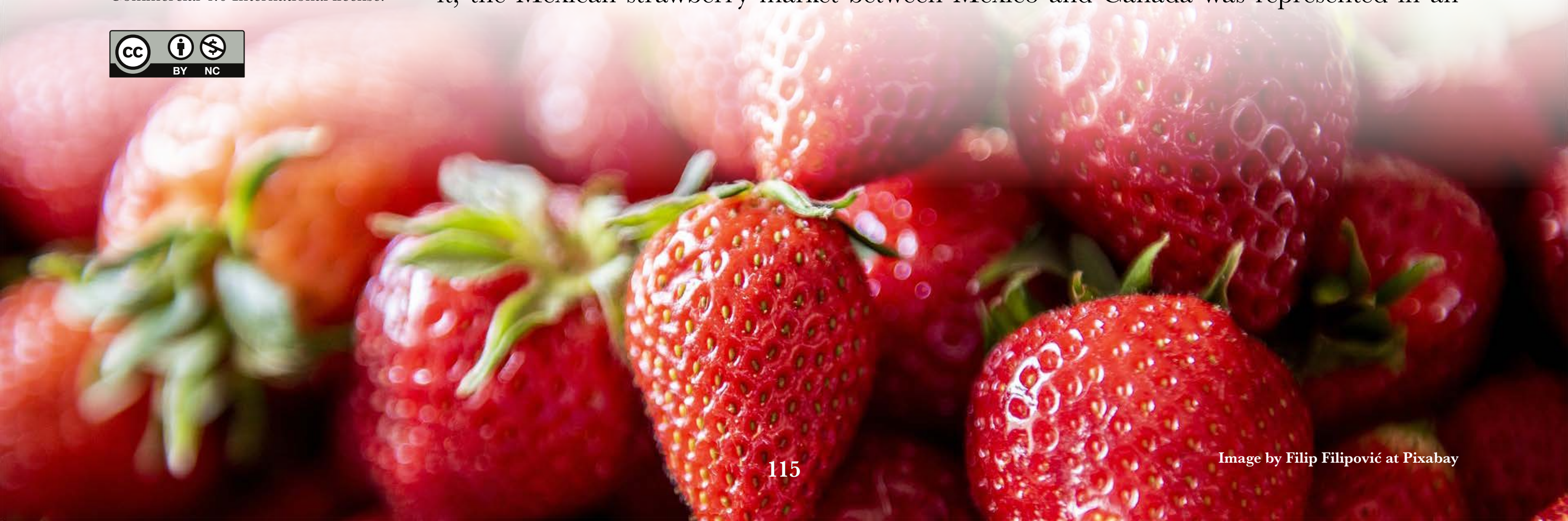

Image by Filip Filipović at Pixabay

econometric model to calculate the price flexibility of demand and to carry out a partial equilibrium analysis of the international market for a good between two economies [1, 2, 3, 4, 5, 6, 7]. This calculation allows the simulation of an increase in the quantity traded. Thus, it is possible to affirm that the study is explanatory, since to carry it out it is necessary to establish the effect that an increase in the quantity exported of Mexican strawberries to Canada causes on the price. Then, the increase in the quantity exported imply two effects, and it is necessary to determine the final effect. The work is quantitative since the market is represented through an econometric model, considering the relationship between the variables that exist for the international trade of strawberries between Mexico and Canada to take place [8, 9].

## MATERIALS AND METHODS

The econometric model that represents the strawberry market between two economies was made up of two main equations: The first one was a demand function for Mexican strawberry imports, in which  is the CIF real unit price, and operates as the dependent variable, and was influenced by:

$$PIFMCan_t = \beta_1 + \beta_2 QIFMCan_t + \beta_3 PPFMR_t + \varepsilon_1 \quad (1)$$

The second one was a supply function for strawberry exports in Mexico, in which the real unit price of $PEFM_t$ was the price of strawberry exports in Mexico, and operates as the dependent variable, and was influenced by: $QEFM_t$, which was the quantity exported of strawberries in Mexico, and by $PPFMR_t$, which was the real unit price of the strawberry to the producer in Mexico:

$$PEFM_t = \beta_{20} + \beta_{21} QEFM_t + \beta_{31} PPFMR_t + \varepsilon_2 \quad (2)$$

At this point, it is necessary to say that the method of 3-Stage Least Squares (3SLS) was applied to the simultaneous equations model (supply and demand in the international market) with the purpose of calculating the coefficients $\beta_1$-$\beta_{31}$. In this regard, the $\beta$ coefficients were calculated simultaneously based on the relationship of the variables in the market, and they were represented inside the model [8]. Likewise, it is important to mention that the $PIFMCan_t$, $QIFMCan_t$, $PEFM_t$ and $QEFM_t$ variables were built with data from the Internet Tariff Information System Via Internet (SIAVI, by its Spanish acronym) of the Mexican Ministry of Economy [10], while the variable $PPFMR_t$ was built with information from the Food and Fisheries Information Service (SIAP) of the Mexican Ministry of Agriculture [11].

### The partial equilibrium analysis

In order to apply the partial equilibrium model, the following assumptions were established:

1. The international market for a good: Mexican strawberries for export to Canada.

2. An international market between two nations: Mexico as the country that exports strawberries to Canada; and Canada as the importing country of Mexican strawberries.
3. For this analysis, strawberry exports in Mexico were equal to the excess supply in the international market.
4. For this analysis, Mexican strawberries imports in Canada were equal to the excess demand in the international market.
5. Monetary values in Canadian dollars Can$.
6. Values and prices in real terms.
7. A 50% increase in the quantity of Mexican strawberries imported in Canada in 2022 compared to the quantities imported in 2021.

Likewise, an increase in the quantity imported of strawberries in Canada was expressed in the international market as a displacement of the excess supply curve from its starting position $ES_0$ to position $ES_1$, as can be seen in Figure 1. This change causes a decrease in the international price from the starting position $IP_0$ towards position $IP_1$, and this, in turn, causes an increase in the quantity traded from $IQ_0$ towards position $IQ_1$. This decrease in the price from $IP_0$ to $IP_1$ also causes an increase in the excess demand $ED$ for strawberries in the international market.

Now, the response of a change in price to a change in the quantity traded is given by the price flexibility of demand, which can be calculated as follows:

$$F^{PIFMCan} = \left(dPIFMCan \,/\, dQIFMCan\right) * \left(QIFMCan \,/\, PIFMCan\right) \tag{3}$$

Thus, through the price flexibility of demand, the percentage by which the international price decreases when the quantity traded (between both countries) increases by 1% can be calculated. The analysis begins (in the first moment) in the real international market for imported Mexican strawberries in Canada in 2021; in this scenario, the international price of imports is $IP_0$ while the quantity imported is given by $IQ_0$. Now, in a second moment, the simulated 2022 scenario considers a 50% increase in the quantity imported

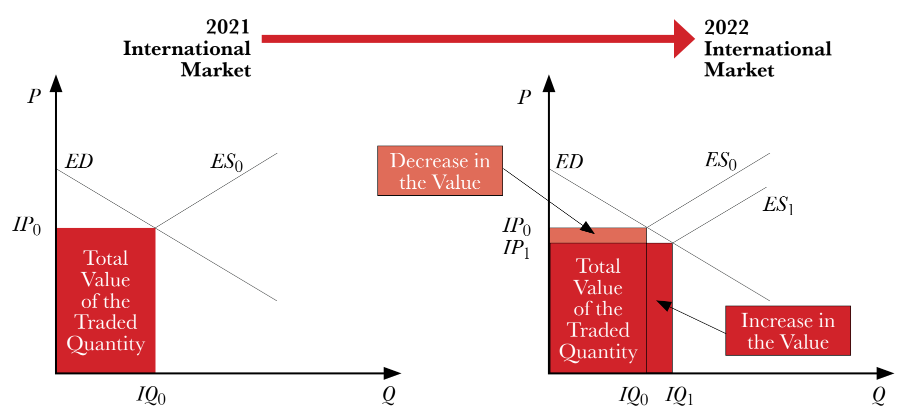


**Figure 1**. Changes in the total value of the quantity traded in the face of an increase in exports.

of Mexican strawberries in Canada, denoted by $IQ_1$ in the international market, while the $IP_1$ price is less than $IP_0$ (in 2021), in a magnitude determined by the price flexibility of demand. Likewise, it can be established that an increase in the amount imported causes an increase in the value of imports, while, on the other hand, a decrease in the price causes a decrease in the mentioned value (as can be observed in the Figure 1). The combined effects of both impacts on the value of Mexican strawberry imports destined for Canada in the international market cause a final impact. The calculation of these effects can be determined as follows:

The increase in value (due to the increase in the quantity imported):

$$Increase\ in\ Value = \left(Q_1 - Q_0\right) * P_1 \tag{4}$$

The decrease in value (due to the decrease in the quantity imported):

$$Decrease\ in\ Value = \left(P_0 - P_1\right) * Q_0 \tag{5}$$

The final effect is the result of both impacts combined, *i.e.*, the difference between the increase in value (of total imports of Mexican strawberries in Canada) due to the increase in quantity minus the consequent decrease in value due to the decrease in price; *i.e.*, this final effect can be calculated as follows:

$$Increase\ in\ Value = Decrease\ in\ Value = Final\ \ Effect \tag{6}$$

The calculation of this final effect is the result of the simulated 50% increase in the quantity imported. So, to determine the viability of this increase in the quantity of strawberries traded between Mexico and Canada in the international market, the criteria are: 1. If the Increase in Value is greater than the Decrease in Value, the difference will have a result with a positive sign, which means that the total value of the quantity traded will increase. This result allows us to establish that a 50% increase in the quantity imported of Mexican strawberries in Canada is viable from an economic perspective; 2. If the Increase in Value is less than the Decrease in Value, the difference will have a result with a negative sign, which means that the total value of the quantity traded will decrease. This result allows us to establish that a 50% increase in the quantity imported of Mexican strawberries in Canada is not viable from an economic perspective.

**The profitability in the producing areas**

Both impacts (50% increase in quantity and the consequent 9.16% decrease in price) can be transferred to the context of strawberry producers in Michoacan, Baja California and, Guanajuato to determine the final effect that a 50% increase in the quantity exported of Mexican strawberries to Canada over the profitability of production. That profitability in production can be expressed through the *Benefit*/*Cost* Ratio [12] and calculated as follows:

$$B / CR = Benefits / Costs \tag{7}$$

In order to determine the profitability for the producer, the first criterion can be expressed as follows:

$$B / CR > 1 \text{ It is profitable} \tag{8}$$

In this sense, equation 8 shows that: to determine the profitability of carrying out a productive activity, the $B / CR$ is greater than 1, this result means that the income is greater than the expenses, so that the performance of the determined activity is profitable. When applied to the case of the 2022 simulated scenario, this result would mean that under the specified conditions, an increase in production with the purpose of increasing the quantity exported of Mexican strawberries to Canada by a magnitude that represents an annual increase of 50% in 2022 compared to 2021, it would be profitable for the producer in Mexico:

$$B / CR = 1 \text{ There are no profits no losses} \tag{9}$$

Now, Equation 9 shows that: to determine the profitability of carrying out a productive activity the  is equal to 1, this result means that the income is equal to the expenses, so that the performance of the determined activity does not represent no profit or loss. When applied to the case of the 2022 simulated scenario, this result would mean that under the specified conditions, an increase in production with the purpose of increasing the quantity exported of Mexican strawberries to Canada by a magnitude that represents an annual increase of 50% in 2022 compared to 2021, for the producer in Mexico there would be no profit or loss:

$$B / CR < 1 \text{ It is not profitable} \tag{10}$$

Now, equation 10 shows that to determine the profitability of carrying out a productive activity, the $B / CR$ is less than 1; this result means that the income is less than the expenses, so that the performance of the determined activity is not profitable. When applied to the case of the 2022 simulated scenario, this result would mean that under the specified conditions, an increase in production with the purpose of increasing the quantity exported of Mexican strawberries to Canada by a magnitude that represents an annual increase of 50% in 2022 compared to 2021, it would not be profitable for the producer in Mexico.

## RESULTS

Based on the results of the application of 3-Stage Least Squares to the econometric model, the $\beta$ coefficients were calculated, as can be seen in Table 1.

Based on the results, t-Student tests were accomplished. To carry them out, it is necessary to mention that the critical value of t-Student for a significance level of 0.05 (5%) is equal

**Table 1**. Coefficients $\beta$ of the function of demand.

| Variable | Coefficient | Value | Standard Error* | t-Student value* | Pr>\|t\| |
|---|---|---|---|---|---|
| Intercept | 1 | 2490.00200 | 509.844100 | 4.88 | 0.0005 1** |
| $QIFMCan_t$ | 2 | −4.33127 | 1.880600 | −2.30 | 0.0418 * |
| $PPFMR_t$ | 3 | 0.0000000054 | 0.000000001174 | 4.60 | 0.0008 ** |

* Note: It is significant at the level of 0.05. ** Note: It is significant at the level of 0.01.

to 1.7613, while the critical value of t-Student for a significance level of 0.01 (1%) is equal to at 2.6245. In this way, in the hypothesis test, the value of t-Student for the coefficient $\beta_1$ was equal to 4.88, therefore, it was greater than 2.6245, so the probability of the respective t-Student test is (0.0005) is less than 0.01**. In the same sense, the t-Student value for the coefficient $\beta_2$ was equal to −2.30, that is, it was less than −1.7613, so the probability of the respective t-Student test (0.0418) was less than 0.05*. Likewise, the value of t-Student for the coefficient $\beta_3$ was equal to 4.60, that is, it was greater than 2.6245, so it was possible to interpret that the probability of the corresponding t-student test (0.0001) was less than 0.01**. Then, with these results, it can be established that the estimated values of $\beta_1$, $\beta_2$ and $\beta_3$ were statistically significant. With the estimation of the values of the coefficients $\beta$'s, it was possible to build the demand equation:

$$PIFMCan_t = 2490.002 - 4.33127\ QIFMCan_t + 0.0000000054\ PPFMR_t + \varepsilon_1 \quad (11)$$

In order to calculate the price flexibility of demand, the partial derivative of the demand function (11) was developed with respect to the quantity $QIFMCan_t$

$$\left(dPIFMCan \,/\, dQIFMCan\right) = 4.33127 \quad (12)$$

With this estimation, price flexibility of demand was calculated:

$$F^{PIFMCan} = \left(-4.33127\right)\left(189.3375 \,/\, 4474.207123\right) = -0.183288751 \quad (13)$$

Thus, it was possible to establish that if the quantity demanded increases 1%, the price decreases 0.18%. So, based on this flexibility, it is possible to affirm that if the quantity demanded increased by 50% for the simulated scenario of 2022 (compared to 2021), this situation would cause a decrease of −9.16% in the price of Mexican strawberries in Canada, compared to the price in 2021, as can be seen in Table 2.

**Table 2**. Estimates of price flexibility of Mexican strawberry demand in Canada.

| Increase in the quantity imported of Mexican strawberry in Canada | Decrease in the price of the Mexican strawberry imports in Canada* |
|---|---|
| 1% | −0.183288751% |
| 50% | −9.164437532% |

*Adapted from the results of the Econometric Model.

Now, an increase in the quantity imported of Mexican strawberries in the Canadian market from $IQ_0$ to $IQ_1$ causes the value of the quantity traded to increase. However, there is also a second effect, a decrease in price from $IP_0$ to $IP_1$, as can be seen in Figure 1. The result of both effects is an increase in the total value of the quantity traded in Can$403,542.07 (see in Table 3).

Now, the value of the areas referred to in Figure 1 is estimated, resulting in an increase of Can $403,542.07 in the total value of the quantity imported of Mexican strawberries in Canada (as can be seen in Table 4).

Then, with these results, the $B/CR$ was estimated to determine the profitability of producing strawberries in Michoacan to export to the Canadian market for the year 2021. It is important to say that, in 2021, 90% of strawberry production in Baja California it was destined for the US market, while 10% was destined for the national market. Likewise, in 2021, the state of Guanajuato allocated 30% of its production to the US market approximately. That is to say, Baja California and Guanajuato did not export strawberries to Canada.

The Table 5 shows that, in 2021, the $B/CR$ of strawberry production in Michoacan was 1.1961, so it was possible to affirm that producing strawberries to export to Canada in Michoacan was profitable. Now, with the purpose of establishing the hypothetical 2022

**Table 3**. Total value of the Mexican strawberry imports in Canada if the quantity increases 50%.

| Qt | Pt | Total Value Qt*Pt |
|---|---|---|
| $Q_{2021}$ = 192.81 | $P_{2021}$ = 5,772.98 | $Q_{2021}$*$P_{2021}$ = Can$1,113,116.84 |
| $Q_{2022}$ = 289.22 | $P_{2022}$ = 5,243.92 | $Q_{2022}$*$P_{2022}$ = Can$1,516,658.91 |
| Increase | | Can$403,542.07 |

*Adapted from Sistema de Informacion Arancelaria Via Internet [10]. Ministry of Economy, 2022.
**Adapted from Sistema de Informacion Agropecuaria y Pesquera [11]. Ministry of Agriculture, 2022.

**Table 4**. Increase in the total value of the Mexican strawberry imports in Canada.

| | | |
|---|---|---|
| Increase in the value due to the increase in the quantity* | $(Q_{2022}-Q_{2021})*P_{2022}$ | Can$505,552.97 |
| Decrease in the value due to the decrease in the in the price* | $(P_{2021}-P_{2022})*Q_{2021}$ | Can$102,010.90 |
| Final increase | | Can$403,542.07 |

*Adapted from Sistema de Informacion Arancelaria Via Internet [10]. Ministry of Economy, 2022.

**Table 5**. Determination of the  of producing strawberries in Mexico to export to Canada.

| State | Quantity* t | Unit price** | Unit cost** | Income | Expenses | B/C R |
|---|---|---|---|---|---|---|
| Michoacán | 192.81 | MXN19,616.72 | MXN16,401.00 | MXN 3,782,397.87 | MXN 3,162,358.81 | 1.1961 |
| Baja California | 0 | MXN31,029.61 | MXN16,391.00 | 0 | 0 | 0 |
| Guanajuato | 0 | MXN12,057.64 | MXN15,976.00 | 0 | 0 | 0 |

*Adapted from Sistema de Informacion Arancelaria Via Internet [10]. Ministry of Economy, 2022.
**Adapted from Sistema de Informacion Agropecuaria y Pesquera [11]. Ministry of Agriculture, 2022.
***Note: Adapted from Agrocostos [13]. Fideicomisos Instituidos en Relacion con la Agricultura, 2022.

scenario with a 50% increase in the quantity exported of Mexican strawberries to the Canadian market, exports equal to 289.22 t were simulated. In this sense, of the total simulated exports, 260.30 t (90%) were assigned to Michoacan; while to include Baja California and Guanajuato in the analysis, which already export strawberries to the US market, 14.46 t (5%) and 14.46 t (5%) were assigned to each state respectively. It is worth mentioning that in order to carry out this scenario, the assumption is a linear function of costs, in this way the cost per t is constant.

Thus, in the simulated 2022 scenario, Table 6 shows that the $B/CR$ for the producers of Michoacan, Baja California and Guanajuato are 1.0865, 1.7196 and 0.6856 respectively. With these results, it was possible to establish that, for producers in Michoacan and Baja California, it would be profitable to produce strawberries to export to Canada in the face of an annual increase in quantity. On the other hand, producing strawberries to export to Canada in Guanajuato would not be profitable, since in the simulated 2022 scenario, given an annual increase of 50% in the quantity exported, the $B/CR$ gets even worse.

**Table 6**. Determination of the  of producing strawberries in Mexico to export to Canada in the simulated scenario.

| State | Quantity* t | Unit price** | Unit cost** | Income | Expenses | B/C R |
|---|---|---|---|---|---|---|
| Michoacán | 260.30 | MXN17,818.96 | MXN16,401.00 | MXN4,638,279.21 | MXN4,269,184.40 | 1.0865 |
| Baja California | 14.46 | MXN28,185.92 | MXN16,391.00 | MXN407,600.12 | MXN237,032.30 | 1.7196 |
| Guanajuato | 14.46 | MXN10,952.63 | MXN15,976.00 | MXN158,387.28 | MXN231,030.93 | 0.6856 |

*Adapted from Sistema de Informacion Arancelaria Via Internet [10]. Ministry of Economy, 2022.
**Adapted from Sistema de Informacion Agropecuaria y Pesquera [11]. Ministry of Agriculture, 2022.
***Adapted from Agrocostos [13]. Fideicomisos Instituidos en Relacion con la Agricultura, 2022.

## CONCLUSIONS

Canada is the third strawberry importer in the world, mainly from the USA. Mexico is the fourth producer, the second exporter in the world, and the second exporter of strawberries to the Canadian market. The results showed that the price flexibility of the demand for Mexican strawberries in Canada was equal to $-0.1833\%$. With this estimate, the simulated scenario showed that an increase of 96.41 t in the quantity exported of strawberries to Canada in 2022 (compared to 2021) would cause a decrease of Can$529.06 per t in the price and causing an effect on income of the producer. The results showed that the final impact on total income would be an increase of Can$403,542.07, therefore, it is possible to establish that the application of mechanisms that increase the quantity exported of Mexican strawberries in Canada in a magnitude that represents annual increases of 50%, would be viable from an economic perspective. Then, transferring the effects calculated in the simulated scenario 2022 to the producer context in Mexico, the results showed that the $B/CR$ for producers in Michoacan, Baja California and Guanajuato would be equal to 1.0865, 1.7196 and 0.6856, respectively. Based on these results, it is possible to affirm that an increase in strawberry production to export to Canada that represents a growth rate of 50% in one year would be profitable for producers in Michoacan and Baja California.

Thus, in Michoacán, strawberry exports to the Canadian market were already carried out, while Baja California exports more than 90% of its production to the US market, since it has the characteristics and quality in the product to obtain Animal and Plant Health Inspection Service (APHIS) certification, an essential requirement to export strawberries to this market. Both states have the technological capabilities to increase the quantity exported; while, regarding Guanajuato, the results show that, for the producer, exporting strawberries to the Canadian market is not profitable, and an increase in the quantity exported in a magnitude that represents 50% in one year would cause that the $B/CR$ got even worse.

Likewise, it is necessary to improve the technological conditions for production of strawberries with the organoleptic and quality characteristics that allow the production developed in Guanajuato to be suitable for entering the Canadian market. In this sense, the technological resources that must be implemented to improve production conditions must include improved seed, fertilizer, adequate mechanisms to combat pests, a controlled environment, as well as the necessary infrastructure for the correct post-harvest handling (food safety, packing and packaging) with the purpose of guaranteeing that the product reaches its destination in optimal conditions, and complying with the demands of the consumer in the international market. Finally, through a partial equilibrium analysis a scenario was built to simulate specific conditions in the market with the purpose of determining effects on the international market, on the economy of the countries, as well as on the technical efficiency for the production and its profitability in the different productive areas. In this way, it is possible to make a forecast of the repercussions of encouraging exports to a specific market and, in this way, determine the feasibility of making the decision to encourage that increase.

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Colegio de Postgraduados

# Evaluation of the influence of pH modification on food proteins structure by FT-IR and AFM

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**ABSTRACT**

**Objective**: The aim of this study was to evaluate the influence of pH variation (3.6, 4.6 and 5.6) on morphometric parameters and the secondary structure of proteins (ovalbumin and gliadin).

**Design/methodology/approach**: The arithmetic mean roughness (Ra) and agglomerate size (AS) of the proteins were analyzed by atomic force microscopy (AFM), while their secondary structure was analyzed by Fourier transform infrared spectroscopy (FT-IR), both at different pH. Subsequently, a correlation analysis of the morphometric changes of the proteins with their secondary structure was performed.

**Results**: Highlighting that it was found that, protein agglomerate size is influenced by changes in $\beta$-sheets and turn conformations.

**Limitations on study/implications**: Effect of pH variation (3.6, 4.6, and 5.6) on morphometric parameters and the secondary structure of proteins (ovalbumin and gliadin).

**Findings/conclusions**: The novelty of this contribution consists in demonstrating that there is a close structure-functionality relationship between the morphometric parameters of proteins and their secondary structure, combining microscopy and spectroscopy techniques. This allows a clear and deep understanding of protein behavior to select the appropriate pH conditions to improve the properties of many foods.

**Keywords**: secondary structure, ovalbumin, gliadin, morphometric.

**Citation**: Chanona-Pérez, J. J., Rojas-Candelas, L. E., Morales-Hernández, J. A., Arredondo-Tamayo, B., Méndez-Méndez, J. V., Díaz-Ramírez, M., & Rayas-Amor, A, A. (2023). *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2608

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

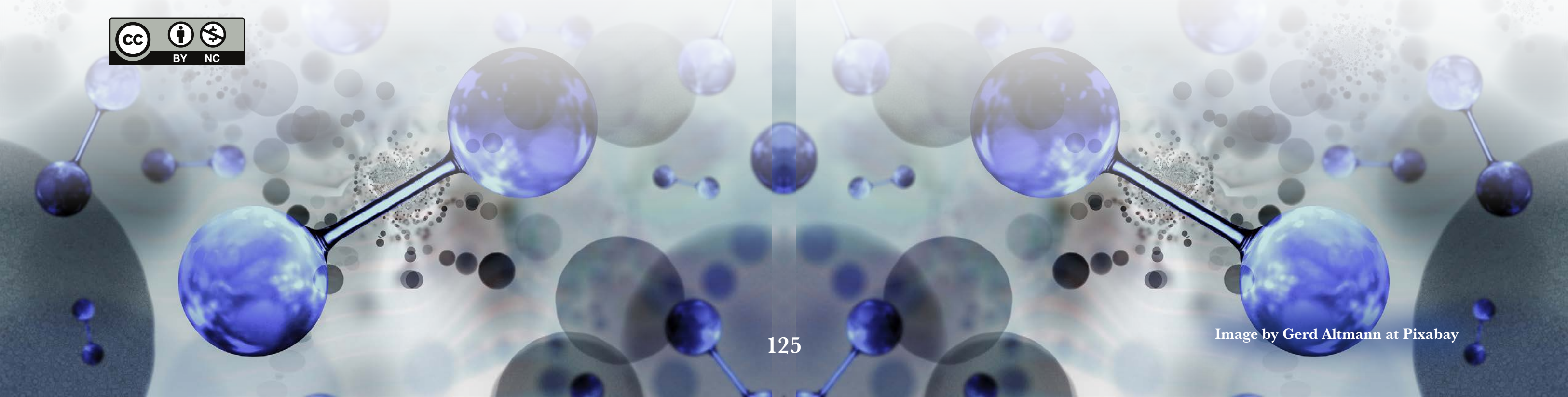
**Received**: March 24, 2023.
**Accepted**: June 20, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 125-134.



## INTRODUCTION

The development of research related to proteins in general plays a fundamental role not only because they are essential for human growth, but also because proteins provide the structural basis for several functional properties of foods that have a profound impact on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food quality. They commonly serve as gelling, binding, emulsifying and foaming agents, which are functions that modify processing and behavior in food systems (Yada, 2007). Although it is important to consider that some protein properties are affected by various factors, such as pH, protein concentration and ionic strength (Chang *et al.*, 2016; Mine *et al.*, 1991). An important protein to study is ovalbumin, the most abundant protein in egg white is useful for its good functionalities, including gelling properties and foaming activities. It is a glycoprotein consisting of 385 amino acids (mostly of which are hydrophobic). Due to its surface hydrophilic nature, it is not a good emulsifier in its raw state (Mine, 1995; Mleko *et al.*, 2007). But the emulsion stability and emulsifying activity of this protein are considerably lower than those of $\beta$-lactoglobulin, bovine serum albumin, $\kappa$-casein, soybean $\beta$-conglycinin and blood globin (Kato *et al.*, 1983; Nakamura *et al.*, 1984). However, the pH-dependent structural changes of ovalbumin lack characterization. Although, it is known that the structural state of ovalbumin is highly sensitive to pH variation because of surface charge alteration (Chen *et al.*, 2018).

Likewise, another protein of importance for its study is gliadin, which is the major storage protein in the byproducts of wheat starch. Wheat gliadins are monomeric, disulfide-bonded proteins, which are abundant in $\alpha$-helices and $\beta$-turns (Tatham & Shewry, 2012). Besides, it should be noted that the peculiar sequence (abundant in prolines and glutamines) and structure of gliadin has already been proven to be responsible for severe anaphylaxis such as celiac disease and food allergy (Kim *et al.*, 2004). Moreover, the gliadin protein has the property of generating foam such as ovalbumin but also has viscoelastic and mechanical properties that are implemented in bread that provide the extensibility to stretch the bread dough without tearing (Quester *et al.*, 2014; Thewissen *et al.*, 2011). But, compared to other proteins, some properties of gliadin are still poorly explored (Wan *et al.*, 2015).

In addition, non-destructive techniques, such as molecular spectroscopy, are available to determine the behavior of proteins at the molecular scale. Different spectroscopy techniques (X-ray diffraction, nuclear magnetic resonance, Fourier transform infrared (FT-IR) and Raman spectroscopy) have been widely used for decades to elucidate the molecular structure of different types of proteins (Horne, 2002; Wang *et al.*, 2017). As a result of the development of high analytical capacity and the ease of acquisition of spectroscopy equipment, its applications have increased dramatically within the food industry. To evaluate the conformational changes of proteins produced by their processing and pH variations, which has been useful to understand their structure-functionality relationship. It should also be noted that FT-IR spectroscopy has been suitable for assessing conformational modifications of secondary and tertiary structures of food-derived proteins (Carbonaro & Nucara, 2010). On the other hand, atomic force microscopy (AFM) has been used to determine protein topography, as reported by McMaster *et al.* (1999), who have shown that the dimensions of A-gliadin fibers can change at different pH values and concluded that fiber shape and size depend on pH. Furthermore, Vié *et al.* (2002) demonstrated by AFM that the diameter of casein micelles is reduced due to their hydrophilic and lipophilic properties. Likewise, Grácia-Juliá *et al.* (2008) studied the dispersion at high pressures of whey protein and reported that the size distributions at 200 MPa ranged between 5 and 8 nm, while at >250 MPa between 60-170 nm and concluded that at pressures >250 MPa the protein agglomerates. Therefore, to have a better understanding of the influence of pH on protein structure and the development of proteins as multifunctional components for the food industry, the conformational and morphometric changes produced in ovalbumin and gliadin were evaluated in the present study. With the main objective of unveiling the importance of pH-dependent structural features for their properties using AFM microscopy and FT-IR spectroscopy as tools to deepen the analysis of this type of biomolecules, because there is a certain complexity of such systems in terms of composition and spatial organization.

## MATERIALS AND METHODS

### Samples

Ovalbumin was acquired by the supplier Hycel (Cat. 568, México) and Gliadin was acquired by Sigma-Aldrich as wheat gliadin (G3375-25G, USA). Gliadin and ovalbumin were used 1% w/v dissolved in MiliQ water; pH adjusted to corresponding using 0.1 M HCl at room temperature (25 °C). The pH values for proteins studied were 3.6, 4.6 and 5.6. The solutions were mixed by a sonicator (VCX130, SONICS Vibra cell™, 90 Newton CT, USA) with 50 % of amplitude at 20 kHz and 130 W with a 120 V generator (CV13) and was used a standard probe of the sonicator (length of 113 mm and diameter of 6 mm).

### Atomic force microscopy

Proteins were observed by AFM (Bruker, Bioscope Catalyst ScanAsyst, USA). AFM images of gliadin and ovalbumin were acquired at different pH values (3.6, 4.6 and 5.6 pH). Samples were placed on a glass slide, dried at room temperature for 10 min and mounted on to equipment. ScanAsyst mode was used, $5 \times 5\ \mu m^2$ scans were performed and 3 images of each sample were selected from the different pH values. The study was carried out at ambient conditions and the cantilevers used in this study were silicon cantilevers (DNP- 10A) with a spring constant of 0.540 $Nm^{-1}$ and a resonance frequency of 1 kHz. For image processing the software NanoScope Analysis v2.0 (Bruker Nano, Santa Barbara, CA, USA) was used (Rojas-Candelas *et al.*, 2019; Rojas-Candelas *et al.*, 2022).

### Fourier transformed infrared spectroscopy

The analysis of the secondary structure of the proteins was carried out by FT-IR spectroscopy (Agilent Cary model 630, USA). A small amount of each sample was placed on the attenuated total reflectance (ATR) diamond crystal of the analyzer. The samples were pressed against the diamond crystal using the attached pressure clamp with a slip clutch press on the clamp that prevents over-tightening. It was operated in the 1000 to 1800 $cm^{-1}$ wavenumber ranges and 64 scans at 4 $cm^{-1}$ of resolution at room temperature (25 °C). For evaluation, a necessary baseline and smoothing correction was performed on the spectrum using OriginPro 8 software (v8.0724, USA). Finally, the deconvolution method was used to evaluate the areas of the regions of interest (Rojas-Candelas *et al.*, 2019; Rojas-Candelas *et al.*, 2022).

### Statistical analysis

Measurements were expressed as mean values with a precision of standard deviation. The data obtained by the previous analyses were compared using the ANOVA-Tukey test, and significant differences were considered significant when $p<0.05$. Both statistical analyses were performed using SigmaPlot v.12 software (Systat Software Inc. USA). In addition, using XLSTAT software (2020.1.3, Addinsoft, USA) a Pearson analysis was performed on all acquired variables and a visualized correlation matrix was produced (Rojas-Candelas *et al.*, 2022).

## RESULTS AND DISCUSSION

### Analysis of morphometric parameters by AFM

Figure 1 shows a selection of AFM height images for protein solution at different pH values: 3.6, 4.6 (close to its isolelectric point) and 5.6. These pH values considered are within the range used in food systems (Mleko *et al.*, 2007; Thewissen *et al.*, 2011). Figures 1(a-c) correspond to AFM height images of ovalbumin, where it is noted that larger agglomerates are formed at pH 4.6 compared to pH 3.6 and 5.6. This is because the protein is quite close to its isoelectric point and therefore most likely has minimal solubility and possesses a neutral charge. While at pH 3.6 and 5.6, although they also present less agglomeration, at these pH values they are mostly soluble, and their surface is more uniform.

The agglomeration size (AS) in a certain way presents relationship, as shown also in Figures 1(a-c), with the average roughness values, where the Ra values are higher at the same pH of 4.6 (4.05±2.60 nm), due to the increase of agglomeration. Since, as observed the Ra at pH 3.6 was 3.40±0.54 nm and that at pH 5.6 was 2.59±0.84 nm. Which suggests that the protein is immobilized with a slight net positive charge (Lahiri *et al.*, 1999).

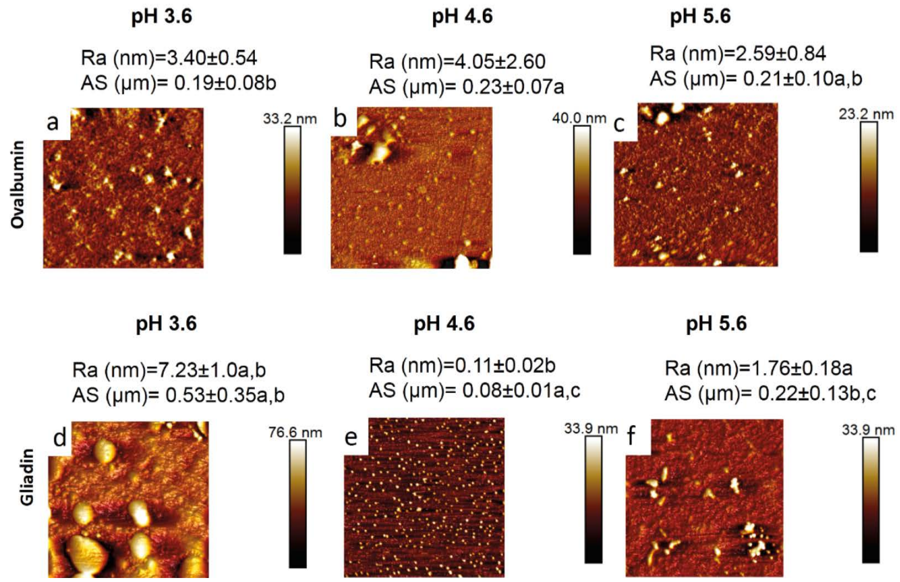


**Figure 1**. a-c: atomic force microscopy (AFM) height images of ovalbumin and, d-f: gliadin scanned at 5×5 $\mu$m at different values pH. Arithmetic average roughness (Ra) and agglomerate size (AS). In AFM images same letters indicate significant difference ($p<0.05$).

Mine, (1996) reported the particle size of ovalbumin with a mean of 317.3 nm to 752.6 nm due to using pH 7.5. And in a different study of the particle size of the mixture of proteins including ovalbumin, finding Ra values of 70-295 nm because the treatment was at pH 3 and with thermosonication. Previous research reported the globular structure of ovalbumin and the size of about 24-25 nm because the protein was subjected to heat treatment (Najbar *et al.*, 2003). Another study likewise confirmed the shape of the globular structure of ovalbumin and the size of 78-143 nm (Taheri *et al.*, 2012).

For gliadin, the same pH values were used and in the AFM height images shown in Figures 1(d-f) it is observed that the largest Ra values were at pH 3.6, since this is where larger and heterogeneous agglomerates are present compared to pH 4.6 and 5.6. When the protein is close to the isoelectric point (pH 4.6) it has homogeneous agglomerates of $0.08 \pm 0.01$ $\mu$m and larger agglomerates than at pH 3.6 and 5.6 due to protein precipitation. The Ra values vary as a function of the number of gliadin agglomerates that are not dissolved in solution. This is consistent with the characteristic size found for each pH condition. Therefore, these results show, that the size of the protein as well as the topographical characteristics depend on the specific pH value used for its precipitation and affect the different properties for food use (Moitzi *et al.*, 2011).

Research has reported average 100 nm sphenoidal structures of gliadin mixed at 70% aqueous ethanol by AFM analysis (McMaster *et al.*, 1999). In addition, work found gliadin aggregates in egg cake substitution around 100-200 nm (Lin *et al.*, 2017). While McMaster *et al.* (2000) found $\alpha$-gliadin fibrils at pH 3 with a width of 15 to 80 mm and a length of 100 nm to 2 $\mu$m. Since the agglomerate size observed in the present work is similar to that reported by previous literature, it is likely that the change in morphology of ovalbumin and gliadin as size and shape at different pH values is associated with reassembly at the molecular level.

**Analysis of secondary structure by FT-IR**

Proteins dissolved at different pH values exhibit modifications in morphology and solubility properties through protein rearrangement at the molecular level, but their correlation remains unknown. Consequently, the secondary structure of proteins was studied to associate these behaviors with their morphometry of the protein evaluated at different pH values. Figure 2 shows the FT-IR spectra with the characteristic bands of the protein. There is information concerning amide vibrational bands and stretching, bending and other values. The first band observed in the spectrum of ovalbumin is due to stretching of the C-C and C-O bonds (1100 cm-1). The corresponding intensity is higher at pH 4.6 compared to those at pH 3.6 and 5.6. (Carbonaro & Nucara, 2010). A similar behavior had to C-H bending (1420 cm-1) the lowest intensity found at pH 5.6 following that at pH 3.6. The band of amide I of ovalbumin shows a reduction of the area under the curve when pH decreases (Figure 3). Amide II has observed a high valley on the far right generated by all peaks.

Table 1 shows that at pH 4.6, ovalbumin decreases the percentage of $\alpha$-helix conformation and increases the $\beta$-antiparallel conformation compared to pH 3.6 and

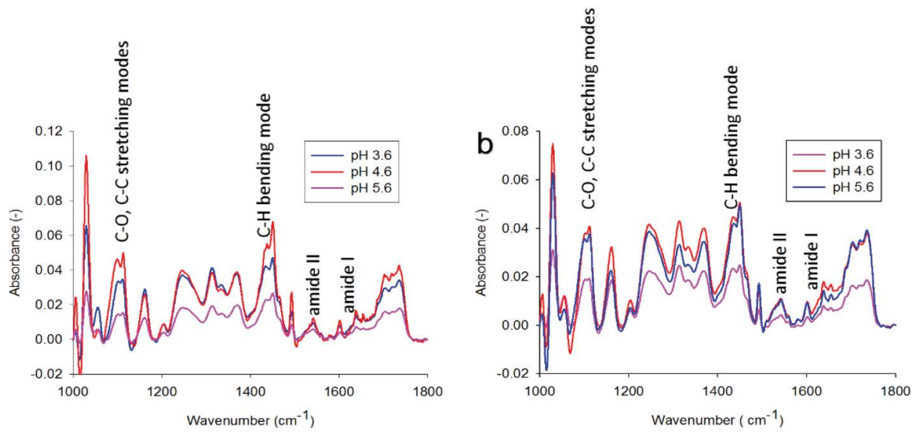


**Figure 2**. Raman spectra of ovalbumin a) and gliadin b) at different indicated pH values.

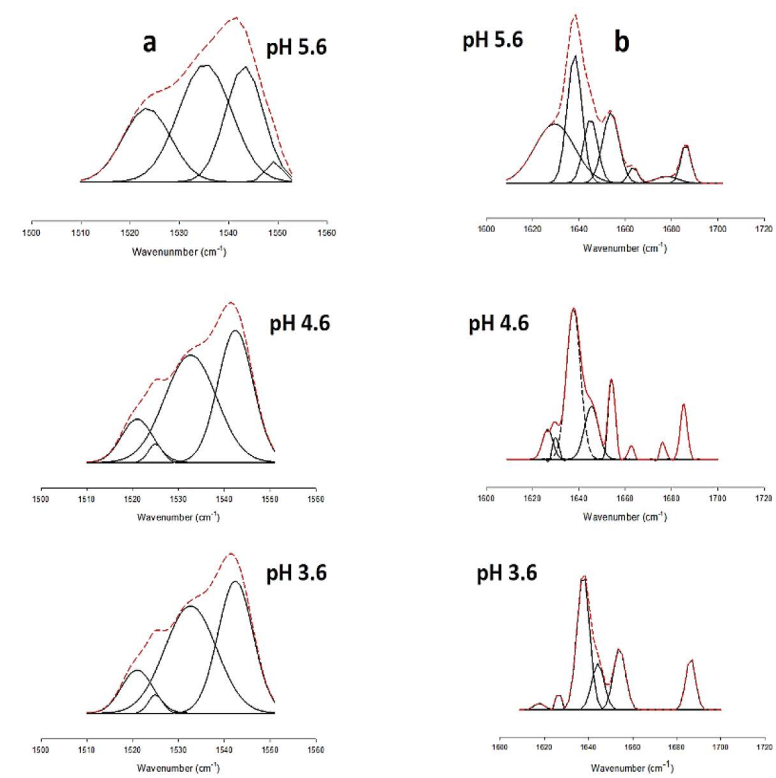


**Figure 3**. Changes in FT-IR of ovalbumin at indicated pH values for the amide II band (a) and amide band I (b). Represents peak-fitting of the second derivative curves of the spectra.

5.6. It is concluded that at pH 4.6 ovalbumin unfolds due to decreased solubility and hydrophobic, covalently bound interactions (Wang *et al.*, 2017).

The previously mentioned characteristics allow water retention and increase gelling properties (Li *et al.*, 2014). Due to the exposure hydrophobic groups and sulfhydryl groups (Campbell *et al.*, 2003). On the other hand, at pH 3.6, the results are different, they do not have a turning percentage but develop a higher content of the $\alpha$-helix structure. This

**Table 1**. Determination of the secondary structure percentages by FT-IR of the ovalbumin solution (0.01%) at different pH values.

| Protein structures | pH 3.6 (%) | pH 4.6 (%) | pH 5.6 (%) |
|---|---|---|---|
| $\beta$-Antiparallel | 2.75 | 21.53 | 16.09 |
| $\beta$-Parallel | 17.26 | 45.46 | 50.14 |
| Turn | 0 | 3.65 | 4.30 |
| Random coil | 15.47 | 16.76 | 12.97 |
| $\alpha$-helix | 20.21 | 12.57 | 16.48 |

most probably means that the protein is folded. This most likely means that the protein is folded and disordered. At pH 5.6, the results are different; it develops a higher content of the $\beta$-parallel and twist structures. There is also a reduction of the $\alpha$-helix conformational structure, so the protein was found to be unfolded.

While in gliadin the bands show the lowest intensity for 3.6 pH compared to 4.6, and 5.6 pH these behaviors affect the bands C-H bending mode, C-O and C-C stretching mode. Gliadin had similar behavior in the amide I and amide II regions. Figure 4 shows that the amide I region at 4.6 pH and 3.6 pH, have similar behavior compared to pH 5.6 which has a heterogeneous distribution. In amide II a high valley was observed at the right end generated by all the peaks.

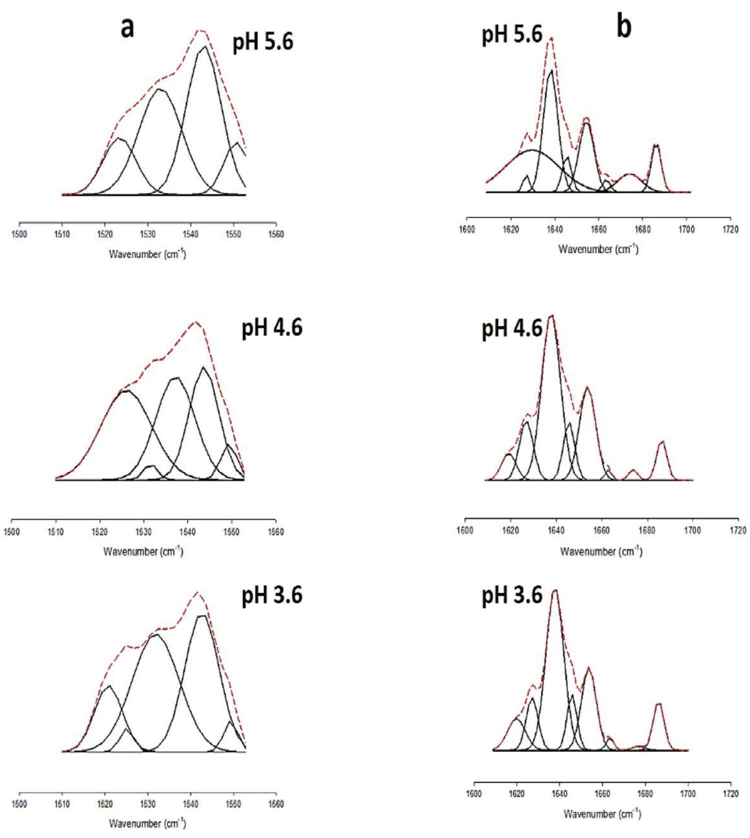


**Figure 4**. Changes in FT-IR of giadin at indicated pH values for the amide II band (a) and amide band I (b) regions. Represents peak-fitting of the second derivative curves of the spectra.

At pH 4.6, ovalbumin increases the $\alpha$-helix but decreases the $\beta$-parallel (Table 2), since the protein was partially disordered and folded and when the protein is folded, it acquires energy transfer properties due to the closeness of protein-protein interaction (Chakraborty & Basak, 2007). While the pH of 5.6 and 3.6 of gliadin decreases the $\alpha$-helix and decreases the $\beta$-parallel conformations. The protein was partially disordered and unfolded.

**Correlation analysis**

Pearson's analysis provided a representation of the correlation of morphometric parameters with protein secondary structure. Figure 5 shows an image of a Pearson correlation matrix, where the range of its correlation coefficients is indicated. The intensity of the green and blue colorings shows which variables have the highest positive or negative correlations (green($\alpha$)=1 to 0.81, red ($\alpha$)=−1 to −0.81) with F (dotted red line). Pearson matrix of ovalbumin observed positive correlation random coil conformation with roughness protein and agglomerate size with $\beta$-Antiparallel, $\beta$-Parallel and turn conformations. The negative correlation agglomerated protein with random coil and $\alpha$-helix conformations. Roughness and agglomerate size also have negative correlation with $\alpha$-helix.

The secondary structures of ovalbumin are correlated with each other, the positive correlation was $\beta$-Parallel with $\beta$-Antiparallel and twist conformations. In addition, the negative correlation was $\alpha$-helix with $\alpha$-Antiparallel. In contrast, the Pearson matrix of gliadin has positive correlation agglomerate size with $\beta$-Parallel conformation, but

**Table 2**. Determination of the secondary structure percentages by FT-IR of the gliadin solution (0.01%) at different pH values.

| Protein structures | pH 3.6 (%) | pH 4.6 (%) | pH 5.6 (%) |
|---|---|---|---|
| $\beta$-Antiparallel | 11.88 | 23.90 | 10.20 |
| $\beta$-Parallel | 58.65 | 42.87 | 60.31 |
| Turn | 2.20 | 2.43 | 8.34 |
| Random coil | 8.47 | 9.34 | 5.07 |
| $\alpha$-helix | 18.78 | 21.43 | 16.05 |

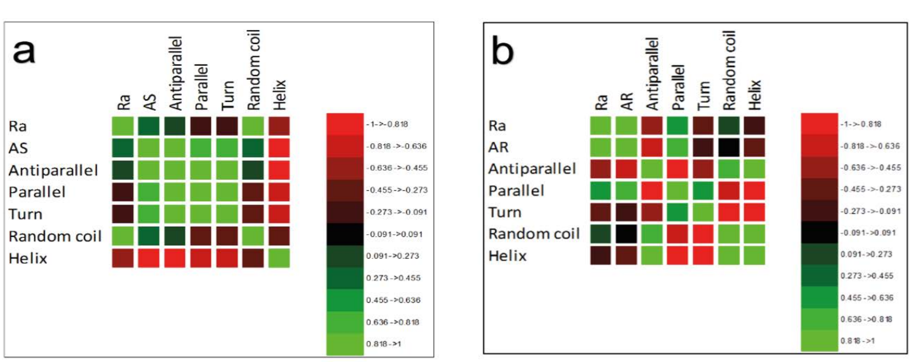


**Figure 5**. Image of the Pearson correlation matrix a) ovalbumin and b) gliadin: using 7 variables: arithmetic mean roughness (Ra), size agglomerate (SA), and the contributions of the secondary conformational structures from different regions ($\beta$-antiparallel, $\beta$-parallel, turn, random coil and $\alpha$-helix).

agglomerate size has negative correlation with $\beta$-Antiparallel. Secondary structures there are correlations with each other, negative correlation was $\alpha$-helix conformation with $\beta$-Parallel and twist conformations, another negative correlation was $\beta$-Parallel with $\beta$-Antiparallel. Positive correlations were $\alpha$-helix with $\beta$-Antiparallel, random coil and $\alpha$-helix, another positive correlation was random coil with $\beta$-Antiparallel.

## CONCLUSION

Changes in pH provided ovalbumin and gliadin with useful information to correlate molecular parameters and topographical parameters. The information collected indicates that the regions of secondary structure with the greatest influence on agglomerate size are the $\beta$-Parallel conformation for proteins. Additionally, for ovalbumin the agglomerate size was $\beta$-Antiparallel and twist conformations. Overall, Ra is shown to have a higher correlation with random coil conformation for ovalbumin. Structural changes at the microstructural and molecular level in proteins and secondary structure at different pH values. This information can be valuable for understanding protein behavior and selecting appropriate pH conditions to improve some food properties.

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Colegio de Postgraduados

# Regionalization of fish production in Mexico based on production value data in MXN pesos

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**ABSTRACT**

**Objective**: Generate a fishing regionalization activity in Mexico based on the economic criteria due to the value of fishery production.

**Design/Methodology/Approach**: Socioeconomic data was taken as well as analyzed from the Statistical Yearbook of Aquaculture and Fisheries of fisheries in Mexico. Subsequently, the findings were organized in a database with geospatial referent reclassified into nominal or ordinal qualitative statistical values. The reclassification process was done through the use of a Geographic Information System, specifically with Arcview 3.2 software, which allowed the generation of geostatistical analysis procedures through the use of the *Kriging* tool.

**Results**: The results are displayed in a visually referenced database shown on a map constructed by data vectorization. The regionalization map of fisheries in Mexico is based on economic criteria of production value classified in four zones with different fishing priority.

**Limitations/implications**: The lack of studies and social, economic and productive indexes of the Mexican fishery is a limitation in the work of regionalization of fishing activity.

**Findings/conclusions**: The efficiency of the use of *Kriging* as a multispecific analysis tool can be proven. The proposed regionalization is based solely on the monetary value, an item that has a greater weight in the decisions made by the institutions, due to its importance in terms of Mexico's Gross Domestic Product. These criteria together with the use of computational tools allowed the geolocalized regional categorization of zones with similar characteristics classified into four fishing regions according to their degree of economic importance: low, medium, high and main.

**Keywords**: Economic regionalization, Geostatistics, Mexican fishing, Value of production.

**Citation**: Magadán-Revelo, L.D., Escalona-Maurice, M. J., Fernández-Ordóñez, Y. M., Aguilar-Ibarra A. (2023). Regionalization of fish production in Mexico based on production value data in MXN pesos. *Agro Productividad*. https://doi.org/10.32854/agrop.v16i7.2626

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 20, 2021.
**Accepted**: April 17, 2023.
**Published on-line**: July 26, 2023.

*Agro Productividad, 16*(6). June. 2023. pp: 135-141.



## INTRODUCTION

Regionalization is the set of integration processes that take place effectively within one or more geographical areas. This phenomenon shares the intensification of relations between state and non-state actors in the same region (Molina, 2007). "It is the intensity of

Image by günter at Pixabay

economic interactions that allows us to speak of the existence of regionalization processes, in which both political and economic interests and underlying ideological-cultural elements converge" (Ibáñez, 1999, 3).

Regionalization refers to the interpretation of processes and space-temporal characteristics existing in locatable geographical points. Data analysis allows us to find differences and similarities when extrapolated to a georeferenced visual projection in a geographical space. They allow us to demonstrate established limits and areas that share topographic, cultural, social, political similarities, etc. The regionalization process is a subjective exercise since the characteristics of the variables confronted are submitted to the needs of the researcher.

The marine areas of our country have been worked under the concept of regionalization by several scholars of the matter using as criteria mere biological and geographical aspects. In Mexico the most emblematic studies are those made by Merino (1987), who distinguishes seven coastal areas with clear geographical and fish-population based differentiation. The studies carried out by Arriaga *et al.* (1998) and Botello *et al.* (2000) recognize seven coastal provinces and five oceanic provinces which are regulated by the Mexican State.

Coastal zones thus regionalized in these studies are defined by exclusively marine features such as bathymetry, coastline, seamounts, fish or related species, ocean currents, etc.; but they leave aside the social action that the fishing communities imprint on the development of these regions.

The Mexican National fisheries institute (Instituto Nacional de la Pesca, 1994) developed their own regionalization based on the exploitation of biotic fishing resources (Figure 1). The fishing resources obtained in the areas described by INAPESCA are the classifying variable.

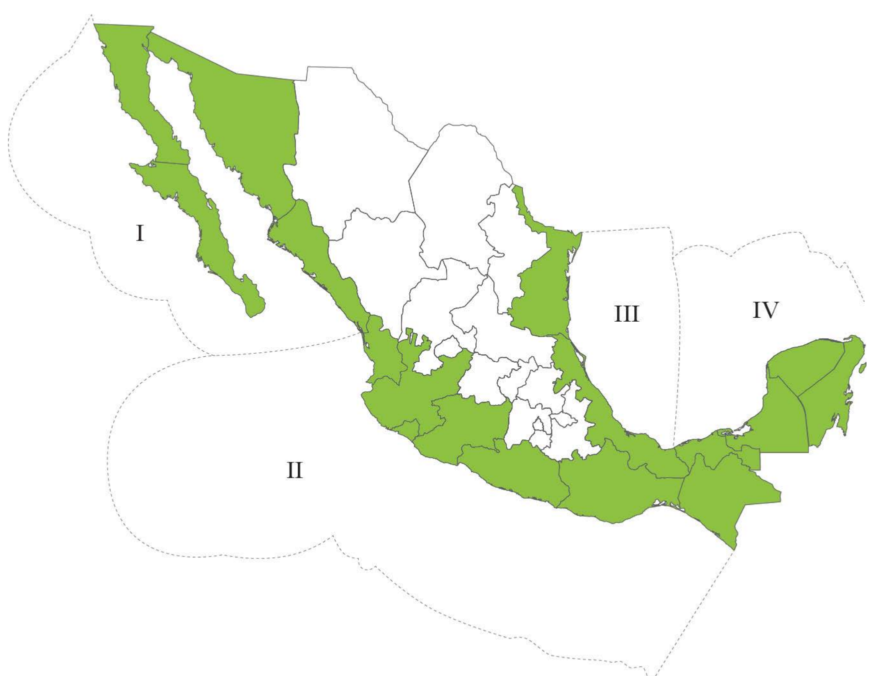


**Figure 1**. Regionalization of fisheries (INAPESCA, 1994).

Fishing activity is limited to a historical-social context; in which the action of the human being as a transformer agent of the world should be present in the regionalization exercises. However, there are not many proposals for socioeconomic regionalization of fishing localities in Mexico since they are not recognized as an inter sectorial entity in economic terms. They are not based on the actions of organized fishing communities, at least.

An attempt at social-based regionalization is the study by Espejel and Bermúdez (2006) that analyzes basic fishing units and propose 178 coastal units as the regionalization of the local coastal regulations that would be developed in Mexico. They mention that in order to regionalize the Mexican seas and coasts, it is necessary to agree on all the existing information expressed in previous efforts. Inside their methodology of study, they do not show or suggest which tools would be the best to make the crossing of those data that authors used as basis for their proposals.

Espinoza *et al*. (2014) mentioned that in the development of an adequate regionalization, data such as: a) new quality controls of fishery, b) supply and demand of fishery products, c) the use of fishing gear, and d) biological and environmental conditions of the fishing area. Once again, reference is made to multiple factors in the regionalization process, leaving aside the actions of the stakeholders who operate fishing.

The lack of socioeconomic data on fisheries in Mexico has been an important limitation to generate a more accurate regionalization of the Mexican coasts. The institutes in charge of fisheries research in Mexico (INAPESCA and CONAPESCA) have put aside the incorporation of social analyses of the communities, and INEGI has only focused efforts on the incorporation of macroeconomic data on extraction and sale, as it is confirmed in the data of the statistical yearbook "Anuarios Estadísticos de Acuacultura y Pesca" provided by CONAPESCA. So it becomes impossible to have a visible reference about community action in the extraction operation of the fishing in Mexico.

The purpose of our study is then, to give two proposals for regionalization of coastal areas in Mexico under social and economic criteria of fisheries, but assigning data relevance to the fisherman as an stakeholder and central node of the activity.

## MATERIALS AND METHODS

As a first study of regionalization of fisheries in Mexico we used the data provided by Mexican National fisheries commission (Comisión Nacional de Pesca) (CONAPESCA, 2015). The official website contains the 2017 production database (Base de datos de Producción Anuario 2017), which was used as the reference source to carry out our first regionalization scenario.

The Anuario estadístico de Acuacultura y Pesca (CONAPESCA, 2017) is the main source of data provided by Mexican institutions. The data presented there were collected by the fisheries offices of the different states in Mexico and these were classified by mere numerical values of production. The data fields with greater representation are: a) Landed weight, b) Live-weight, and c) Market price (MXN pesos).

First, a database was generated where the aquatic aspects were discriminated, since these are not of interest for the purposes of our study. Then, the data of "Market price in

MXN pesos" was classified into conglomerates of subtotals by state. With this, we obtained a quantification of the fishing economic importance for the selected geographical areas.

The classified data were the input, by means of a relational table, of an ArcView 3.2 layer, then georeferenced into a polygonal shape (.shp format).

Values were classified by quartile and reclassified into qualitative variables characterized under the nominal criterion of economic importance (*i.e.* market price), where: 1 = Low, 2 = Medium, 3 = High, and 4 = Main (Table 1).

**Table 1**. Reclassified market price as estimator of the value of fishing production.

| NOM_ENT | VALUE_PROD $ | REG_VALOR | Reclassification |
|---|---|---|---|
| Distrito Federal | 0 | 1 | Low |
| Aguascalientes | 6,459 | 1 | Low |
| San Luis Potosí | 7,949 | 1 | Low |
| Zacatecas | 38,082 | 1 | Low |
| Querétaro | 109,164 | 1 | Low |
| Tlaxcala | 127,181 | 1 | Low |
| Hidalgo | 271,068 | 1 | Low |
| México | 513,068 | 1 | Low |
| Puebla | 602,282 | 1 | Low |
| Nuevo León | 732,804 | 2 | Medium |
| Chihuahua | 792,149 | 2 | Medium |
| Durango | 1,690,242 | 2 | Medium |
| Morelos | 3,168,984 | 2 | Medium |
| Coahuila de Zaragoza | 15,934,130 | 2 | Medium |
| Guanajuato | 24,619,334 | 2 | Medium |
| Quintana Roo | 158,384,456 | 2 | Medium |
| Michoacán de Ocampo | 188,026,961 | 2 | Medium |
| Guerrero | 202,869,230 | 3 | High |
| Jalisco | 226,496,854 | 3 | High |
| Oaxaca | 337,781,744 | 3 | High |
| Colima | 371,477,498 | 3 | High |
| Tabasco | 420,804,949 | 3 | High |
| Chiapas | 485,354,353 | 3 | High |
| Nayarit | 617,446,533 | 3 | High |
| Tamaulipas | 718,197,218 | 3 | High |
| Veracruz de Ignacio de la Llave | 753,500,229 | 4 | Main |
| Baja California | 837,562,938 | 4 | Main |
| Campeche | 1,021,449,948 | 4 | Main |
| Yucatán | 1,211,490,835 | 4 | Main |
| Baja California Sur | 1,318,499,755 | 4 | Main |
| Sonora | 1,961,426,286 | 4 | Main |
| Sinaloa | 2,906,602,355 | 4 | Main |

1 Data from the statistical yearbook (CONAPESCA, 2017).

Once the values were reclassified and entered as fields in the database table of data, we proceeded to convert the visual reference of the polygons of Mexico into central points, with which we generated a display of the values described above as focalized areas of incidence.

Subsequently, the data were analyzed with the *Kriging* geostatistical tool, which offers as results a zoning based on the proximity of similar data in Raster format (information stored by pixel entity), clustering them in specific areas of incidence, and linking the similarity of the data into reclassified and differentiated polygons.

The result obtained from the *Kriging* process must be reconverted into vector data, in order to later make cuts as necessary, based on the limits of the Mexican territory.

## RESULT AND DISCUSSION

Regionalization of Mexican fisheries based on economic values reported by CONAPESCA was generated (Figure 2).

A clear difference in the economic importance of fishing can be observed. The most economically important "Main" areas are in the south-east and north-west regions.

The central states of Mexico reported a "Low" economic importance, contrary to common sense speculations, about this region is the basis of a sector of the population dedicated to the capture of aquatic species, an activity carried out in continental waters such as rivers, lakes, lagoons, or dams of Mexico.

The region with "High" economic importance is comprised by states close to the southern Pacific coast, while the "Medium" importance is located in the northern states, without access to the coasts in their political delimitation where fishing is practiced in continental waters.

This regionalization is carried out only under the economic parameters of fishing, it does not differentiate between artisanal or deep-sea fishing. The foregoing is the product

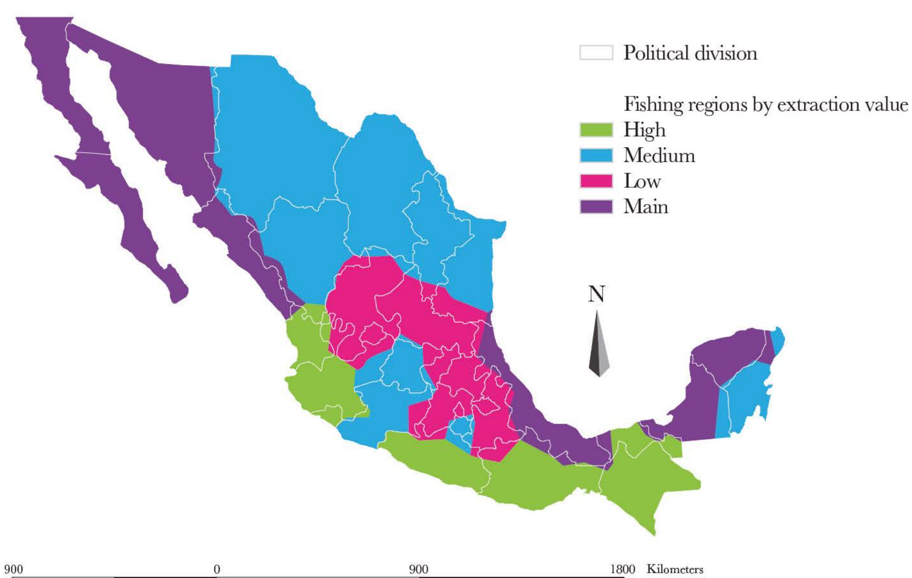


**Figure 2**. Regionalization of fisheries based on monetary value.

of the deficiency that exists in the databases collected by CONAPESCA, or else, it is due to the lack of disclosure and encryption level of data.

However, analyses like this one offer the opportunity to visualize and analyze the reality of fishing in Mexico, and how it affects the socioeconomic policies of the country.

## CONCLUSIONS

Fishing is studied by the institutions in a biased manner; in no official document, social and cultural data of people involved in the development of this activity are considered.

This regionalization proposal is based merely on monetary value (market price), an item that has a greater weight in decisions taken by the institutions, because of its importance in terms of Mexico's Gross Domestic Product (GDP).

Four fishing regions are characterized by their degree of economic importance; this means, by the value obtained from the sale of fishing resources. Those characterized regions are classified as: low, medium, high and main.

The visualization of the results through maps provides greater clarity when analyzing the information. The fishing regions characterized as main and high economic importance are located in the coastal areas of the country. Moreover, fishing in continental waters in these areas is also a substantial part of the activity.

The fishing regions characterized as medium and low are concentrated in products obtained in continental waters such as dams, rivers, lakes and lagoons, which further highlights the importance of these bodies of water.

The regionalization of the economic factors of fishing would be specified, if data at the municipal or local level were taken as the base unit of work. This would lead to a longer and specialized process, which is perfectly achievable.

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