

# Handbook T-XXI

## CIERMMI Women in Science

Biological, Humanities and Social Sciences

**MARROQUÍN-DE JESÚS, Ángel**

**CASTILLO-MARTÍNEZ, Luz Carmen**

**OLIVARES-RAMÍREZ, Juan Manuel**

**OLGUÍN-LÓPEZ, Norma** *Coordinators*

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# **ECORFAN CIERMMI Women in Science**

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## **Volume XXI**

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The Handbook will offer volumes of selected contributions from researchers who contribute to the scientific dissemination activity of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C. in their areas of research in Biological Sciences. In addition to having a total evaluation, in the hands of the directors of the Colegio de Ingenieros en Energías Renovables de Querétaro A.C., the quality and timeliness of its chapters, each individual contribution was refereed to international standards (RESEARCH GATE, MENDELEY, GOOGLE SCHOLAR and REDIB), the Handbook thus proposes to the academic community, recent reports on new developments in the most interesting and promising areas of research in the Biological Sciences.

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**MARROQUÍN-DE JESÚS, Ángel. PhD**  
**CASTILLO-MARTÍNEZ, Luz Carmen. MsC**  
**OLIVARES-RAMÍREZ, Juan Manuel. PhD**  
**OLGUÍN-LÓPEZ, Norma. PhD**

Coordinators

**CIERMMI Women in Science**  
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## Prologue

Science has two main objectives, to understand and to improve, we seek to understand how our environment works, but also how to improve our expectations and quality of life. and quality of life.

Each discovery, scientific and/or technological breakthrough, the understanding of our social behavior has social behavior, has been contributing to make our daily life better and better, either by It may be through medical advances, accessibility to drugs that improve our health and why not, that of our pets; through modifications or additions in foods that modify their quality or resistance, evaluations that prove that they are better for our consumption; the use of new technologies that make procedures more dynamic and effective, in addition to the fact that every day we are generating a society that is more aware of human feelings, where social skills improve the experience of everything that involves us, but never forgetting sustainable development, taking care of the planet we live in.

All the above is part of this compendium, which, with effort, dedication and effort, the teachers-researchers effort, the professors-researchers, have captured in the form of scientific articles, their advances and discoveries. advances and discoveries. Collaborating one more year with the CIERMMI congress and with this Handbook, where they share with all of us their science and their passion for it.

So, I extend the invitation to read each one of these papers, so vast and enriching, to increase our knowledge of the enriching, increase our knowledge, and continue doing what we are passionate about: Science. My most sincere congratulations and thanks to each of the contributors.

*María Mayola Giselle Galván Mondragón*  
*Universidad Tecnológica de San Juan del Río*

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## Chapter 1 Basic social skills in the training of dentists

### Capítulo 1 Habilidades sociales básicas en la formación de odontólogos

ZÁRATE-DEPRAECT, Nikell Esmeralda\*†, GARCÍA-JAU, Rosa Alicia, MORENO-TERRAZAS, Efigenia and LEVET-VELASCO, Hortensia

*Universidad Autónoma de Sinaloa*

ID 1<sup>st</sup> Author: *Nikell Esmeralda, Zárate-Depraect* / **ORC ID:** 0000-0002-7374-1606

ID 1<sup>st</sup> Co-author: *Rosa Alicia, García-Jau* / **ORC ID:** 0000-0002-7221-3764

ID 2<sup>nd</sup> Co-author: *Efigenia, Moreno-Terrazas* / **ORC ID:** 0000-0002-68191-8350

ID 3<sup>rd</sup> Co-author: *Hortensia, Levet-Velasco* / **ORC ID:** 0009-0003-0299-4248

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N. Zárate, R. García, E. Moreno and H. Levet

\* [nikell.zarate@uas.edu.mx](mailto:nikell.zarate@uas.edu.mx)

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## **Abstract**

Objective: To highlight the importance of basic social skills in the training of second-year public university dentistry students. Methodology: Qualitative research with a hermeneutical historical approach that applies the techniques: questionnaire and workshop to 25 students who voluntarily decide to participate in this study. Informed consent applies. Data are categorized and analyzed in Microsoft Excel and descriptive results are reported. Contribution: They recognize how important basic social skills are necessary for good clinical performance and identify which of them were strengthened after completing the "Basic Social Skills Strengthening Program". They realize that these skills are developed in everyday life, acquired from childhood through observation, but that they can also be learned and modified over time. They also realize that they positively influence their interpersonal relationships.

## **Students, Dentistry, Basic social skills**

### **Resumen**

Objetivo: Destacar la importancia de las habilidades sociales básicas en la formación de los estudiantes de segundo curso de Odontología de las universidades públicas. Metodología: Investigación cualitativa con enfoque histórico hermenéutico que aplica las técnicas: cuestionario y taller a 25 estudiantes que voluntariamente deciden participar en este estudio. Se aplica consentimiento informado. Los datos se categorizan y analizan en Microsoft Excel y se reportan resultados descriptivos. Contribución: Reconocen lo importante que son las habilidades sociales básicas necesarias para un buen desempeño clínico e identifican cuáles de ellas se fortalecieron después de realizar el "Programa de Fortalecimiento de Habilidades Sociales Básicas". Se dan cuenta que estas habilidades se desarrollan en la vida cotidiana, se adquieren desde la infancia a través de la observación, pero que también se pueden aprender y modificar con el tiempo. También se dan cuenta de que influyen positivamente en sus relaciones interpersonales.

## **Estudiantes, Odontología, Habilidades sociales básicas**

### **1.1 Introduction**

The ultimate goal of any teaching model is to develop or improve students' abilities, capacities and skills. Every person must have self-awareness (self-knowledge) and must be clear that he/she is influenced by the social framework in which he/she is immersed. This influence occurs through socialization and interaction with diverse situations and the plurality of social environments to which he or she is exposed (Figueroa and Mijangos, 2018). However, it is not always easy to be aware of one's self and one's actions, when this happens it hinders the achievement of personal, social and professional objectives and goals; therefore, the person must express him/herself clearly both verbally and corporally and realize that his/her environment directly influences his/her performance (Sales et al., 2018).

Learning is acquired through social interaction, which guides behavior in certain situations (communicating ideas, respecting rules, etc.) Thus, basic social skills, emotional intelligence and inter- and intrapersonal intelligence allow human beings to know themselves and know how to relate to their environment in order to act harmoniously.

As we are constantly facing changes in the scientific, technological, cultural and, above all, political spheres, we need people with well-developed basic social skills (listening, initiating and maintaining a conversation, asking questions, introducing themselves and giving compliments) that allow them to have personal and social well-being.

Most people have basic social skills, they just do not internalize them, i.e., most people exercise them automatically. This leads to the assumption that, if one listens, understands, thanks or acknowledges, one exercises "active listening"; therefore, there is no effective communication, i.e., what is required is not answered or it has been understood in another sense. Most people think that hearing is the same as listening, seeing is the same as observing, assuming instead of asking or clarifying.

For the above mentioned, it is essential that students have basic social skills for their interactions to be of quality, since they are present in everyday life, which makes it necessary to incorporate programs to strengthen these skills as an improvement of school contexts and therefore of their professional future.

## 1.2 Methodology to be developed

Qualitative hermeneutic research, which aims to highlight the importance of basic social skills (listening, initiating and maintaining a conversation, asking questions, introducing oneself and giving compliments) in the training of 25 second year dental students of a public university who decided to participate on a voluntary basis. The following techniques are used: Goldstein's questionnaire (1989), "Basic social skills strengthening program" and open-ended questionnaire.

The qualitative approach invites to construct the problem and approach it through the observation of society, it studies an integrated whole that forms a unit of analysis and that makes something, to be what it is; it identifies the deep nature of realities, in this case through behavior. In this research approach one observes and describes; and data are taken from people's own words whether spoken or written, or through the behavior of the participants in the study.

Whereas hermeneutics allows the interpretation of social and communicative influence, rather than of a text. It refers to interpreting, explaining or translating through reflection that seeks to unravel the truth. It focuses on the development of interpretative sensitivity to everything related to the object of study.

The data were collected with prior informed consent.

First, the Goldstein questionnaire of basic social skills was applied, then the workshop entitled "Program for strengthening basic social skills" was implemented and, at the end, the Goldstein questionnaire, the open-ended questionnaire and the semi-structured interview were applied again. The questions of the Goldstein questionnaire, are the following:

- 1 Do you pay attention to the person who is talking to you and make an effort to understand what they are saying to you?
- 2 Do you talk to others about unimportant topics and then move on to more important ones?
- 3 Do you talk to others about things that interest others?
- 4 Do you determine what information you need and ask for it from the right person?
5. Do you let others know that you appreciate favors?
6. Do you make yourself known on your own initiative?
7. Do you help others in the group get to know each other?
8. Do you like some positive aspect of the other person or activity they do and tell them about it?

The maximum score is that which is close to the value "five" and is in relation to the skill that is most developed. That is, it indicates to what extent the participant is competent or deficient in the use of a certain basic social skill. Scores 1 and 2 indicate a deficiency in these skills.

The "Program for strengthening basic social skills" was also designed and implemented, which consisted of 10 sessions that addressed the following topics: communication, attentive listening, empathy, nonverbal communication and conversational skills. The program sessions were designed under the four-step model proposed by Goldstein (1989):

1.- Modeling: in it, participants pay attention to the situation presented to them and are asked to pay more attention to the behavior they should reproduce.

Role-playing: in this step the participant is asked to represent a certain basic social skill and it is also made clear beforehand that, for this step to work well, the participant's availability, commitment and spontaneity are necessary, together with the teacher's reinforcement.

Reinforcement: The teacher and the other participants praise the positive behaviors represented that are close to the basic social skill to be reinforced and also describe those that need to be improved.

Generalization of the training; the participant implements what he/she has learned in daily life.

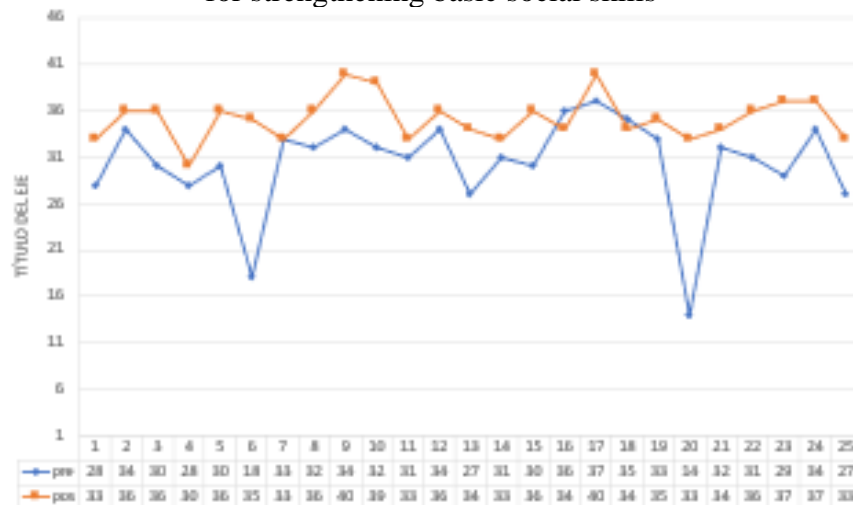
An open-ended questionnaire was also applied with 3 questions related to the importance they give to the development of basic social skills for the practice of the dental profession.

1. 1.- From your point of view, what basic social skills should you apply when taking a clinical history?
2. 2.- How do you consider that basic social skills improve your dental training?
3. 3.- What do you think are the effects that the program for strengthening and developing basic social skills has had on you?

### 1.3 Results

Twenty-five second-year dental students from a public university participated in the study. Of these, 23 were women and 2 were men.

**Graphic 1.1** Results of the applications of Goldstein's questionnaire (before and after) of the "program for strengthening basic social skills"



Source: own elaboration, 2022

Graphic 1.1 shows the difference in the acquisition of basic social skills before (pre) and after (post) the implementation of the "Basic Social Skills Strengthening Program". It should be noted that all participants increased these skills after the program.

As for the results of the open-ended questionnaire, applied at the end of the "Basic social skills strengthening program", the answers were categorized in (verbal and non-verbal communication, active listening, assertiveness and empathy) and the following results were obtained:

- 1.- From your point of view, what basic social skills should you apply when taking the clinical history?

In this question the basic social skill that prevailed the most was "Active listening". The participants refer to the following:

- A) I would use the listening method which is mainly the most complete method where we have more communication with the patient or the person we are interacting with.
- B) Introduce yourself, listen, form questions, conversations and give thanks.
- C) Know how to listen and have a good communication in order to understand in the best way what they are trying to tell us.
- D) To be assertive, to be empathetic with patients. Achieve a good doctor-patient communication. Asking questions is also important because it helps us to ask in the right way. Giving thanks is also of utmost importance since we should always give thanks for helping us, being grateful is everything. Introducing yourself is also important because it builds confidence in them and calms their nerves.
- E) I think that all of them are important to use since first of all you have to introduce yourself in a correct way, you have to know how to start the conversation and then listen, you also have to ask questions that the patient can understand and finally thank them in a kind way.
- F) Active listening to the patient, initiate and maintain a conversation with the patient, it is important to ask questions to find out how the patient is doing, to thank and motivate the patient and to receive or give praise, why not.
- G) Of the social skills that I think we should apply in the clinical history I consider that listening is the basis of this, having an active listening, paying attention, being assertive in what we want to communicate and what they want to communicate to us, knowing how to maintain a good conversation from the beginning to the end of the conversation with the patient, and that during this conversation questions are asked with assertiveness that manage to capture our ideas to the patient and this will make the collection of information more efficient. A good presentation and saying thank you whenever possible will motivate and make the person listening to us feel how he/she feels.

Regarding the question How do you think basic social skills improve your dental training? The students responded as follows:

- A) They form students capable of understanding the power of language, not only for their daily life, but also for professional practice, I consider that the BSS are the key to success, to perform an optimal job.
- B) I believe that they have a great influence because in many things we had different thoughts or we thought in a different way, at the time of the course maybe I had a notion, but already taking into account the course made things clearer and even helped us to improve what we already knew.
- C) The doctor-patient relationship within the office or practice is important because it will allow you to have more information about the patient and the HSB will give you the tools to achieve it, in addition to generating a space of trust and security and as a doctor you will gain good comments.
- D) I think this greatly improves the training of us as students since we must know how to treat people, be tactful and treat them as we would like to be treated, I feel it is very important because it forms us not only as professionals but also as people.
- E) It helps us to behave and communicate properly with others, this will allow us to have a better interaction with our patients and work team.
- F) It is beneficial for us because we learn to express ourselves better and understand other people, take into account the needs, feelings and interests of others.
- G) It makes us more socially skilled, more empathetic and kind to people, and assertive when communicating. This is something we will need throughout our career.

H) In the question "What do you think are the effects that the program of strengthening and development of basic social skills left on you?"

The participants responded as follows:

- A) I feel able to deal with a patient, to obtain good results and to make the patient feel comfortable. Today I feel more confident when speaking and with the ability to generate an optimal environment for work development and communication.
- B) That now I have strengthened the actions that forge us as a person.
- C) More tools to have a better doctor-patient interaction, more confidence in your arguments.
- D) It changed many things in me, it made me realize that there were things about my behavior towards people that I had to change, it helped me to be more empathetic and have more patience with people. I really liked this course because it helped me to be a better person.
- E) Personally I feel that it helped me, and I learned many things to have a better relationship and communication with others, although I still have many things to apply what I learned, little by little I am developing these skills.
- F) From my point of view, it seems to me that there are no negative points or effects, because everything that was seen is for the good of ourselves and to be able to understand people better.
- G) More confident, empathetic and assertive people when communicating.

Regarding the analysis and discussion, this research was carried out with the aim of highlighting the importance of basic social skills (listening, initiating and maintaining a conversation, asking questions, introducing oneself and giving compliments) in the training of dentists. With the results obtained, it can be affirmed that these skills should be fostered in the family and reinforced in the educational context as mentioned by López (2014)

The application of basic social skills as rules of coexistence for the implementation of various learning activities, help the student to reflect on their importance to be assumed in the clinical context. In this way, the importance of living together with respect for oneself and others is highlighted, as referred to by the Ministry of Education of Peru (2015).

With this study, it is shown that even at the university level, the development of basic social skills remains an area of opportunity; which coincides with international findings (Henriquez, 2021; Huaman 2020; Guerra et al., 2019; Condor, 2018; Tapia, 2018; Huertas, 2017; Mendieta, 2016; Monzon, 2014; Cabrera, 2013; Santos, 2012) And which in turn has an impact on poor interpersonal relationships (Sanabria, 2017; Romo et al., 2018).

With the development of the same, it is inferred that interpersonal relationships in the educational context are improved, matching what was commented by Barrón (2018) and Pérez and Fililella (2019).

If programs similar to the one used in this study were implemented consecutively, kindness, courtesy, cooperation, companionship, sincerity and honesty could be strengthened when participants share their thoughts, as commented by (Flores et al., 2016; Valdés and Gómez, 2019).

With the results obtained, it is inferred that it is of utmost importance to mention that the family (upbringing system), is the beginning of the formation of these skills. It is in the family where the person learns values, norms and behavioral models. The family, is the first formative agent, which has direct influence on the socio-affective development that influences the behavior that will be expressed in the following stages of the life cycle (adolescence, adulthood, etc.); as mentioned by (Muñoz, 2009; Vargas et al., 2017).



## Conclusions

It can be said that, based on the results obtained in this study, basic social skills were developed in the students. They refer that through the "Program for strengthening basic social skills" they visualize the importance of social skills in their dental practice. The students recognize that:

- They had no notion of what "active listening" was; they used to interrupt and not pay full attention when dialoguing.
- They frequently interrupted the sender to give their personal opinion.
- They heard their senders, but did not listen to them correctly.
- They were in the habit of talking to someone and they were doing something else, either on their cell phone or something else, and did not show interest in the conversation.
- They did not understand how to make my non-verbal language match what I was saying.
- They were not confident enough to express to the sender if something was bothering them during the dialogue.
- They did not attach much importance to thanking.

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## Chapter 2 Short-term association between morbidity and daily concentrations of O<sub>3</sub> and PM<sub>10</sub> in the Bajío region: A time series study

### Capítulo 2 Asociación a corto plazo entre la morbilidad y las concentraciones diarias de O<sub>3</sub> y PM<sub>10</sub> en la región del Bajío: Un estudio de series de tiempo

UC-CHI, Martha Patricia†´, CERÓN-BRETÓN, Rosa María´\*, LARA-SEVERINO, Reyna del Carmen´´ and CERÓN-BRETÓN, Julia Griselda´

´ *Universidad Autónoma del Carmen, Facultad de Química. México.*

´´ *Universidad Autónoma del Carmen, Facultad de Ciencias de la Salud. México.*

ID 1<sup>st</sup> Author: *Martha Patricia, Uc-Chi* / **ORC ID:** 0000-0002-9480-6034, **CVU CONAHCYT ID:** 1187081

ID 1<sup>st</sup> Co-author: *Rosa María, Cerón-Bretón* / **ORC ID:** 0000-0001-8647-022X, **CVU CONAHCYT ID:** 30106

ID 2<sup>nd</sup> Co-author: *Reyna del Carmen, Lara-Severino* / **ORC ID:** 0000-0001-6173-0187, **CVU CONAHCYT ID:** 357254

ID 3<sup>rd</sup> Co-author: *Julia Griselda, Cerón-Bretón* / **ORC ID:** 0000-0003-1551-7988, **CVU CONAHCYT ID:** 122903

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M. Uc, R. Cerón, R. Lara and J. Cerón

\* rceron@pampano.unacar.mx

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## Abstract

Short-term effects of air pollution on the health of residents in the region of Bajío in Guanajuato, Mexico were assessed from 2012-2015 using a time-series approach. Irapuato showed the highest number of exceedances (884) to the maximum allowable limit established by NOM-020-SSA1-2021, followed by Silao (477) and Salamanca (53), respectively. With respect to PM<sub>10</sub>, all municipalities showed significant exceedances to the maximum allowable limit established by NOM-025-SSA1-2021; Celaya with 518, León with 281 and Salamanca with 210 exceedances, respectively. Comparing both pollutants, we concluded that the pollution due to PM<sub>10</sub> is a hotspot in the Bajío region in comparison with pollution due to O<sub>3</sub>. The association between PM<sub>10</sub> and morbidity was positive and significant, since when PM<sub>10</sub> concentrations increased, the risk values also increased: Irapuato with 0.32%, Silao with 0.24%, Celaya with 0.20% and León with 0.02%. In the case of ozone, correlations found were positive but not significant; therefore, we concluded that there was not a significant risk of morbidity by ozone exposure. Population between 0 and 59 years was identified as the most vulnerable age subgroup, suggesting that, activities of people played an important role in the exposure to these pollutants, since, people in this group comprises workers and students of all ages, who develop their activities outside home, just in the hours in which O<sub>3</sub> and PM<sub>10</sub> reach their peak levels as a result of industrial activity and mobile sources.

## Morbidity, association, exposure, contaminants, vulnerable

### Resumen

Los efectos a corto plazo de la contaminación del aire en la salud de los residentes de la región del Bajío en Guanajuato, México, se evaluaron entre 2012 y 2015 mediante un enfoque de series de tiempo. Irapuato presentó el mayor número de excedencias (884) al límite máximo permisible establecido por la NOM-020-SSA1-2021, seguido de Silao (477) y Salamanca (53), respectivamente. Con respecto al PM<sub>10</sub>, todos los municipios presentaron excedencias significativas al límite máximo permisible establecido por la NOM-025-SSA1-2021; Celaya con 518, León con 281 y Salamanca con 210 excedencias, respectivamente. Comparando ambos contaminantes, concluimos que la contaminación por PM<sub>10</sub> es un punto caliente en la región del Bajío en comparación con la contaminación por O<sub>3</sub>. La asociación entre PM<sub>10</sub> y morbilidad fue positiva y significativa, ya que al aumentar las concentraciones de PM<sub>10</sub> también aumentaron los valores de riesgo: Irapuato con 0.32%, Silao con 0.24%, Celaya con 0.20% y León con 0.02%. En el caso del ozono, las correlaciones encontradas fueron positivas, pero no significativas; por lo tanto, concluimos que no hubo un riesgo significativo de morbilidad por exposición al ozono. Se identificó a la población de 0 a 59 años como el subgrupo etario más vulnerable, sugiriendo que las actividades de las personas juegan un papel importante en la exposición a estos contaminantes, ya que en este grupo se encuentran trabajadores y estudiantes de todas las edades, quienes desarrollan sus actividades fuera de casa, justo en las horas en que el O<sub>3</sub> y el PM<sub>10</sub> alcanzan sus niveles máximos como consecuencia de la actividad industrial y fuentes móviles.

## Morbilidad, asociación, exposición, contaminantes, vulnerable

### 2.1 Introduction

Air pollution is one of the main causes of death and disease worldwide. It is widely supported by scientific evidence that air pollution is the major global public health risk factor (Bodor et al. 2022). According to WHO statistics, more than 80% of urban residents in Metropolitan Areas are exposed to air quality levels which exceeds the established permissible maximum limits in the regulations. The increase of atmospheric pollution caused by the combustion of fossil fuels, transport and industry, is responsible for the increasing morbidity and mortality, especially by respiratory and circulatory diseases. Chen et al. (2022) reports that short-term exposure to air pollutants is associated with elevated risk for myocardial infarction, stroke, heart failure and arrhythmic. Bergmann et al. (2020) reported that the exposure to high concentrations of air pollutants such as PM<sub>10</sub> and O<sub>3</sub> is associated with both, mortality and morbidity diseases.

Due to differences in size, chemical composition and concentration, some atmospheric particles are very dangerous to human body. Particulate matter penetrates deep in pulmonary system, causing adverse effects on human health, particularly on respiratory and circulatory systems (Bagherian et al. 2016). The increased PM<sub>10</sub> and PM<sub>2.5</sub> concentrations are associated with an increased morbidity in the European Union, reducing the average life span by 8.6 months (Bodor et al. 2022).

In addition to effects of PM<sub>10</sub>, there is an increasing evidence that ozone may play a significant role in generating adverse health effects. Atmospheric ozone levels have become a growing public health concern, since O<sub>3</sub> is a powerful oxidant. Epidemiological studies have reported a positive and significant correlation between ozone levels and cardiovascular mortality and morbidity in different regions of the world (Lim et al. 2019; Raza et al. 2019; Yang et al. 2018; Yin et al. 2017). Biological mechanisms involved in O<sub>3</sub>-induced cardiovascular diseases have been studied. Besides the impact on systemic inflammation and oxidative stress, O<sub>3</sub> exposure also have effects on lipid metabolism (Li et al. 2019).

Consequently, air pollution constitutes a significant threat to the population, particularly to population sub-groups, which can be considered as vulnerable such as children, elderly, asthmatic people or people with pre-existing conditions. Children are susceptible to poor air quality since their lungs are still developing (Ibrahim et al. 2021). Studies about exposure to air pollutants and health effects on children are well documented (Ab Manan et al. 2018; Cheng et al. 2021; Horne et al. 2018; Mazonq et al. 2017). On the other hand, older adults have increased their vulnerability to cardiovascular diseases. Therefore, it is important to get supporting evidence to develop pollution control policies in order to protect elderly.

In spite of, there is enough evidence supporting relationship between air pollution and morbidity (Carugno et al. 2021); studies about sex differences are scarce. Khan et al. (2019) found a higher risk of circulatory effects for women, approximately twice that for men, in response to short-term exposure to O<sub>3</sub> and PM<sub>10</sub>.

Regarding to climate and seasonal patterns, some authors report that the synergy of heat or cold and air pollution may affect health diseases more severely than the single factor of heat or cold, or air pollution itself, suggesting that the effects on health associated to some pollutants could show a seasonal behavior (Wang et al. 2023). Mannucci et al. (2015) concluded that the combined exposure to heat and air pollution affect both, physical and mental health and recent studies suggest that short- and long-term exposure to PM<sub>10</sub> and PM<sub>2.5</sub> may be responsible for onset or worsening of depressive symptoms (Buoli et al. 2018).

Evidence of the effects of the short-term exposure to air pollutants are conclusive for respiratory and circulatory mortality, and for hospital admissions (morbidity). Because air pollution imposes a significant number of mortalities and morbidities on society, it is very important to assess the extent of its effects on health (Calle-Martínez et al. 2023; Leili et al. 2023; Li et al. 2023; Pu et al. 2023; Wagner et al. 2023; Naghan et al. 2022). In the last years, many studies have applied time-series methods to evaluate these associations. In this way, Time-series studies are useful to provide valuable information that can be used by decision makers and health professionals to establish new air quality standards or regulations, or to change the current permissible maximum limits.

In this regard, some epidemiological studies have been carried out in Mexico to assess the association between atmospheric pollutants and morbidity; however, excepting Mexico City, the available data and information are not enough to get a diagnosis about the prevailing situation in the remaining metropolitan areas of the country. Therefore, the aim of this study was to assess the association between short-term exposure to PM<sub>10</sub> and O<sub>3</sub> and morbidity during 2012-2015 in 5 Metropolitan Areas of the Bajío region in Guanajuato, Mexico, considering age sub-groups (0-59 years and >60 years), gender, all causes and specific cause (respiratory and circulatory diseases), seasonal trends in air quality data and meteorological variables. In addition, in this study, we assessed the effects derived from a hypothetical scenario in which daily mean concentration of atmospheric pollutants increases in 10%, in order to estimate the resulting association in a future scenario. Results obtained here, allowed us to quantify the number of exceedances to regulation for each pollutant, to identify in which region of the Bajío these exceedances are higher, and to identify in which areas of this region, relative risk indexes were higher, as well as the age sub –group more vulnerable.

## 2.2 Methodology

### 2.2.1 Study Area

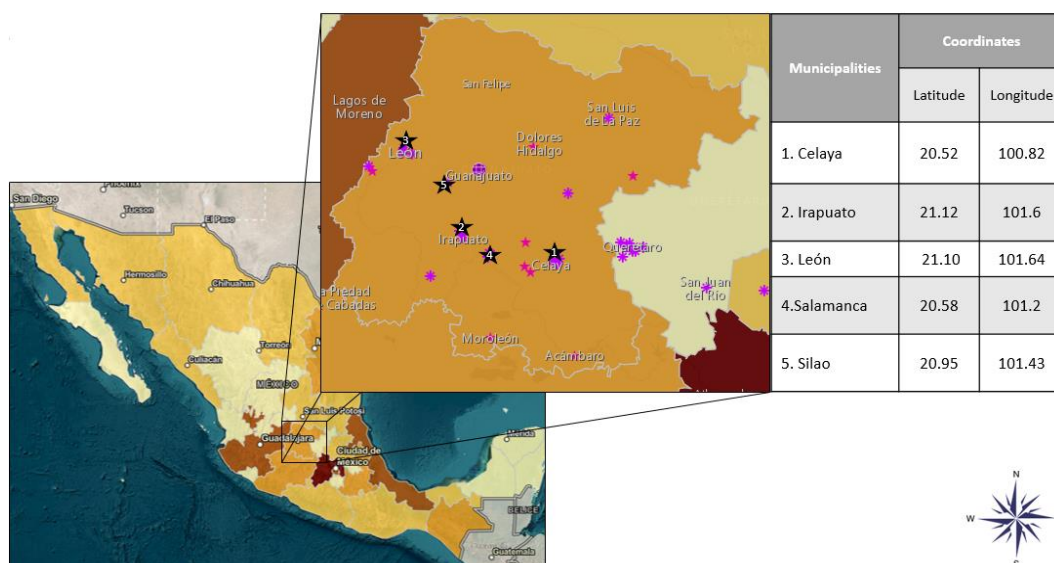
For this study, the municipalities of León, Silao, Irapuato, Celaya and Salamanca in the state of Guanajuato, Mexico were considered. These five municipalities constitute the Bajío corridor, which comprise a total of 2, 652, 893 habitants.

León is the most populated city in the state of Guanajuato, and the fourth-most populated city in the country, with 1,578,626 habitants. León is part of the macro region known as Bajío, located in the Central Mexican Plateau. The main economic activity in León is shoe and leather industry, but in the last decade, automotive industry has been developed, with several industrial parks in this sector.

In the case of Silao, this municipality is part of the Metropolitan Area of León with a total of 83, 352 habitants, and their main productive activities are agriculture and automotive. Salamanca has a population of 160, 682 habitants, being a region very industrialized, the main industries are energy, petrochemical, automotive, food and electronic. The municipality of Celaya is an important crossroad of trade toward the Northern and Western side of the country. Celaya has a total of 378, 143 habitants, and the main productive activities are agriculture, livestock, energy, electric, pharmaceutical, paper, chemical, steel and processed food. Finally, Irapuato is the second biggest municipality in Guanajuato, with 452, 090 habitants, being their main economic activities textile industry, automotive industry, and agriculture for exportation.

Locations of these municipalities are shown in Figure 2.1.

**Figure 2.1** Location of municipalities of the Bajío region considered in this study



Source: Own elaboration

The Bajío region has a humid subtropical climate ( $C_{wa}$  in the Köppen classification) with rains occurring along the summer. However, its climate closely borders on a semi-arid climate, with an average annual temperature of 19.9 °C, where the warmest month is May (with a maximum average of 31.7°C) and the coolest month is January (with a minimum average temperature of 7.7 °C).

### 2.2.2 Air quality and meteorological data

Air quality (For  $O_3$  and  $PM_{10}$ ) and meteorological data (Temperature and Relative Humidity) measured by from automatic monitoring stations in Metropolitan Areas of León, Celaya, Salamanca, Silao and Irapuato were obtained from SINAICA (National System of Air Quality Information) during 2012-2015. Methods used to measure criteria air pollutants concentrations are standardized methods. In the case of  $O_3$ , the principle of operation of the analyzer Photometry UV; whereas in the case of  $PM_{10}$ , is Gravimetric.

### 2.2.3 Applicable regulations

Reference values to protect public health are established by Mexican Federal Government who is the responsible to monitor compliance, the reference values are published in the Mexican Official Regulations (NOM) being obligatory at a national scale. Applicable NOM's for each criteria air pollutant are presented in Table 2.1.

**Table 2.1** Applicable NOM's for each criteria air pollutant

Criteria Air Pollutant	Exposure time used for the assessment	Kind of Exposure	Allowed frequency	Maximum permissible limit value	Reference (NOM)
Ozone (O <sub>3</sub> )	Hourly data (1 h)	Acute	It is not allowed	0.090 ppm	NOM-020-SSA1-2021
	Mobil average of 8 h		It is not allowed	0.065 ppm	
	Annual		Once a year	0.021 ppm	
PM <sub>10</sub>	24 h	Acute	It is not allowed	70 µg/m <sup>3</sup>	NOM-025-SSA1-2021
	Annual	Chronic	--	36 µg/m <sup>3</sup>	
	24 h			0.040 ppm	

*Source: NOM-020-SSA1-2021 for Ozone (O<sub>3</sub>) and NOM-025-SSA1-2021 for PM<sub>10</sub>*

From air quality data and meteorological data set, time series for each pollutant and meteorological variable were integrated considering both, daily mean and daily maximum concentrations. From the reference values established for each criteria air pollutant, air quality was assessed and the exceedances were estimated. Missing data and the continuity in the records were assessed, in some cases; it was required to complete the databases for a given station. Therefore, it was necessary to establish some criteria to include or not data in the databases: First, to decide which stations would be included (only values from monitoring stations showing a valid data percentage >75% were considered). Second, if one or more stations showed missing data, to define how missing data would be imputed (to complete the database in which, despite complying with valid data percentage, missing data were isolated or intermittent, they were completed by using NIPALS approach).

### 2.2.4 Epidemiological data

Although National System of Air Quality Information (SINAICA) integrates air quality information from several cities in Mexico; in the case of health data, there is no a system which integrates morbidity data. However, it was possible to obtain this information (hospital admissions for Bajío region) during the study period from SINAIS (National System of Health Information). Databases in a monthly and annual base was requested to the corresponding authorities in the health sector for purposes of this study. Morbidity database from SINAIS is based in the international classification of diseases established by World Health Organization (WHO) revision CIE-10/2 considering respiratory system diseases (J00-J99) and circulatory system diseases (I00-I99). Morbidity data were assessed by hospital admission cause (all causes, respiratory and circulatory), gender (male and female), and age group (<1 year, 1-4 years, 5-59 years, 60-74 years and >75 years).

### 2.2.5 Time series analysis design

Time series study developed in the present research work involved the following stages:

- Assessment of temporal variations in morbidity rate in a monthly basis for all population, by age, by gender and by specific cause and by municipality during the study period (from January 1, 2012 to December 31, 2015).
- Assessment of temporal variations of O<sub>3</sub> and PM<sub>10</sub> in a monthly basis by municipality for the study period (from January 1, 2012 to December 31, 2015).
- Assessment of air quality: Analysis of exceedances to reference values established as maximum permissible limits in NOM-020-SSA1-2021 for O<sub>3</sub>, and in NOM-025-SSA1-2021 for PM<sub>10</sub>.

- Estimation of the magnitude of the association between morbidity by all causes and by specific cause, and atmospheric pollution concentrations by municipality for each population sub-group. In this stage, meteorological variables were included (temperature and relative humidity).
- a) Study subject: Hospital admissions occurring in the residents of the Bajío region during the period from January 1, 2012 to December 31, 2015.
- b) Variables:
  - Response variables: Number of monthly hospital admission during the study period by all, respiratory and circulatory causes.
  - Explanatory variables: Criteria air pollutants (quantitative explanatory variable), monthly average concentration for O<sub>3</sub> and PM<sub>10</sub> during the study period.
  - Meteorological variables (quantitative explanatory variable), monthly average values for maximum temperature, minimum temperature and relative humidity during the study period.
  - Gender (qualitative explanatory variable), number of hospital admissions by gender.
  - Age (qualitative explanatory variable), number of hospital admissions by age group.
  - Control variables (seasonality): seasons were classified as cold months (from November to February) and warm months (from May to August).
  - Confusion variables: Temperature and Relative Humidity.

Since the hospital admission did not occur at the same time in which the study subject is exposed to a given air pollutant concentration, is necessary to consider a certain delay time. Time delay was estimated from cross correlations of the series (mortality vs temperature, and mortality vs relative humidity) by using Infostat software v. 2008 (Di Rienzo et al. 2008), and selecting time delays according to their significance level.

In addition, it was required to carry out a pre-treatment of the time series, in this case, epidemiological data series were smoothed, by applying a non-parametric method (LOWESS: LOcally WEighted regression Scatterplot Smoothing); whereas in the case of air quality data, series were smoothed by using ARIMA method (Autoregressive Integrate Moving Average). The smooth procedure of time series was carried out by using statistical software XLSTAT v. 2017 (<https://www.xlstat.com/es/>).

### **2.2.6 Estimation of the association between morbidity by all causes and by specific cause, and criteria air pollutants concentrations for each population sub-group**

Once, both time series, epidemiological and air quality were treated and smoothed; a Poisson model was applied to reduce Pearson residuals. However, it was necessary to apply a multivariate analysis to decide which variables will be added to the base model.

A principal component analysis (ACP) and a linear regression analysis (RLM) by using XLSTAT v. 2017 were applied to data series for morbidity; criteria air pollutants and meteorological variables. From ACP, the principal components contributing to the major percentage of data variability were obtained, considering those showing the major load of factor and a major statistical significance. A first approach was carried out with the Poisson basal model including those variables which contributed with significant information to explain the variability of dependent variable (daily morbidity).



### Relative Risk Index (RRI) of daily morbidity associated to atmospheric pollution

To apply the Poisson model, the methodology was the same than APHEA (Katsouyanni, 1996) and EMECAN (Ballester et al., 2002) projects. This methodology is described in detail by Cerón-Bretón et al. (2018) and Ídem et al. (2021). Once, basal model has been established, the model is extended for each pollutant and its time delays. The construction of the auto-regressive Poisson model let to determine if the response variable depends or not on other variables. This effect is assessed by beta coefficient of each independent variable in the Poisson regression model. The general model to relate the response variable with different independent variables is the following:

$$\ln(E_y) = \beta_0 + \sum_{i=1}^n \beta_i x_{t,i} \quad (1)$$

Where  $E_y$  is the expected number of cases,  $\beta_0, \beta_i$  are the model constants, and  $x_{ti}$  are the explanatory variables.

The next step is to obtain the relative risk index (RRI) from beta coefficients as follows:

$$RRI_i = e^{\beta_i} \quad (2)$$

Where, RRI is the relative risk index associated to the explanatory variable  $i$  by increment unit of this variable, and  $\beta_i$  is the regression coefficient associated to the explanatory variable  $i$  in the model. Poisson regression analysis was carried out by using the statistical software XLSTAT v. 2017 (<https://www.xlstat.com/es/>). In this study, besides the Poisson model corresponding to the current atmospheric pollution conditions, a hypothetical scenario in which the concentration of each pollutant was increased in 10% separately was considered. In this case, again the regression parameters were obtained, a Poisson distribution was applied considering this increase and keeping the rest of variables unchanged. From obtained  $\beta_i$  values again, relative risk index was estimated for morbidity considering an increase of 10% in the magnitude of each explanatory variable. It let us to determine the effect on risk derived from a future hypothetical scenario.

#### 2.2.7 Mapping of relative risk index for each municipality of the Bajío region in Guanajuato

The relative risk indexes (RRI) of daily morbidity by all causes associated to  $O_3$  and  $PM_{10}$  in the Bajío region were mapped by using a Geographic Information Systems QGIS v. 2.14.7 (QGIS, 2017).

## 2.3 Results

### 2.3.1 Air Quality

Descriptive statistical for  $O_3$  and  $PM_{10}$  of data registered from automatic monitoring stations in León, Celaya, Silao, Irapuato, and Salamanca is shown in Tables 3.1 and 3.2. From Table 3.1 it can be observed that for all period 2012-2015, the mean concentration for  $O_3$  was 26.6224 ppb, reaching a maximum concentration of 73.6725 ppb in Irapuato. León and Celaya showed the lowest mean concentrations for ozone; whereas Irapuato and Silao showed the maximum levels of  $O_3$ .

**Table 2.2** Descriptive Statistical for  $O_3$  during the study period in the Bajío region

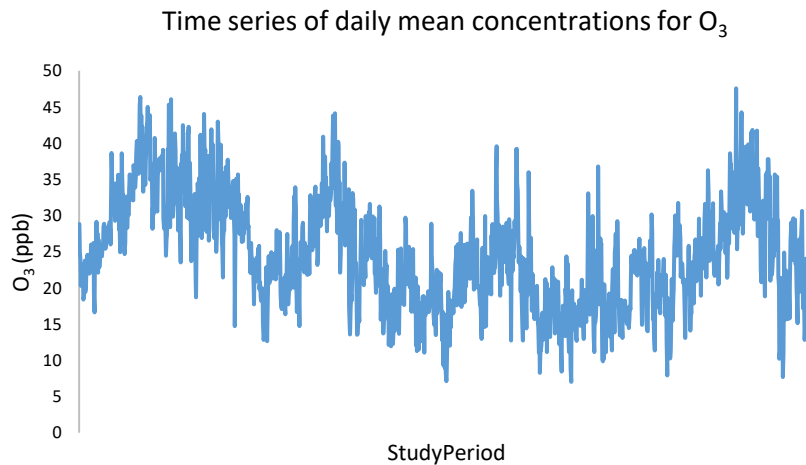
León				
Pollutant	Mean Concentration	Standard deviation	Minimum	Maximum
$O_3$ (ppb)	24.7017	7.5610	7.0235	47.5736
Celaya				
Pollutant	Mean Concentration	Standard deviation	Minimum	Maximum
$O_3$ (ppb)	25.2340	7.9457	6.5232	47.5068
Silao				
Pollutant	Mean Concentration	Standard deviation	Minimum	Maximum
$O_3$ (ppb)	28.8402	9.0672	7.5000	63.1788
Irapuato				
Pollutant	Mean Concentration	Standard deviation	Minimum	Maximum
$O_3$ (ppb)	29.0217	12.9467	5.7182	73.6725
Salamanca				
Pollutant	Mean Concentration	Standard deviation	Minimum	Maximum
$O_3$ (ppb)	25.3144	7.9857	8.7379	50.7264

Source: Own elaboration

The highest concentrations were observed during 2012 and 2015, whereas the lowest concentrations were registered during 2014. Ozone concentrations were in general higher during the warm months in comparison with the winter season, being 22% higher than those observed during cold months.

Time series for daily mean concentrations for O<sub>3</sub> during the period 2012-2015 in the Bajío region, are shown in graph 2.2. It can be observed a seasonal pattern, in which, the highest values of ozone concentration occurred during the summer months whereas, the lowest concentrations occurred during winter months.

**Graph 2.1** Time-series for daily mean concentrations for Ozone during the study period in the Bajío region in Guanajuato



*Source: Own elaboration*

From Table 2.3 it can be observed that for all period 2012-2015, the mean concentration for PM<sub>10</sub> was 54.1229  $\mu\text{g m}^{-3}$ , reaching a maximum concentration of 160.9764  $\mu\text{g m}^{-3}$  in the municipality of Celaya. The lowest mean concentrations were found for Silao and Irapuato; whereas the maximum values were registered in Celaya and Salamanca.

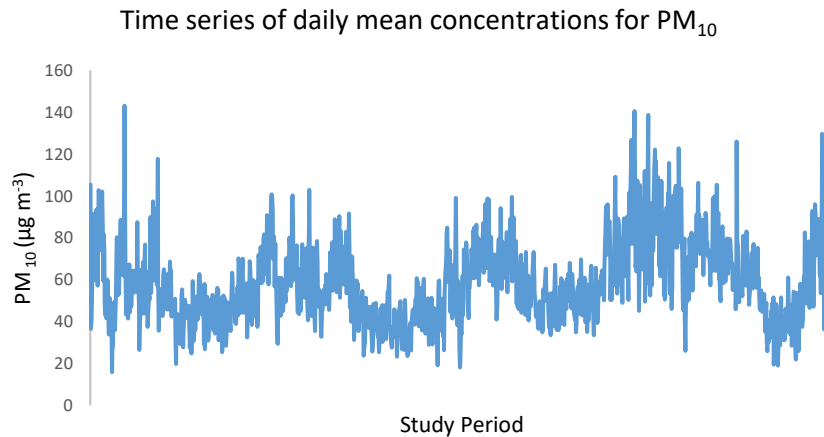
**Table 2.3** Descriptive Statistical for PM<sub>10</sub> during the study period in León, the Bajío region in Guanajuato

<b>León</b>				
<b>Pollutant</b>	<b>Mean Concentration</b>	<b>Standard deviation</b>	<b>Minimum</b>	<b>Maximum</b>
PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	58.8244	19.3295	15.8481	143.1591
<b>Celaya</b>				
<b>Pollutant</b>	<b>Mean Concentration</b>	<b>Standard deviation</b>	<b>Minimum</b>	<b>Maximum</b>
PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	69.1963	25.9990	18.9079	160.9764
<b>Silao</b>				
<b>Pollutant</b>	<b>Mean Concentration</b>	<b>Standard deviation</b>	<b>Minimum</b>	<b>Maximum</b>
PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	41.9583	16.3670	8.7742	104.2083
<b>Irapuato</b>				
<b>Pollutant</b>	<b>Mean Concentration</b>	<b>Standard deviation</b>	<b>Minimum</b>	<b>Maximum</b>
PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	45.5014	17.6875	8.8098	130.4270
<b>Salamanca</b>				
<b>Pollutant</b>	<b>Mean Concentration</b>	<b>Standard deviation</b>	<b>Minimum</b>	<b>Maximum</b>
PM <sub>10</sub> ( $\mu\text{g m}^{-3}$ )	55.1345	19.6767	14.9325	152.6935

*Source: Own elaboration*

The highest concentrations were observed during 2015, whereas the lowest concentrations were registered during 2013 and 2014. PM<sub>10</sub> concentrations were in general higher during the cold months in comparison with the summer season, being 1.29 times higher than those observed during warm months. Time series for daily mean concentrations for PM<sub>10</sub> during the period 2012-2015 in the Bajío region are shown in graph 2.2. It can be observed a seasonal pattern, in which, the highest values of PM<sub>10</sub> concentration occur during the winter months whereas, the lowest concentrations occur during the summer months.

**Graphic 2.2** Time-series for daily mean concentrations for PM<sub>10</sub> during the study period in the Bajío region in Guanajuato



*Source: Own elaboration*

### 2.3.2 Epidemiological data

Descriptive statistical for the 5 municipalities in the region of Bajío was estimated, it was found that León (5.73) and Irapuato (2.94) showed the highest number of hospital admissions by all causes during the study period. On the other hand, Salamanca (1.09) and Silao (1.40) showed the lowest.

Morbidity rate is an epidemiological concept that refers to the number of persons which have a disease regarding to a given population and period. The total of population for each municipality was obtained from National Institute of Statistics, Geography and Informatics (INEGI). In the case of morbidity rate, the estimation considered groups of 1000 habitants.

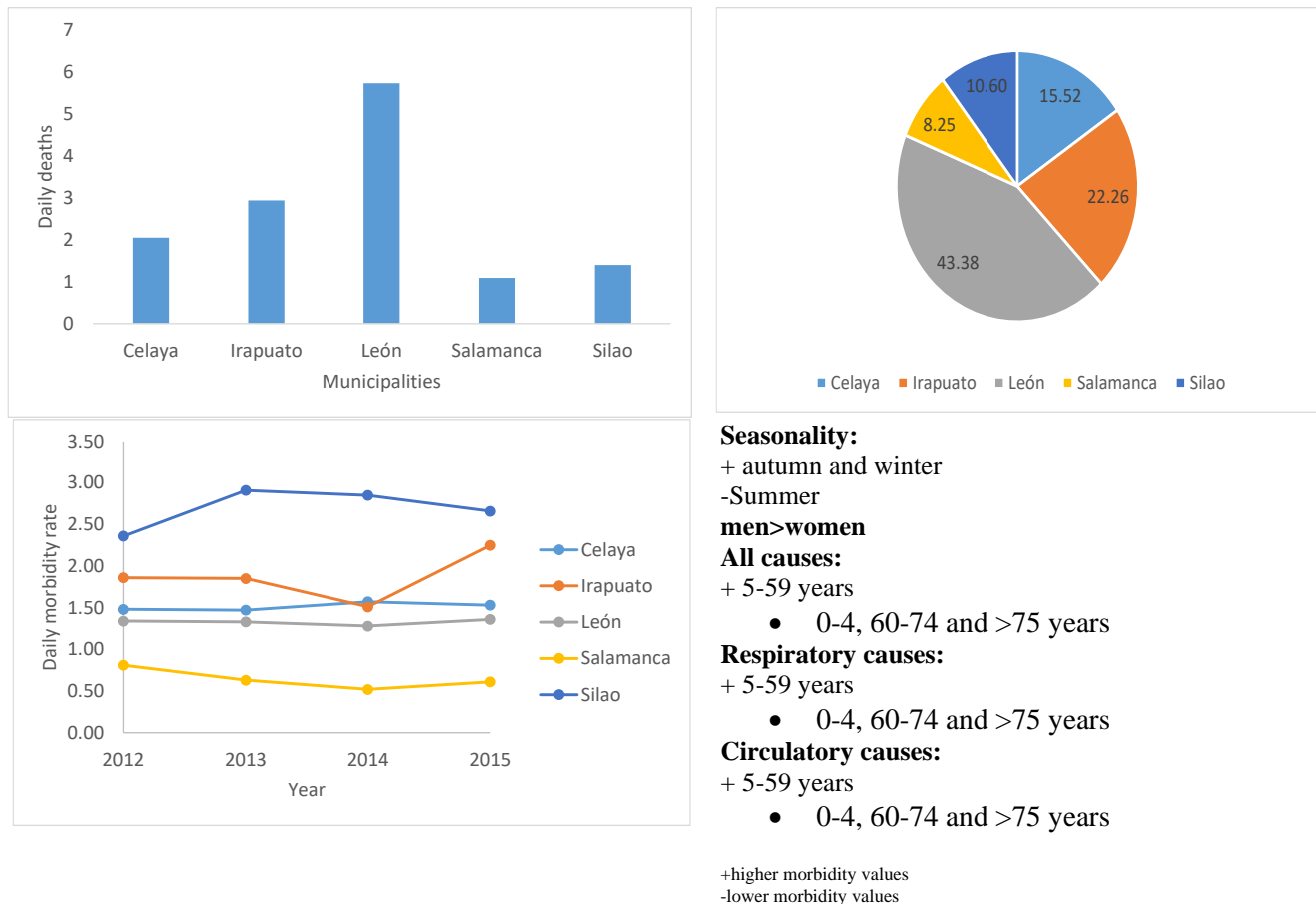
Silao (2.36-2.91) and Irapuato (1.51-2.25) showed the highest morbidity rate, whereas, Salamanca (1.22-1.71) showed the lowest. It is important to mention that in spite of León had the highest values of morbidity, its morbidity rate was low. It was found some uniformity in the results for all municipalities studied, for example, the highest values of the relative maximum for daily morbidity were found during November, December and January. In addition, it was observed a marked and evident seasonality, with the number of hospital admissions by all causes being higher during autumn and winter seasons. However, it was not observed any inter-annual trend.

Regarding to age sub-group. It was possible to identify people between 5 and 59 years as the population group with the higher number and frequency of admissions; whereas the sub-group of 60-74 years and >75 years showed the lowest. In terms of gender, in León and Irapuato the number of admissions registered was similar in both, women and men. On the other hand, in Silao and Salamanca, women showed greater hospital admissions in comparison with men. Finally, in the municipality of Celaya, the number of admissions was higher in men.

When respiratory cause was considered as the specific cause of hospital admission the highest number of admissions was found for people between 5 and 59 years, the same was observed when circulatory diseases were considered as specific cause. Regarding to the gender, in the sub-group of 5-59 years, 17.4 and 23.8% were men; whereas, between 21.8 and 24.2% were women.

From Graphic 2.3, it can be observed that León (43.38%) and Irapuato (22.26%) contribute in a great proportion of the total morbidity in the region of Bajío in Guanajuato; registering also the highest number of daily hospital admissions.

**Graphic 2.3** Integrated information of daily morbidity during the study period in the Bajío region in Guanajuato

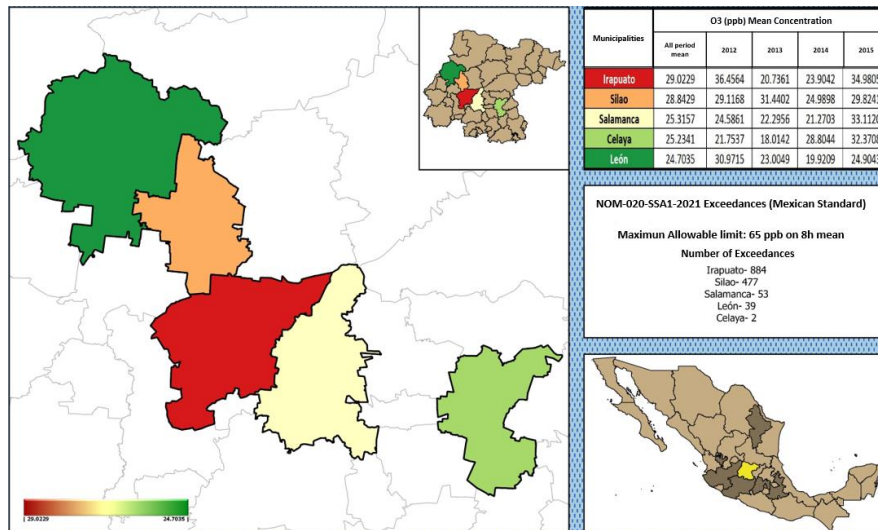


Source: Own elaboration

### 2.3.3 Exceedances to reference values established by NOM's

In Graphcis 2.4 and 2.5, integrated information about the number of exceedances for O<sub>3</sub> and PM<sub>10</sub> for the Bajío region in Guanajuato is shown. From Graph 3.4, it can be observed that, Irapuato, Silao and Salamanca presented the highest mean concentration values for O<sub>3</sub> during the all period. However, Irapuato showed the highest number of exceedances (884) to the maximum allowable limit established by NOM-020-SSA1-2021, followed by Silao (477) and Salamanca (53), respectively. It is to say, zones located in the middle part of the Bajío presented more photochemical pollution.

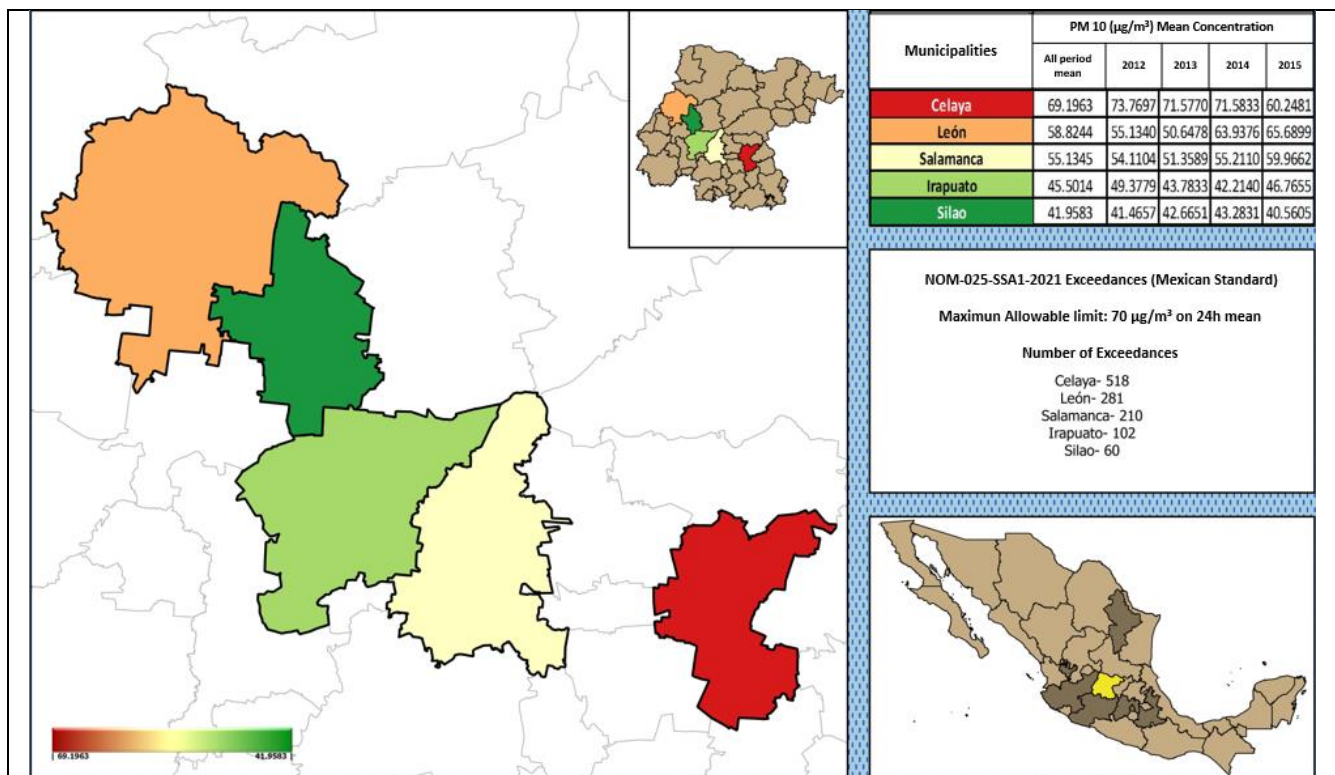
**Graphic 2.4** Integrated information about the number of exceedances for O<sub>3</sub> in the Bajío region in Guanajuato



Source: Own elaboration

From Graph 3.5, it can be observed that, Celaya, León and Salamanca presented the highest mean concentration values for PM<sub>10</sub> during the all period. All municipalities showed significant exceedances to the maximum allowable limit established by NOM-025-SSA1-2021; Celaya with 518, León with 281 and Salamanca with 210 exceedances, respectively.

**Graphic 2.5** Integrated information about the number of exceedances for PM<sub>10</sub> in the Bajío region in Guanajuato



Source: Own elaboration

### 2.3.4 Estimation and mapping of the relative risk index (RRI) for each pollutant by municipality

*Bi-variate analysis, multivariate analysis and multiple regression of daily mortality data with explanatory variables.*

#### - *O<sub>3</sub>-All causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for Celaya and Salamanca. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Irapuato, León and Salamanca were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that ozone variable provided significant information to the prediction model for daily morbidity.

#### - *PM<sub>10</sub>-All causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for León and Irapuato. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Celaya, Irapuato and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that PM<sub>10</sub> variable provided significant information to the prediction model for daily morbidity.

#### - *O<sub>3</sub>-From 0 to 59 years*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for Celaya and Salamanca. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), León, Salamanca and Celaya, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that ozone variable provided significant information to the prediction model for daily morbidity.

#### - *PM<sub>10</sub>-From 0 to 59 years*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for Irapuato, Celaya and Silao. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Celaya, Irapuato and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that PM<sub>10</sub> variable provided significant information to the prediction model for daily morbidity.

#### - *O<sub>3</sub>->60 years*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for León and Silao. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), León, Salamanca and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that ozone variable provided significant information to the prediction model for daily morbidity.

- *PM<sub>10</sub>->60 years*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for León and Salamanca. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), León and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that  $PM_{10}$  variable provided significant information to the prediction model for daily morbidity.

- *O<sub>3</sub>- Respiratory causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for Salamanca, Silao and León. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Irapuato, León, Salamanca and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that ozone variable provided significant information to the prediction model for daily morbidity.

- *PM<sub>10</sub>- Respiratory causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for Irapuato, León and Celaya. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Celaya, Irapuato, León, Salamanca and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that  $PM_{10}$  variable provided significant information to the prediction model for daily morbidity.

- *O<sub>3</sub>- Circulatory causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for León and Silao. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), León and Silao, were the municipalities which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that ozone variable provided significant information to the prediction model for daily morbidity.

- *PM<sub>10</sub>- Circulatory causes*

The association of this pollutant with daily morbidity considering bi-variate relations (Pearson coefficient) showed significant values only for León. In the case of multiple regression analysis, the null hypothesis states that explanatory variable contributes significantly to the model, considering Fisher statistical test. If  $F < 0.0001$ , it means that the risk of assuming the null hypothesis as incorrect is lower than 0.01%. From multiple regression analysis (RLM SC Type III), Salamanca was the only municipality which showed the lowest values of the statistical test ( $F < 0.0001$ ), therefore, we can conclude that  $PM_{10}$  variable provided significant information to the prediction model for daily morbidity.



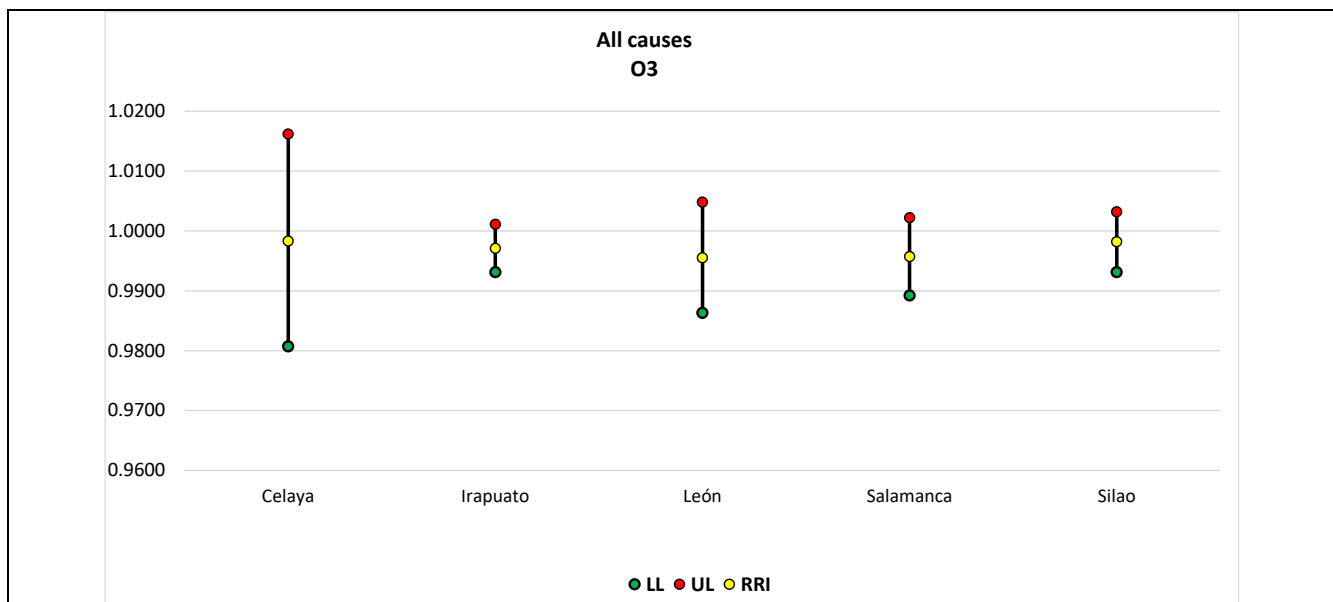
### Estimation of Relative Risk Index (RRI)

Results for relative risk index (RRI) and the confidence interval (lower limit and upper limit) at 95% of confidence corresponding to an increase of 10% in the concentration of each pollutant are shown in Graphs 3.6-3.10 for  $O_3$  and Graphs 3.11-3.15 for  $PM_{10}$  for morbidity by all causes, age groups (0-59 years and >60 years), respiratory and circulatory causes.

#### $O_3$

In Graphs 2.6-2.10, relative risk indexes (RRI) corresponding to an increase of 10% in daily mean ozone concentrations are shown. Celaya showed the highest significant correlations for morbidity by respiratory causes (IRR=1.0002) [IC95%: 0.9759-1.0250] during warm months. On the other hand, Silao showed the highest RRI values for morbidity by respiratory causes (IRR=1.0020) [IC95%: 0.9937-1.0104] and during the cold months [IC95%: 0.9726-1.0564] with an IRR=1.0136. The association between morbidity by all causes and ozone concentrations showed positive correlations in all municipalities, but this associations was not significant, being Celaya the highest with an IRR=0.9983 [IC95%: 0.9807-1.0162]. Regarding to the age-group, the age range between 0 and 59 years showed correlations not significant in all municipalities, excepting Silao which did not present any correlation. Finally, the association between morbidity and ozone concentrations in people major than 60 years, showed correlations not significant in León, Salamanca and Silao; whereas Celaya and Irapuato no did not present any correlation.

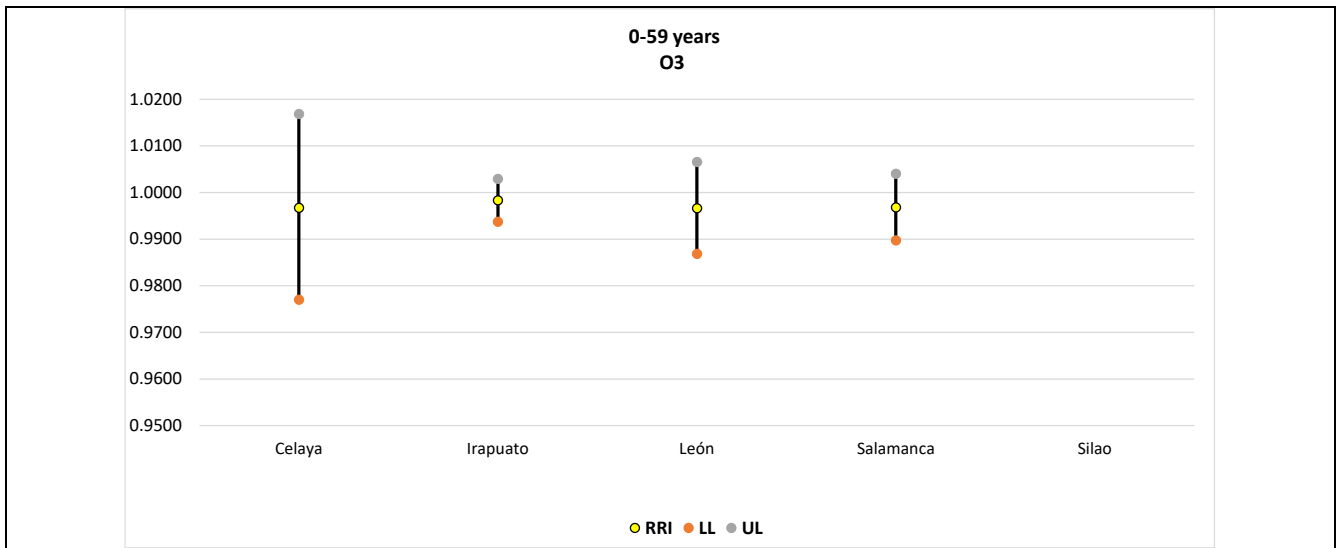
**Graphic 2.6** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for  $O_3$  considering morbidity by all causes in the Bajío region in Guanajuato



Source: Own elaboration

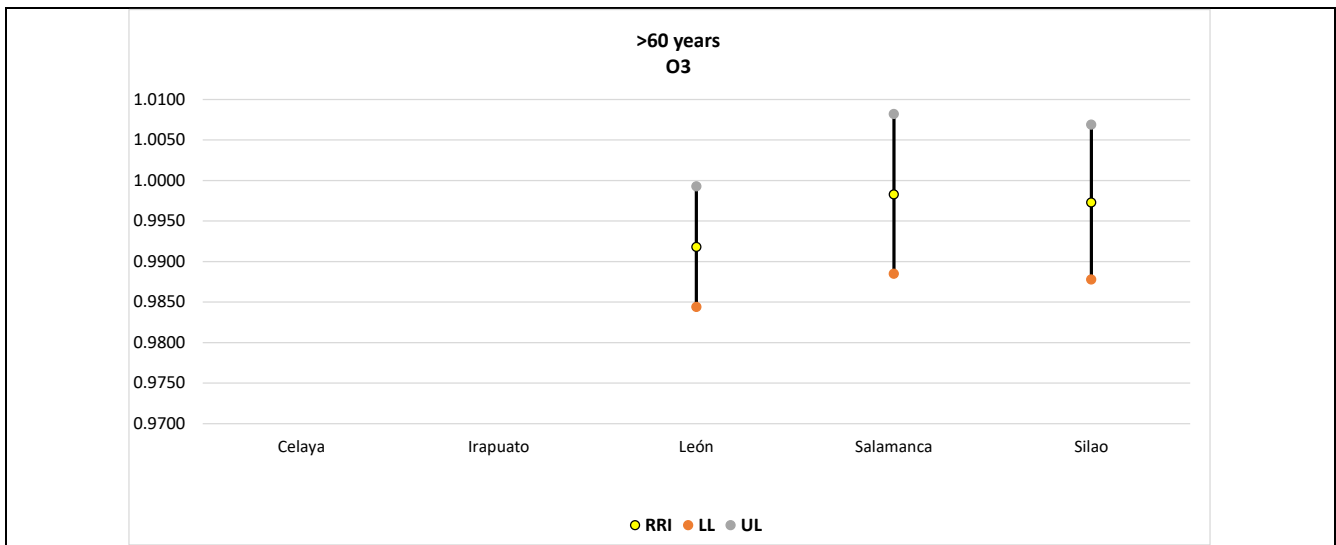


**Graphic 2.7** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for O<sub>3</sub> considering the age group of 0-59 years in the Bajío region in Guanajuato



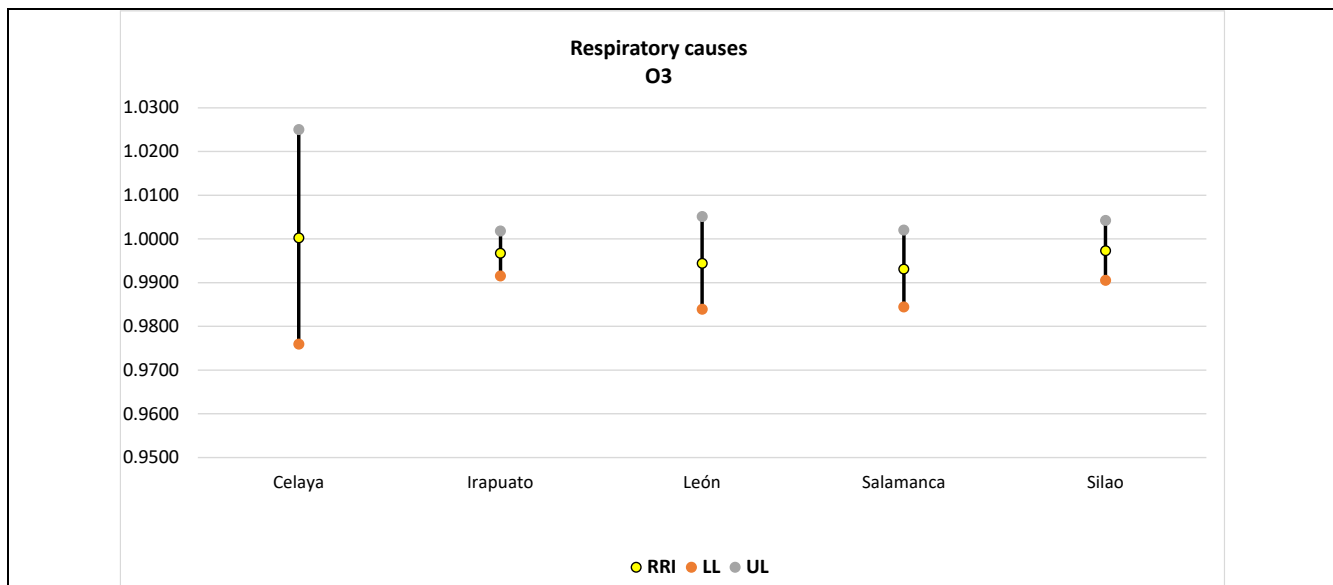
Source: Own elaboration

**Graphic 2.8** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for O<sub>3</sub> considering the age group >60 years in the Bajío region in Guanajuato



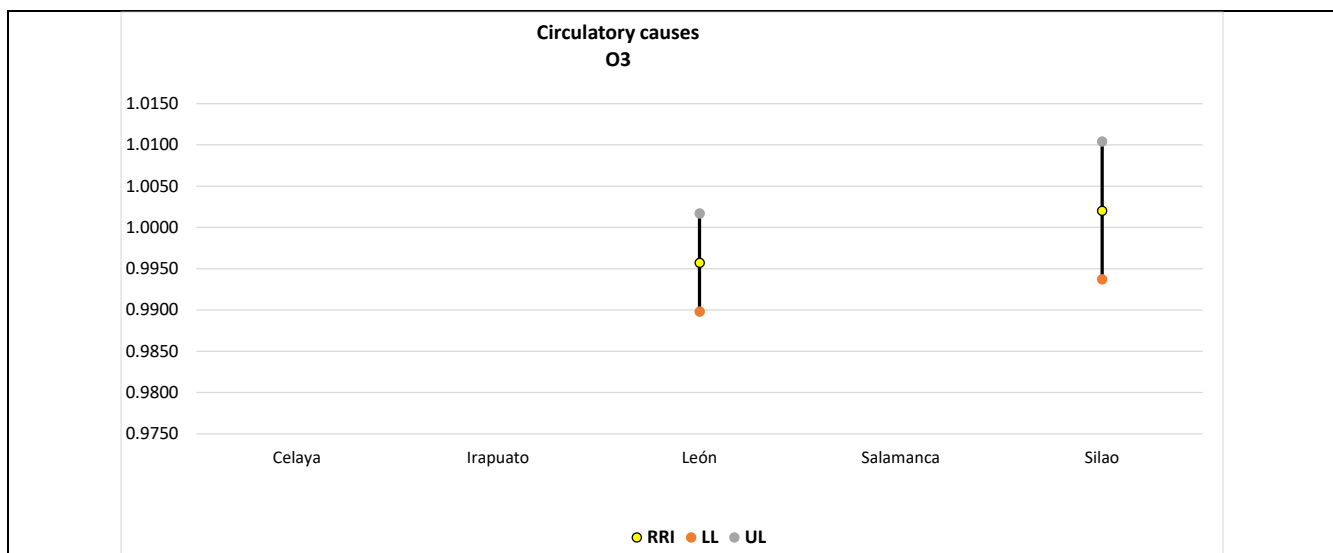
Source: Own elaboration

**Graphic 2.9** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for O<sub>3</sub> considering morbidity by respiratory causes in the Bajío region in Guanajuato



Source: Own elaboration

**Graphic 3.10** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for O<sub>3</sub> considering morbidity by circulatory causes in the Bajío region in Guanajuato

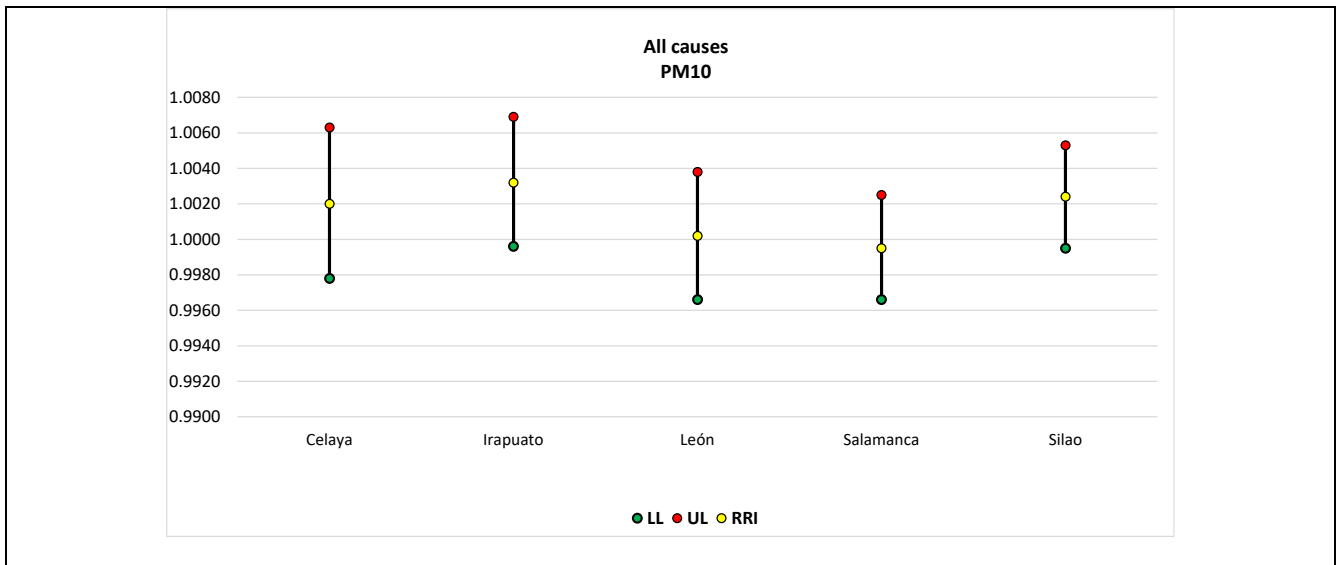


Source: Own elaboration

### PM<sub>10</sub>

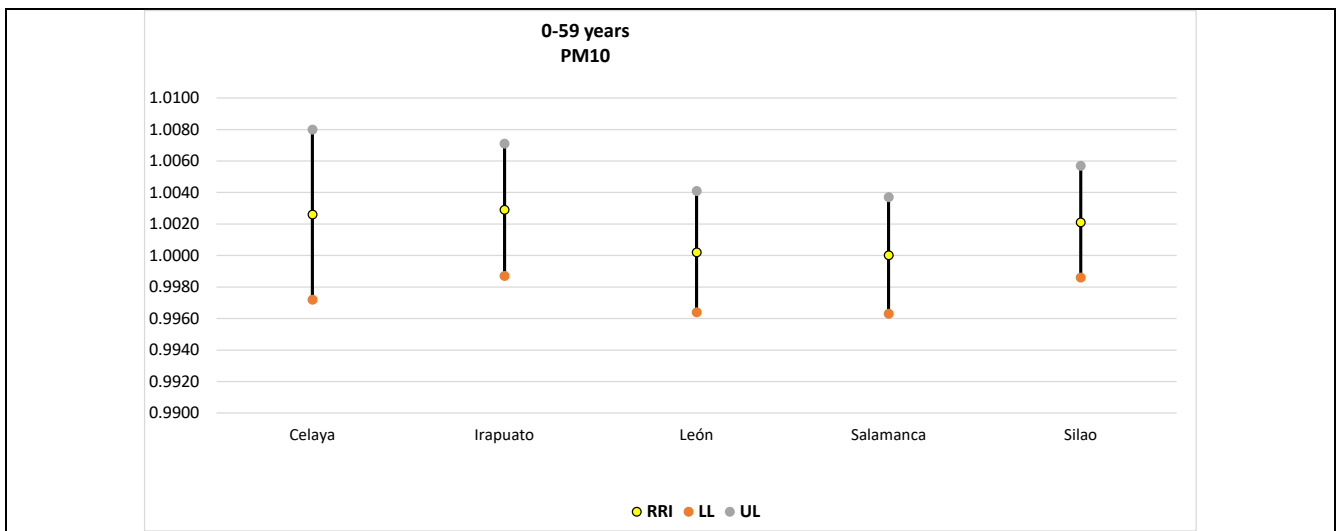
In Graphcis 3.11-3.15, relative risk indexes (RRI) corresponding to an increase of 10% in daily mean PM<sub>10</sub> concentrations are shown. The highest RRI values were found for Irapuato for morbidity by all causes (IRR=1.0032) [IC95%: 0.9996-1.0069]; in the age range from 0 to 59 years (IRR=1.0029) [IC95%: 0.9987-1.0071] and for morbidity by respiratory causes (IRR=1.0034) [IC95%: 0.9988-1.0081] as can be observed in graphs 3.11, 3.12 and 3.14, respectively. In addition, this municipality also showed the highest risk during the cold months [IC95%: 0.9999-1.0094] with an IRR=1.0047. The association between morbidity and PM<sub>10</sub> concentrations considering people major than 60 years [IC95%: 0.9985-1.0036], and morbidity by circulatory causes [IC95%: 0.9978-1.0023] were the highest for the municipality of León with IRR values of 1.0011 and 1.0001, respectively. Silao showed the highest risk during the warm months (IRR=1.0022) [IC95%: 0.9918-1.0128].

**Graphic 2.11** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for PM<sub>10</sub> considering morbidity by all causes in the Bajío region in Guanajuato



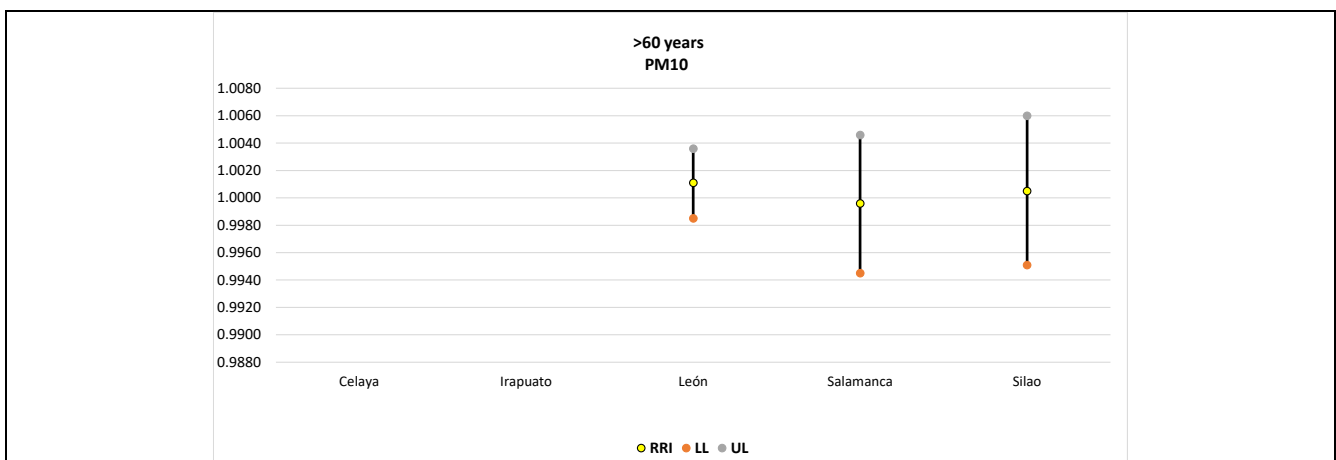
Source: Own elaboration

**Graphic 2.12** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for PM<sub>10</sub> considering the age group of 0-59 years in the Bajío region in Guanajuato



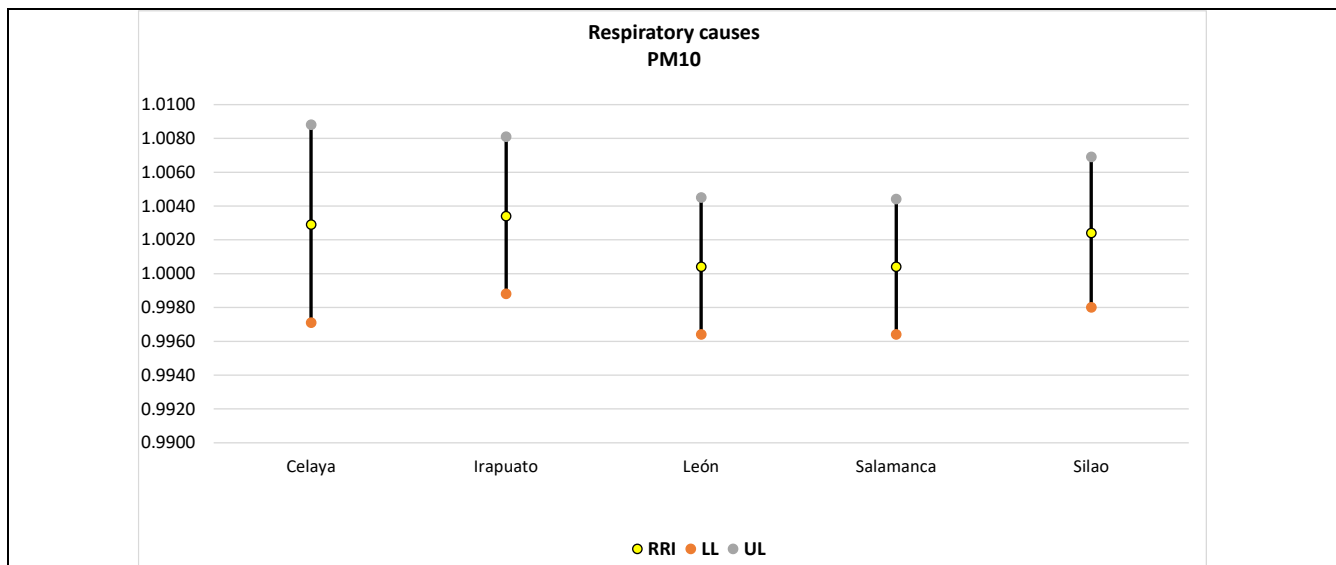
Source: Own elaboration

**Graphic 2.13** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for PM<sub>10</sub> considering the age group >60 years in the Bajío region in Guanajuato



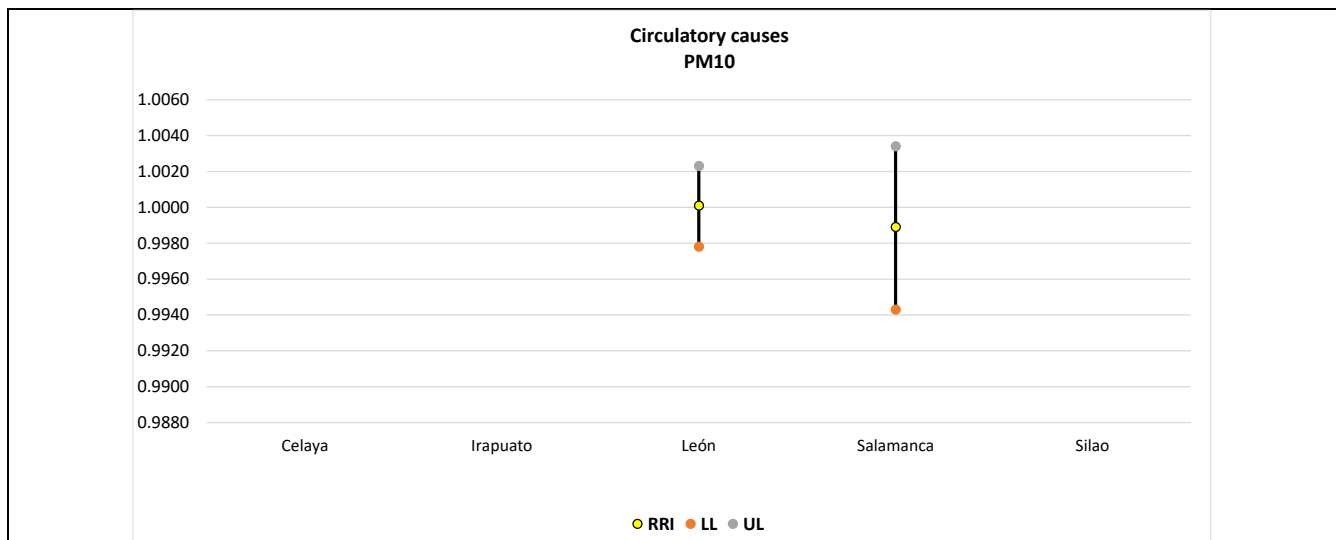
Source: Own elaboration

**Graphic 2.14** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for PM<sub>10</sub> considering morbidity by respiratory causes in the Bajío region in Guanajuato



Source: Own elaboration

**Graphic 2.15** Integrated information about relative risk index (RRI) and confidence interval (LL and UL) for PM<sub>10</sub> considering morbidity by circulatory causes in the Bajío region in Guanajuato

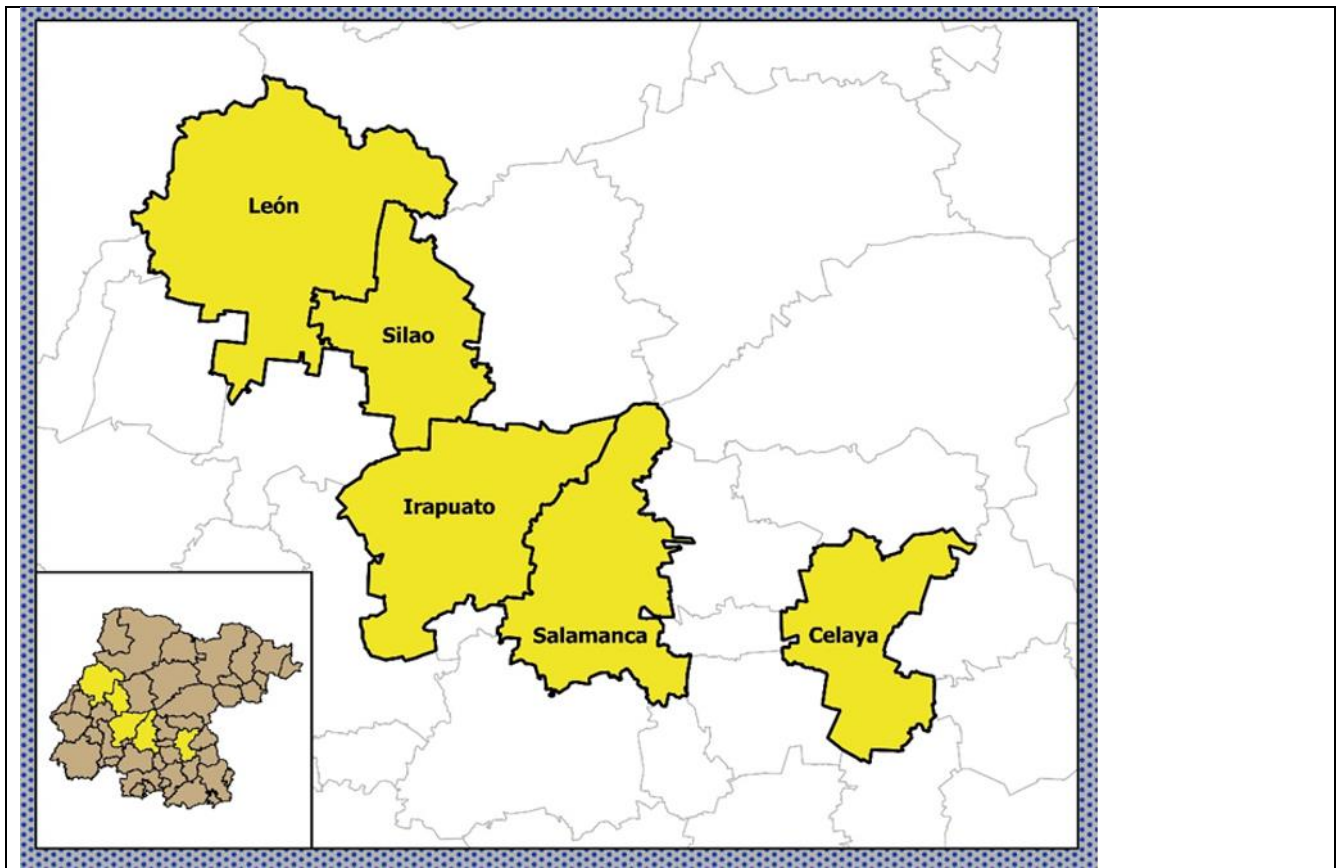


Source: Own elaboration

#### *Integrated Mapping of Relative Risk Index for each pollutant.*

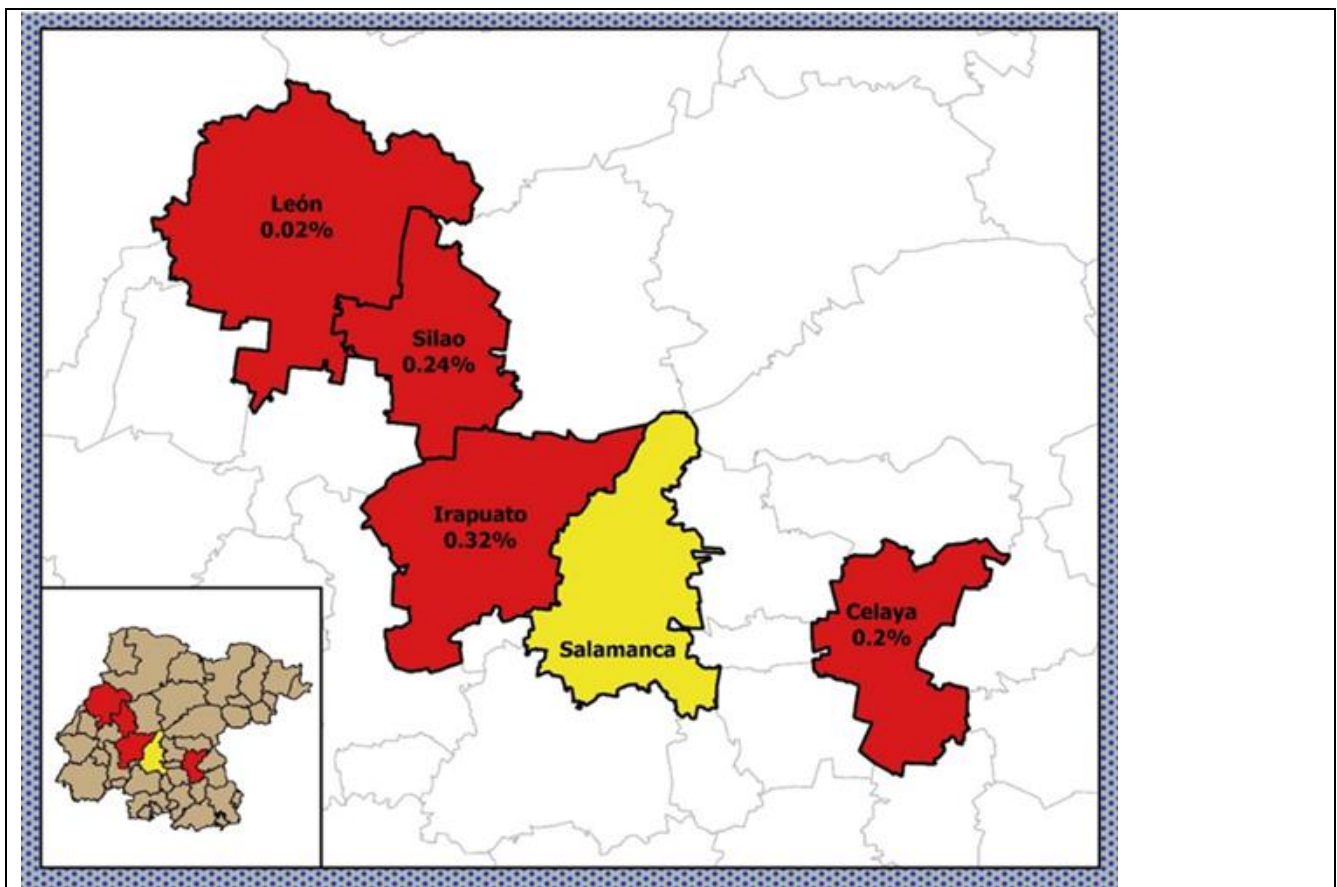
As can be observed in Graphic 2.16, an increase of 10% in the daily mean concentrations of ozone does not constitute a significant risk of morbidity in the municipalities studied, for this reason, all municipalities are in yellow, suggesting that there was a correlation, but this correlation was not significant. On the other hand, from Graphic 2.17, it can be observed that because of an increase of 10% in PM<sub>10</sub> daily mean concentrations, morbidity showed an increase in Irapuato (0.32%), Silao (0.24%), Celaya (0.20%) and León (0.02%). These municipalities are in red, suggesting that there was a correlation, and this correlation was significant. In the case of Salamanca, which is presented in yellow, indicates that there was a not significant correlation.

**Graphic 2.16** Integrated Mapping of Relative Risk Index (RRI) considering the hypothetical scenario in which O<sub>3</sub> concentrations increases in 10% in the Bajío region



Source: Own elaboration

**Graphic 2.17** Integrated Mapping of Relative Risk Index (RRI) considering the hypothetical scenario in which PM<sub>10</sub> concentrations increases in 10% in the Bajío region



Source: Own elaboration

## 2.4 Conclusions

Regarding to air quality, Irapuato, Silao and Salamanca presented the highest mean concentration values for O<sub>3</sub> during the all period. However, Irapuato showed the highest number of exceedances (884) to the maximum allowable limit established by NOM-020-SSA1-2021, followed by Silao (477) and Salamanca (53), respectively. It is to say, zones located in the middle part of the Bajío presented more photochemical pollution. With respect to PM<sub>10</sub>, Celaya, León and Salamanca presented the highest mean concentration values for PM<sub>10</sub> during the all period. All municipalities showed significant exceedances to the maximum allowable limit established by NOM-025-SSA1-2021; Celaya with 518, León with 281 and Salamanca with 210 exceedances, respectively. Comparing both pollutants, we can conclude that the pollution due to PM<sub>10</sub> is a hotspot in the Bajío region in comparison with pollution due to ozone.

Silao (2.36-2.91) and Irapuato (1.51-2.25) showed the highest morbidity rate, whereas, Salamanca (1.22-1.71) showed the lowest. It is important to mention that in spite of León had the highest values of morbidity, its morbidity rate was low. It was found some uniformity in the results for all municipalities studied, for example, the highest values of the relative maximum for daily morbidity were found during November, December and January. In addition, it was observed a marked and evident seasonality, with the number of hospital admissions by all causes higher during autumn and winter seasons. However, it was not observed any inter-annual trend.

Regarding to age sub-group. It was possible to identify people between 5 and 59 years as the population group with the higher number and frequency of admissions; whereas the sub-group of 60-74 years and >75 years showed the lowest. In terms of gender, in León and Irapuato the number of admissions registered was similar in both, women and men. On the other hand, in Silao and Salamanca, women showed greater hospital admissions in comparison with men. Finally, in the municipality of Celaya, the number of admissions was higher in men.

From relative risk indexes found in this study; we can conclude that an increase of 10% in the daily mean concentrations of O<sub>3</sub> does not constitute a significant risk of morbidity in the municipalities studied; however, the scenario was very different with respect to PM<sub>10</sub>, since when PM<sub>10</sub> concentrations were increased, the risk values also increased: Irapuato with 0.32%, Silao with 0.24%, Celaya with 0.20% and León with 0.02%.

Population between 0 and 59 years was identified as the most vulnerable age sub-group, suggesting that, the habits and activities of people played an important role in the exposure to these pollutants, since, people in this group comprises economically active population and students of all ages, who develop their activities outside home just in the hours in which O<sub>3</sub> and PM<sub>10</sub> reach their peak levels as a result of industrial activity and mobile sources. Therefore, decision makers can use reported data in this work to propose or improve regulations, programs or actions focused to protect population health between 0-59 years against atmospheric pollution effects in these municipalities.

## 2.5 Financing

This work has been financed by PNUD-INECC. Project No.: 85488. Sixth National Communication. United Nations Framework Convention on Climate Change.

## 2.5 References

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### Chapter 3 Consumption condition of Chihuahua type cheese: case study

#### Capítulo 3 Condición de consumo del queso tipo Chihuahua: estudio de caso

MIRELES-MEDINA, Antonia†\*, MIRELES-MEDINA, Elma Alejandra, MIRELES-MEDINA, Manuel Patricio and MIRELES-MEDINA, Jesús

*Empresa Villa Guadalupe and Tecnológico Nacional de México (Campus Zacatecas Norte) /Instituto Tecnológico Superior Zacatecas Norte*

ID 1<sup>st</sup> Author: *Antonia, Mireles-Medina* / **ORC ID:** 0000-0001-9773-9108, **CVU CONACYT ID:** 299436

ID 1<sup>st</sup> Co-author: *Elma Alejandra, Mireles-Medina* / **ORC ID:** 0009-0008-2651-1032, **CVU CONACYT ID:** 1292760

ID 2<sup>nd</sup> Co-author: *Manuel Patricio, Mireles-Medina* / **ORC ID:** 0000-0002-8179-9752, **CVU CONACYT ID:** 1064500

ID 3<sup>rd</sup> Co-author: *Jesús, Mireles-Medina* / **ORC ID:** 0009-0000-3258-1944, **CVU CONACYT ID:** 1299679

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A. Mireles, E. Mireles, M. Mireles and J. Mireles

\* mirelesmed\_7@hotmail.com

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## **Abstract**

This study consists of obtaining information regarding the conditions of consumption of Chihuahua type cheese in Río Grande, Zacatecas, México (analysis unit), due to the fact that currently in the municipality in question the information is scarce, so it is necessary to know what the characteristics are (age, sex, socioeconomic level, among others); your consumption and purchase habits; your knowledge of the competition, brands, etc.; in addition to their consumption and purchase habits. The background of the unit of analysis is described, as well as the study of current concepts and research regarding consumption conditions; the type of social, economic, cultural and academic repercussions. Also, a comparative analysis is made with another unit of analysis, which in this case is the colony La Honda, Miguel Auza, Zacatecas, Mexico, establishing the reasons that led to said confrontation. The final result is the profile of the consumer of Chihuahua type cheese in Río Grande, Zacatecas, México, prepared with the case documentation obtained from the analysis of the consumption conditions through the present study, which will support further investigations for the generation of marketing plans, strategies and tactics that will support the producers of Río Grande, Zacatecas, México. The purpose is to establish the bases of the analysis of the consumption condition that exists among the consumers of Chihuahua type cheese in the municipality of Río Grande, Zacatecas, Mexico from the comparative analysis.

## **Consumer profile, Chihuahua type cheese, Strategies, Consumption**

### **Resumen**

Este estudio consiste en obtener información con respecto de las condiciones de consumo del queso tipo chihuahua en Río Grande, Zacatecas, México (unidad de análisis), debido a que en la actualidad en el municipio en mención la información es escasa, por lo que es necesario saber cuáles son las características (edad, sexo, nivel socioeconómico, entre otras); sus hábitos de consumo y compra; su conocimiento de la competencia, las marcas, etc.; además de sus hábitos de consumo y de compra. Se describen los antecedentes de la unidad de análisis, así como el estudio de conceptos e investigaciones actuales con respecto a las condiciones de consumo; el tipo de repercusiones sociales, económicas, culturales y académicas. También, se hace un análisis comparativo con otra unidad de análisis que en este caso es la colonia la Honda, Miguel Auza, Zacatecas, México estableciendo los motivos que llevaron a efectuar dicha confrontación. Se encuentra como resultado final el perfil del consumidor de queso tipo chihuahua en Río Grande, Zacatecas, México, elaborado con la documentación del caso obtenida del análisis de las condiciones de consumo a través del presente estudio, el cual apoyará a posteriores investigaciones para la generación de planes, estrategias y tácticas de mercadotecnia que apoyarán a los productores de Río Grande, Zacatecas, México. La finalidad es establecer las bases del análisis de la condición de consumo que existe entre los consumidores de queso tipo chihuahua en el municipio de Río

## **Perfil del consumidor, queso tipo chihuahua, estrategias, consumo**

### **3.1 Introduction**

Markets today are more challenging and demanding, this leads companies or organizations to seek strategies that allow them to generate competitive advantages over others. (Vázquez, 2012) says that "today the most successful organizations in the world have reached a level of consumer satisfaction, transforming the entire organization to serve them and stay close to them. To achieve this goal, these companies have generated a consumer approach, where they have first determined what they want to be able to design, produce and market products or services with the highest quality and at reasonable prices". In the companies producing chihuahua-type cheese in the municipality of Río Grande, Zacatecas, Mexico, the product is made without having knowledge of the consumer profile, which means that they are less competitive in the market.

(Corona, Paz Gómez, & Camacho Gómez, 2016) mention that in México there are 3,724,019 family businesses throughout the country, of which 49.9% (1,858,550 are concentrated in commerce), services 36.7% (1,367,287) and manufacturing industries 11.7% (436,851) according to information from (INEGI, 2014). A family business is one that has been founded by a family member and has been passed on, or is expected to be passed on, to their descendants. The descendants of the original founder or founders will have ownership and control of the company. In addition, family members work and participate in the company (Mucci, 2008). On the other hand, the dairy agroindustry in Mexico takes on great importance not only because it produces products with nutritional and gustatory benefits for consumers, but also because of the economic value represented by the processing activity, its ability to generate and maintain employment (Gante & Cervantes Escoto, 2011).

Currently, in the municipality of Río Grande, Zacatecas, México there are producers of chihuahua cheese that are characterized by being family businesses, which are characterized by being founded by a family member, and has been transmitted or is expected to be transmitted to their descendants. Where the production activity has been learned and transmitted from generation to generation in an empirical way and is carried out by the members of the families of the producers, due to this situation, the production of is considered as a trade and a tradition.

The industrialization of chihuahua cheese in Río Grande, Zacatecas, México is smaller in scale, because they are family businesses where the lack of business strategies to be more competitive is evident, because, although there is a good product that they market in local establishments and there is a high demand in the regional market, there is a lack of business guidelines. As well, there are producers who have started their family business and due to ignorance of the market in relation to consumption, they have not managed to be competitive, a situation that has led them to failure.

As for the media, advertising and promotion, they are null since the producers make direct sales to the consumer and in local establishments, without seeking that their final consumer differentiates their product from other brands, ignoring consumption habits and how to achieve consumer loyalty towards their brand of cheese they produce.

It is the case that small producers in Río Grande, Zacatecas, México also face competition from transnational and national companies, who do have defined strategies and knowledge of the consumption condition of their market, such as Lala, Chilchota, Alpura, Sigma, as well as dairy producers in the Northern Region of the State of Zacatecas. who stand out with greater knowledge of the market of the dairy sector of chihuahua cheese and consumption factors, among which stand out to mention some Productos Lácteos Pomas S.A. de C. V., Derivados Lácteos Menonitas S.A. de C.V., Tres Estrellas, El Saucito and Quesería San Lorenzo.

The producers of Río Grande, Zacatecas, México do not know what the characteristics of the consumer are, that is, they do not know their age, where they live, when they buy, their sex, their socioeconomic level (purchasing power), among other important characteristics to take actions aimed at exploiting their market opportunities (Consumer Profile).

Therefore, it is important that the producers of Río Grande, Zacatecas, México have knowledge about the factors of consumption, in order to potentiate the dairy sector in the municipality, because these family businesses are generators of employment and contribute to the economic growth of the municipality, state and the country, since these companies give economic sustenance to the producing families, as well as at the same time generating jobs; thereby contributing to the development of the municipality.

As well as, setting precedents on the consumption condition of the dairy sector in the region of Río Grande, Zacatecas, México since there is little information with this sector and in recent years, in the municipality of Río Grande, Zacatecas, México the supply with respect to dairy has increased, thereby generating saturation in the market without there being a market penetration due to lack of knowledge of the consumption condition of the dairy product.

It is important to identify market opportunities, tastes, preferences and consumer profile. This will allow to potentiate the commercial opportunities of dairy products and at some point, position, create or exploit their competitive advantages in the market.

It is because of this, that it is necessary to look for the means or means that allow to take advantage of the commercial opportunities of the chihuahua cheese. However, due to the lack of information regarding the environment and the characteristics of the market, specifically the consumer, it is necessary to know and identify the profile of the consumer, first of all, due to its importance, the tastes and preferences of this and in this way know to whom to direct the efforts of the company.

From the academic point of view, this study represents a challenge, because it is intended to give a practical contribution, according to the knowledge acquired during vocational training and thereby contribute to the analysis of situations that arise day by day in the environment, as the production chain is analyzed, where the lack of business strategies are evidenced, Although you have a good product and there is a high demand in the regional market, there is a lack of business guidelines.

From the cultural point of view, this problem has an impact, since it can be said that the chihuahua-type cheese in the municipality of Río Grande, Zacatecas, has a deep territorial roots and recognition of consumers, because society registers the cultural and historical heritage of the producers and implicitly recognizes the transmission of the knowledge of its elaboration from generation to generation.

## **3.2 Objectives**

"The objectives must be clearly expressed to avoid possible deviations in the research process" (Sampieri, et al., 2014). Every project must have clear and concise objectives, as well as a defined guideline, which allow them to be measurable and above all achievable. Next, the general and specific objectives are stated, by virtue of the wide range of dairy products, the present work delimits the study to only one product, the chihuahua cheese of bovine milk, the above for being the product derived from the milk more produced in the region of Río Grande, Zacatecas, México as stated in this chapter.

### **3.2.1 General Objective**

The general objective "It should reflect the essence of the problem statement and the idea expressed in the title of the research project" (Bernal, 2006). The objective of this project is to:

1. To lay the foundations for the analysis of the consumption condition that exists among consumers of chihuahua cheese in the municipality of Río Grande, Zacatecas, México.
2. Determine the consumer profile of chihuahua-type cheese, in order to contribute to the improvement of the competitive position of chihuahua-type cheese producers in Río Grande, Zacatecas, México.

### **3.2.2 Specific objectives**

"The specific objectives are derived from the general one and must be formulated in such a way that they are oriented to the achievement of the general objective" (Bernal, 2006).

The specific objectives to be achieved in this study are mentioned below:

1. To determine the socioeconomic variables of the consumer of chihuahua cheese from bovine milk such as: age, sex, place of residence, academic level and occupation.
2. To determine the internal variables of the consumer of chihuahua cheese from bovine milk in the city of Río Grande, Zacatecas, México such as: reasons for purchase, perceptions and expectations.
3. Determine external variables of the consumer of chihuahua type cheese from bovine milk: reason why he buys a certain brand of cheese, influence groups, price, purchasing capacity, frequency of consumption.
4. Contribute to the rooting of customs and traditions, since chihuahua cheese represents one of the traditional dairy products, most consumed in the region of Río Grande, Zacatecas, México by preserving an artisanal production of the processed products.

### 3.3 Justification

The justification of all research consists in the exposition of its reasons (the why? and/or for what? of the study). All research is carried out with a definite purpose (Sampieri et. al., 2014), because as the authors mention it is not a whim of a person, since all research has a purpose; which is sufficient to carry out the investigation. For the above, the reasons for this study are subsequently written:

Dairy producers in Río Grande, Zacatecas, México face a great challenge to be competitive in the face of the globalized economy and the changing desires of the market. Reasons that create the need to maintain a competitive advantage in order to maintain their market share. That is why we are interested in analyzing and studying alternatives to improve the satisfaction of its consumers.

According to (Economía, 2012) the dairy industry is the third most important activity in Mexico within the branch of the food industry, after corn and meat; However, it has shown a higher growth rate in recent decades. Today there is an increase in the concentration of the population in Río Grande, Zacatecas, which causes an increase in the demand for a variety of foods, among which are milk and its derivatives.

For this reason, it is of great importance to know the needs of consumers of dairy products, in order to satisfy them through quality products that meet the expectations of consumers, which will serve to guide the production of the company. The supply of dairy products in Río Grande, Zacatecas, México has been increasing, causing competitiveness among its producers, who often do not know market strategies with which they can be more competitive against their competition, such as knowing the consumer profile of dairy products. The increase in the supply of dairy products in Río Grande, Zacatecas, México is due to population growth, and the lack of opportunities in the labor market, because in search of opportunities by families to have an economic livelihood, small dairy companies are founded, in order to generate their economic sustenance for their family (self-employment); therefore the economic and social importance of this study, because by determining the consumption status of dairy products will be providing information to dairy producers in Río Grande, Zacatecas, México who often only produce their products considering having an income to support the needs of their family, and not having knowledge of the profile of their consumers have not managed to position their product, since not knowing their market end up disappearing.

This research will serve to set a precedent and obtain information on the consumer of dairy products in Río Grande, Zacatecas, México and with them the producers of dairy products, have elements to make decisions regarding the dairy products offered in the market, since dairy producers in Río Grande, Zacatecas, México require more momentum for their growth, otherwise, they can disappear at any time in the face of well-established competition. It is essential to obtain this information for the identification of elements for the generation of marketing plans, sales among others and in this way give a greater boost to the activity, elaboration and commercialization of dairy products.

With this study we will have information on the market and the consumer of dairy products, since there is no current and much less published information in the municipality of Río Grande, Zacatecas. In case of not obtaining this information and in addition to not having sufficient elements to face industrialized competition and if it continues with ignorance of the consumer, it will surely have a failure in the short or medium term. For this reason, we want to address the issue of the consumer profile of dairy products, this being the object of study.

That is why the present study of analysis of the condition of consumption of dairy products of Río Grande, Zacatecas, México aims to strengthen the market through the knowledge of strategies that face the global and competitive environment, and thus contribute to the development of the economic, social and cultural environment of the region of Río Grande, Zacatecas, México since this sector retains a deep territorial roots and recognition of consumers, since society registers the cultural and historical heritage of the producers and the transmission of the knowledge of its elaboration from generation to generation is implicitly recognized.

### 3.4 Frame of reference

#### *Cheese*

The milk of domestic mammals has been an important part of man's food since ancient times. This has been processed into other products that are also relevant in human consumption, one of them is cheese.

The origins of cheese making will never be known with certainty, but it is known that the cheese was consumed in Asia. It was known to the ancient Egyptians, Greeks and Romans. In the first great civilization of which there are detailed indications (Egypt, 4000 years before our era (ANE)), dairy farming arises to reach a high degree of development. In the history of Babylon, 2000 years ANE, reference is made to milk, butter and cheese. In the hymns of the Vedas in India, en el periodo 3000-2000 años ANE, se proclamaba que el matar una vaca era un pecado y se refiere al uso de la leche y la mantequilla como alimento (INAES, Quesos, s.f.). "There is a great variety of cheeses and each class has a specific production technology" (Luna, 1981). For the purposes of this document, chihuahua cheese is considered.

#### *Definition of chihuahua cheese*

"It is the product obtained from pasteurized whole cow's milk subjected to coagulation processes, cut, drained, fermented, salted, pressed and matured for a minimum period of 7 days at controlled temperature and humidity; without fats or proteins not derived from milk having been used in its preparation" in accordance with the Mexican Standard NMX-F-209-1985.

#### *Characteristics of chihuahua cheese*

Likewise, with respect to the Mexican Standard NMX-F-209-1985, the following characteristics are identified that chihuahua cheese must have, within the sensory characteristics are considered the color, which must be white or slightly yellow; the taste, characteristic free of strange flavors; the smell, free of strange odors and the consistency, semi-hard and slicable.

And as physical, chemical and microbiological characteristics it is said that it must not contain pathogenic microorganisms, microbial toxins, and microbial inhibitors or other toxic substances that may affect the health of the consumer or cause deterioration of the product, therefore it must comply with the following physical and chemical specifications as indicated in Table 3.1:

**Table 3.1** Physical and chemical specifications of chihuahua cheese

Specifications	Minimal	Maximum
Humidity in %		45.0
Fat (Butyric), %	26.0	
Protein of lactic origin, in %	22.0	
Total solids, in %	55.0	
pH	5.0	5.5
Total ash in %		6.5
Sodium chloride in%		3.0

Source: Own elaboration of data obtained from the Mexican Standard NMX-F-209-1985

In addition to the physical and chemical specifications, Chihuahua cheese must meet the following microbiological specifications as shown in the Table 3.2:

**Table 3.2** Microbiological specifications of chihuahua cheese

Specifications	UFC/g Maximum
Coliformes	10,000
<i>Staphylococcus aureus</i>	100
<i>Escherichia coli</i>	1,000
<i>Salmonella</i> en 25 g	Negative

Source: Authors, data consulted in the Mexican Standard NMX-F-209-1985



### Additives for chihuahua cheese

The additives allowed by the Ministry of Health and Assistance according to the Mexican Standard NMX-F-209-1985, are:

1. Lactic cultures
2. Sodium chloride
3. Vegetable or animal rennet
4. Annatto (annatto seed and carotene in proportion with greater than 0.06%).
5. Calcium chloride (CaCl<sub>2</sub>) in a proportion not greater than 0.02%.
6. Sorbic acid or its sodium or potassium salts 1 g/kg.
7. Sodium or potassium nitrate in proportion not greater than 0.03%.
8. Pimaricin in a maximum concentration of 300 mg/kg in the solution.

Another important aspect with respect to cheese is consumption, this occurs at all socioeconomic levels, and reaches the amount of 2.8 kg per capita per year average according to (Huezo & Durán Lugo, s.f.).

In México, according to figures from the ( Secretaría de Agricultura y Desarrollo Rural, 2019) that the annual production of bovine milk in the period from 1999 to 2018, is as shown in the Table 3.3.

**Table 3.3** Annual production of bovine milk

Year	Production	Annual growth (%)
2018	12,008,239	2.0
2017	11,767,556	1.4
2016	11,608,400	1.9
2015	11,394,663	2.4
2014	11,129,622	1.5
2013	10,965,632	0.8
2012	10,880,870	1.5
2011	10,724,288	0.4
2010	10,676,695	1.2
2009	10,549,037	-0.4
2008	10,589,481	2.4
2007	10,345,982	2.6
2006	10,088,550	2.2
2005	9,868,302	0.0
2004	9,864,302	0.8
2003	9,784,355	1.3
2002	9,658,279	2.0
2001	9,472,293	1.7
2000	9,311,444	4.9
1999	8,877,314	6.8
1998	8,315,711	6.0
1997	7,848,105	3.4
1996	7,586,422	2.5
1995	7,398,598	1.1
1994	7,320,213	-1.1
1993	7,404,078	6.3
1992	6,966,210	3.7
1991	6,717,115	9.4
1990	6,141,545	10.1

Source: Authors, information consulted in (Secretaría de Agricultura y Desarrollo Rural, 2019).

From the table above, it can be noted that the dairy sector in the last decade year after year, has shown a growth in its production, since for the year 2018 the production of bovine milk was 12,008,239 with 2% compared to 2017.

At the end of 2018, the production of dairy derivatives and ferments such as cheese, cream and yogurt, reached a volume of one million 150 thousand tons, with a value of 52 thousand 104 million pesos. For its part, the cheese industry produced 418 thousand 650 tons with a market value of 21 thousand 293 million pesos as can be seen in the Table 3.4:

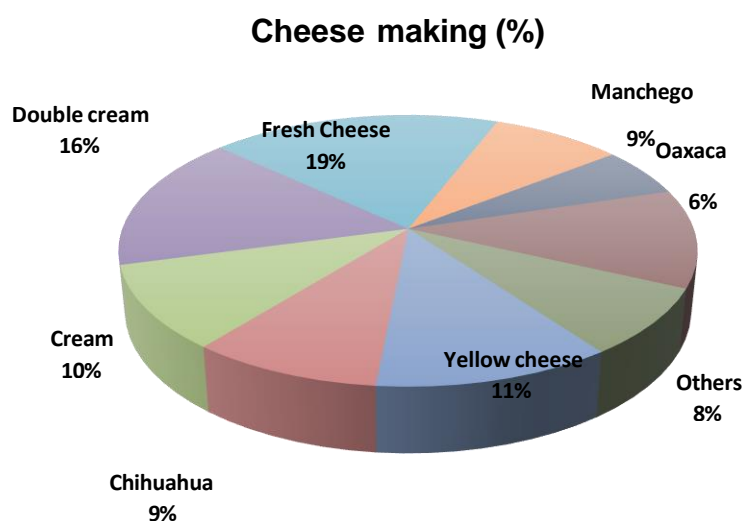
**Table 3.4** National cheese production

Year / Month	Cheese making (Tons)									Total
	Yellow	Chihuahua	Cream	Double cream	Cheese Fresh	Manchego	Oaxaca	Panela	Other	
2018	48,254	38,625	41,965	67,597	79,279	35,799	24,427	48,947	33,757	418,650
January	4,047	3,438	3,943	5,188	5,590	3,075	2,097	3,887	2,465	33,730
Febr	3,675	3,231	2,809	5,138	5,970	2,839	1,921	4,030	2,599	32,212
March	4,068	3,405	3,458	5,682	6,565	2,960	2,112	4,334	2,996	35,580
April	4,168	3,353	3,470	5,513	6,329	2,948	2,036	4,009	2,799	34,625
May	4,016	3,296	3,449	5,640	6,613	3,195	2,059	4,208	2,821	35,297
June	3,848	3,253	3,663	5,593	6,475	3,109	1,951	4,240	2,819	34,951
July	4,039	3,303	2,722	6,089	7,042	2,806	2,056	4,159	2,857	35,073
August	4,080	3,199	3,575	5,743	6,795	2,827	2,154	4,085	3,016	35,474
September	4,014	3,010	3,342	5,568	6,664	2,864	1,959	4,214	2,906	34,541
October	4,122	2,949	4,306	5,791	6,891	2,990	2,002	4,003	2,869	35,923
November	4,104	3,045	3,526	5,453	6,594	2,864	1,981	3,847	2,793	34,207
December	4,073	3,143	3,702	6,199	7,751	3,322	2,099	3,931	2,817	37,037

Source: Authors, information from the (Secretaría de Agricultura y Desarrollo Rural, 2019)

Graphic 3.1 shows the share of cheese production by type, for the period January-December 2018 according to the analysis of the data in the table above, which shows that Chihuahua-type cheese represents nine percent with respect to other cheeses.

**Graphic 3.1** Share of cheese production



Source: Authors, information from the (Secretaría de Agricultura y Desarrollo Rural, 2019).

"In México, dairy products such as cheeses and yogurts, as well as industrialized milks: pasteurized, ultra-pasteurized and powdered, occupy the first places of commercialization, manifesting a tendency towards the supply of urban areas, since these have accessible communication routes and concentrate groups with higher income levels. in contrast to non-urban areas, where dairy consumption is mainly limited to raw milk and artisanal products" according to (Ministry of Economy, 2012).

(Castro Castillo & others, 2013) mention that cheese production in the country is an important activity within the food industry; (Almanza-Rubio et al., 2013) They say that "the lack of a denomination of origin that protects Chihuahua cheese has motivated the large cheese factories in México and abroad to imitate the cheese produced by the Mennonites."

Chihuahua cheese is made with whole cow's milk with a minimum fat content of 3%, standardized, pasteurized and added lactic ferments such as *Streptococcus lactis* and *Streptococcus cremoris*. Its elaboration requires the use of pasteurized milk, management of lactic cultures, additives such as calcium chloride and potassium nitrate, pressing and maturing of the product according to (Montañez et al., 2006).

### *Consumer definition*

The consumer according to (Hernández & Méndez, 2020) is a person who satisfies one of his needs by using until its end and destroying a product or a good. It is the one who has the purchasing power (money) to be able to buy or acquire goods and services (food, electricity, clothing, etc.). Each existing person is a consumer, who has different tastes and preferences according to each of their personal characteristics that they have been acquiring or were becoming part of themselves as time went by from birth to death.

### *Types of need*

(Soto Dueñas & Mafaldo Rengifo, 2018) consider human needs to be states of perceived lack. These can be of different types:

1. Physical needs: food, clothing, warmth and security.
2. Social needs: these refer to affection and belonging to a group.
3. Individual needs: these are those of knowledge and oral expression.

### *Concept of desire*

As they point out (Kotler & Armstrong, 2013) desires are forms that human needs take, which have been determined by: culture, the individual personality of the consumer and the society to which he belongs. In a more concrete and clear way, it can be said that they are objects that manage to satisfy needs, which become demand when there is a financial capacity to acquire these objects. It is said that by acquiring objects that can satisfy needs, the consumer obtains various benefits thanks to the attributes that these objects have, which causes the consumer to give him a greater or lesser value and satisfaction.

### *Demand*

There are several criteria when defining demand, according to marketing experts it is a preponderant factor in the life of companies, so for (Kotler & Keller, Marketing Management, 2012) it is a human desire that is determined by a specific purchasing power.

According to (Fischer & Espejo, 2011) demand refers to the amount of product that consumers are willing to buy at possible market prices.

### *Consumer behavior*

To talk about consumer behavior, reference must be made to customers called final consumers, that is, those who use the product at the end of the day. End customers are those who acquire goods and services for their personal consumption. This group of consumers is called the consumer market. All consumers, around the world vary according to their age, income, educational level and tastes, therefore, depending on each of these aspects their behavior of each one is different. Taking into account (Kotler & Keller, Marketing Management, 2012) the analysis of consumer behavior is the study of how individuals, groups and organizations choose, buy, use and dispose of goods, services, ideas or experiences to satisfy their needs and wants. In addition, (Delgado Estrada et al., 2018) mention that consumer buying behavior is influenced by cultural, social and personal factors.

(Arteaga Cabrera & Herrera Vargas, 2018) taking into account Jorge Elicier address the classification of consumer behavior according to four theories:

1. Psychological theories: These theories indicate that consumer behavior is dominated by psychological motivations, instinctive impulses, and other emotional and physiological issues with little relation to rational or logical aspects.
2. Learning theories: For these theories, consumers choose based on previous consumer experiences without taking into account the price of products as is the case with consumers with brand loyalty (for example, consuming only Adidas sneakers).
3. Sociological theories: This set of theories indicates that what defines consumer behavior is mostly determined by the pressures of context and other social relations that lead the individual to act according to dominant social standards. For example, consumption for the purpose of belonging to a community or following a fashion.
4. Economic Theories: These theories are based on the assumption that consumers seek to achieve their maximum level of personal satisfaction of a good or services conditional on their purchasing power or budget constraint.

(Arteaga Cabrera & Herrera Vargas, 2018) from the point of view of Schiffman and Kanuk, the four sets of theories provide different perspectives to explain consumer behavior that can be used to have a better understanding of the purchasing decisions of individuals. On the side of psychological theories there are several aspects to highlight. The authors propose motivations and personality as an important psychological component of the formation of consumer behavior. Motivation is understood as all those forces within an individual that make him decide to take a particular action. Generally, this inner force occurs as a result of a need that has not been fully satisfied.

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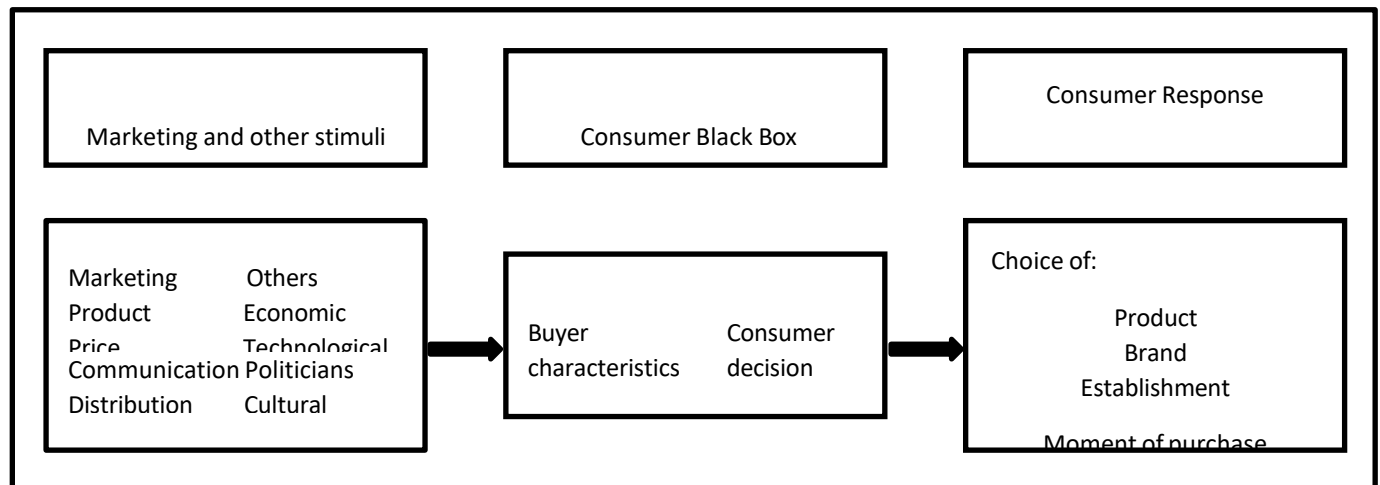
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#### *Factors influencing consumer decisions*

To understand a little about the decision-making that a client presents, (Kotler & Armstrong, 2013) suggest that it is necessary to take into account the aspects that affect the purchase decision of a consumer, which are highlighted in Figure 3.1.

**Figure 3.1** Consumer behavior model



*Source: Own elaboration (2023), information consulted in (Kotler & Armstrong, Fundamentals of Marketing, 2013)*

From the previous model, it can be identified that the consumer's purchase decision is influenced by different aspects, of which some of them can be noticed in the previous figure, are denominated as marketing stimuli and others such as marketing, the physical product itself (colors, flavors, presentations, etc.); the price (whether it is cheap or expensive compared to the competition); its advertising (both of the brand that is to be chosen and that of the competition), whether or not there is a purchase of it (distribution); whether you have the purchasing power (economic stimuli); whether it is the most current or modern version of the product (technological stimuli); or even if the product is used normally or if it is used or known to use or consume (cultural stimuli).

However, the existence of these stimuli intervene the characteristics of the consumer such as age, sex and socioeconomic level, all this causes him to start and be affected his purchase decision, to finally be able to reach an end in his reasoning. In addition, the consumer's purchase decision is also affected by various factors among which are aspects related to the culture acquired or taught, the society in which it operates, personal aspects and psychological aspects that are part of each of the consumers.

### *Culture*

Citing (Fischer & Espejo, 2011) culture is considered as the representation of a series of factors such as: knowledge, beliefs, values, art, morals, laws, customs and habits acquired by man as a member of a society.

Because this aspect varies from country to country, as well as from neighborhood to neighborhood, it is considered a more complex aspect than it seems, therefore, it is necessary that companies adapt to these situations and aspects, so you must be aware of the changes that are suffered in this aspect since consumers can modify tastes, Wants, preferences and needs in consequence of cultural changes.

### *Subculture*

As expressed (Kotler & Armstrong, 2013) each culture has small cultural groups inside, that is, groups that share common values and experiences, so they include: nationalities, religions, racial groups and geographical regions. In the same way, we must be attentive to these aspects since they are part of the characteristics of the consumer and therefore of his purchase decision.

### *Social class*

As noted (Kotler & Armstrong, Fundamentals of Marketing, 2013) the members of this relatively permanent and orderly division of society share similar values, interests and behaviors (social stratification).

In the case of Mexico, it is determined and identified as socioeconomic level, which is made up of groups of people with similar characteristics to each other, and according to the (Mexican Association of Market Intelligence and Opinion Agencies (AMAI), 2018), there are 7 socioeconomic levels considering the following six characteristics of the household:

1. Schooling of the head of household.
2. Number of bedrooms.
3. Number of full bathrooms.
4. Number of employed persons aged 14 and over.
5. Number of cars.
6. Internet tenure.

### *Family*

As they say (Kotler & Keller, 2012) the family is the most important consumer purchasing organization in society, and its members constitute the reference group with the greatest primary influence.

This group can have a great influence on the consumer's purchasing decision. Each member of the family can have a different influence on buyers, this depends largely on the product or product category that is intended to buy, as well as the changes that are taking place in the lifestyle of each of the members.

They point out (Fischer & Espejo, 2011) that there are two special situations in the family, but very common in the Mexican family: the extended family and the composite family. The extended family is one that has in its bosom, in addition to father, mother and siblings, a blood relative, it can be a cousin who came to study in the city, or the beloved grandmother. The composite family is one that has within its bosom a friendship living temporarily, it can be the godson who came to try his luck in the city and while he is placed he lives in the house of the godparents.

### *Personal factors*

Based on (Kotler & Keller, Marketing Management, 2012) purchasing decisions will also be influenced by issues such as age, the phase of the life cycle that the consumer is living, profession, economic situation, lifestyle, personality and self-concept.

### *Lifestyle*

Kotler & Armstrong, (2013) argue that lifestyle is the pattern that follows his life of a person, expressed according to his psychographics, that is, considering activities, such as: work, entertainment, shopping, sports, social activities; interests such as food, fashion, family, leisure, etc.; opinions about themselves, social and commercial matters and products.

### *Psychological factors*

In the words of (Kotler & Armstrong, 2013) a person's purchasing choices are further influenced by four psychological factors: motivation; perception; learning and beliefs; and attitudes. Those described below:

#### *Motivation*

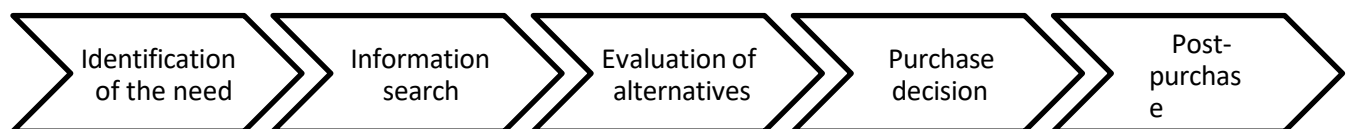
Everyone has needs, these can be biological, due to states of tension such as hunger, thirst or some type of discomfort, some are psychological due to the need for recognition, appreciation or belonging. He states (Kotler & Armstrong, (2013) that a need can become a motive when the intensity is sufficient. Pursuing a need on the part of a person is when the need is compelling enough and therefore is a motive. Mention (Kotler & Armstrong, 2013) that the theories about the needs of Abraham Maslow and Sigmund Freud are the best known. That is why only his theories are addressed below:

1. Sigmund Freud said that people are not aware of what motivates them to behave in one way or another, in the same way he thought that each person suppressed various impulses, which causes these to grow which were not eliminated only "kept a little in control", but emerged in dreams, that is, in lapsus linguae, in neurotic and obsessive behaviors, that is, in psychosis. So a person doesn't really understand his own motivation.
2. The techniques to study the motivation of a consumer can range from completing sentences, interpreting ink stains, to describing the typical users of a brand or creating a family about brands or purchase situation.

### *The purchase decision - process*

The purchase decision process may vary depending on the author, however, according to (Kotler & Armstrong, Fundamentals of Marketing, 2013) this process basically consists of five phases as shown in Figure 3.2:

**Figure 3.2** Purchase decision process



*Source: Own elaboration (Kotler & Armstrong, 2013)*

In normal purchases, consumers skip some of the phases or change the order. Each of the points mentioned above in the figure is described in the following points.

### *Definition of segmentation*

As expressed (Kotler & Armstrong, Fundamentals of Marketing, 2013) is called the division of market into smaller groups of buyers with common needs, characteristics or behaviors that might need specific products or marketing combinations, that is, similarities and combinations between age, sex, purchasing power, lifestyle, etc. Taking into account (Kotler & Armstrong, 2013) the four main steps of designing a customer-oriented marketing strategy are the following:

1. **Market segment.** It is a group of consumers who respond in a similar way to a given series of marketing stimuli, that is, their reactions to advertising, samples, etc. are similar between one consumer and another because their characteristics (age, sex, attitudes, etc.) are the same or very similar to each other.
2. **Target audience.** It is the process through which the evaluation of the attractiveness presented by each market segment and selection of one or more segments to which the company is going to be directed is carried out, that is, it is that group of consumers to whom the product is directed and that have characteristics well defined by the company.
3. **Positioning.** It is the establishment of the competitive position of the product and creation of a detailed marketing mix or, in other words, it is the place that a brand occupies in the mind of the consumer.
4. **Competitive advantage.** It is that advantage that you have over competitors that is obtained by offering consumers a greater value, for example: through low prices or generating greater benefits that justify higher prices. In short, they are those attributes or benefits that distinguish a product above all others, especially from its direct competition.

### *Market segmentation*

(Foullon Inzunza, 2020) citing (Bonta, 2016) mentions that "Market segmentation can be called the way in which an organization divides the market, determining this in small portions, taking into consideration the characteristics that help the organization to comply with all the proposed plans, when the respective segmentation process is carried out, all the resources can be directed talking about marketing focused on that segment and with this obtain information from customers".

### *Consumer market segmentation*

Taking into account (Kotler & Armstrong, 2013) there is no single method to segment a market, so the variables must be identified, independently or together, to find the best way to visualize the structure of the market. Because of this, we can talk about four main groups to be able to segment: geographically, demographically, psychographically and behaviorally. The seven socioeconomic levels according to AMAI (Mexican Association of Market Intelligence and Opinion Agencies) are described below in Table 3.5.

**Table 3.5** Socioeconomic levels according to AMAI

<b>A/B</b>	The socioeconomic level A / B is made up mostly of households in which the head of the family has professional or postgraduate studies (82%). 98% of households have fixed internet in the home. It is the level that invests the most in education (10% of spending) and the one that devotes the least to spending on food (28%).
<b>C+</b>	87% of households at this level have at least one transport vehicle and 93% have access to fixed internet in the home. In relation to expenditure, just under a third (32%) is spent on food purchases and 28% on transport and communication.
<b>C</b>	83% of households at this level are headed by a head of household with primary education and 77% have a fixed internet connection in the home. Of the total expenditure in these households, 35% is devoted to food and 7% to education.
<b>C-</b>	About three out of four households (74%) at this level have a head of household with a higher education than primary school. Just over half (52%) have a fixed internet connection in the home. In relation to spending, 38% is dedicated to food and spending on transport and communication reaches 24%.
<b>D+</b>	In just over 6 out of 10 households at this level (62%), the head of household has education beyond primary school. Only 22% of households have a fixed internet connection in the home. Spending on food increases to 42% and spending on education is 7%.



<b>D</b>	In 56% of households at this level, the head of household has education up to primary school. Internet access in the home in these households is very low, only 4%. About half of the expenditure (46%) is dedicated to food and only 16% to transport and communication.
<b>E</b>	The vast majority of households at this level (95%) are headed by a head of household with education up to primary school. The possession of fixed internet in the home is practically zero (0.2%). Just over half of household expenditure (52%) is spent on food and only 11% is used for transport and communication, a percentage similar to that allocated to housing.

Source: Own elaboration according to (Mexican Association of Market Intelligence and Opinion Agencies (AMAI), 2018)

The determination of a social class, is not limited to income, also intervene: education, material possessions, characteristics of the home, among others. For this reason, each social class has differences between their tastes and preferences for products, brands and services.

Results: Conditions of consumption of cheese and its dairy products according to research (Kotler & Gary, 2001) the consumer profile "is the starting point to understand the buyer, it is the stimulus-response model. Environmental and marketing stimuli enter the buyer's consciousness, the buyer's characteristics and the decision process lead to certain purchasing decisions. The role of the marketer is to understand what happens in the buyer's consciousness between the arrival of the external stimulus and the buyer's purchasing decisions."

In addition, they mention (Kotler & Gary, 2003) that the factors that influence consumer behavior are: cultural (culture, subculture, social class); social (reference groups, family, roles and status); personal (age, stage of the life cycle, occupation, economic situation, lifestyle and personality) psychological (motivation, perception, learning, beliefs and attitudes).

Likewise, (López-Díaz & Martínez Ruiz, 2018) point out that the variability in the physicochemical parameters of Chihuahua cheese affects its sensory profile and with it, consumer preferences, which can determine the acceptance or rejection of the product against the consumer

**Table 3.6** Conditions of consumption obtained from research

<b>Who buys?</b>	<ul style="list-style-type: none"> <li>- Cheese is regularly consumed by both men and women.</li> <li>- Emphasizing women are those who regularly occur to make purchases for household consumption.</li> <li>- Regarding the activity carried out by people who buy, it was found that employees, housewives, students, professionals, teachers, activities in the field, so it can be said that cheese is consumed by the majority of the population.</li> <li>- When making consumption strata based on the income of the economically active population, the stratum with the highest purchasing power shows low consumption for medical reasons and the stratum with the lowest purchasing power does so for economic reasons.</li> </ul>
<b>Where do you buy?</b>	<ul style="list-style-type: none"> <li>- Department or self-service stores, but cheese belongs to large companies (strata with higher income).</li> <li>- Stores specializing in the sale of dairy products (strata with higher income).</li> <li>- Market.</li> <li>- Directly to the producer (cheese factories).</li> <li>- Grocery stores (the lowest strata).</li> </ul>
<b>What brand do they buy?</b>	<ul style="list-style-type: none"> <li>- The most consumed is the type and brand of cheese of the region.</li> <li>- Followed by nationally recognized brands.</li> <li>- The strata with greater purchasing power include in their diet more refined (mature) cheeses.</li> </ul>
<b>Factors they take at the time of purchase. Mentioned according to their degree of importance.</b>	<ul style="list-style-type: none"> <li>- Flavor (flavor is given by the physicochemical profile and sensory profile of the cheese).</li> <li>- Price.</li> <li>- Recognized brand.</li> <li>- Container.</li> <li>- Availability.</li> <li>- Properties and reliability.</li> <li>- Hygiene.</li> <li>- Nutritional property.</li> <li>- Caducity.</li> </ul>

<b>How often and how much do you buy?</b>	<ul style="list-style-type: none"> <li>- Consumption is daily, twice a week, and once a week. The purchase is on average, weekly and is acquired around half a kilo because 36% of respondents indicated that they buy the product, while 34% acquire in the same period less than one kilo and 28% of the people surveyed from one to two kilos per week.</li> <li>- The amount you buy is given by the frequency of purchase.</li> </ul>
<b>Consumption use</b>	<ul style="list-style-type: none"> <li>- It is used in a wide variety of dishes, either more elaborate or in those that are prepared quickly or served to calm cravings. The main prepared dishes that require cheese, according to the survey are: enchiladas, quesadillas, chiles rellenos, burritos, etc.</li> </ul>

The Honda Mennonite, is conformed, by thirty-four Mennonite camps, integrated by more than seven thousand inhabitants; and that among its main activities highlights agriculture, livestock, industry and commerce, among others (Bergen, 2015). The livestock to be one of the most important in the colony of the Honda, Miguel Auza, Zacatecas, México is distinguished by the raising of cattle of bovine milk, where the production is stabled, because in each household there is its small stable, taking advantage of the competitive advantage of being producers of fodder for the sustenance of their stables. Derived from the raising of dairy cattle, it is necessary that the Mennonites are producers of milk, and that, in search of commercializing and giving added value to milk, said production is destined to cheese factories producing Chihuahua cheese that are located within the Mennonite fields, which are part of this case study. where the condition of consumption of Chihuahua cheese is identified, among which the following currently stand out:

Pomas company: According to information obtained from (Pomas dairy products, 2015), Pomas is a company specialized in the production of high quality cheeses, our wide range of products, as well as more than 50 years of experience in the market. Legally constituted company, with logo and brand recognized in the region of origin of Mennonites. The production starts from the breeding and feeding of the cattle, making a selection and rotation of this, ensuring with this, a high quality raw material. The Las Pomas stable is located in Campo 20, today this stable has 7,368 heads, milking 2,875 and a total of around 140 workers, the majority of Mexican origin, from the surrounding ranches. It has the most advanced technology and highly qualified personnel trained in quality and hygiene standards. The distribution of this producer is wholesale. It processes around 50,000 liters of milk daily. Within its production are the following products:

1. Pomas: Chihuahua cheese.
2. Pomas: Cheddar type.
3. Pomas: Grill.
4. El Trébol: Chihuahua cheese.
5. La Maravilla: Chihuahua type.
6. Grienthal: Chihuahua cheese.
7. La Carreta Menonita: Chihuahua cheese.
8. El Arcon: Chihuahua cheese
9. Del Campo: Manchego cheese.
10. Universal: Chihuahua cheese
11. Fiesta: Chihuahua cheese.
12. Yogurt
13. Crema Pomas
14. Base for ice cream, aguas frescas and Pomas popsicles

The cheese is marketed mainly in the following cities: Torreón, Coahuila; Durango, Durango; Ciudad Juárez, Chihuahua; Monterrey, Nuevo León; Mexico City; with a distribution branch in each of them. The brands of the line of cheeses distributed in Monterrey, Nuevo León are: Grienthal, la Maravilla, del Campo, Pomas Asadero, Pomas Cheddar, Mennonite cheese, Carreta Menonita distributed mainly in Soriana stores (Pomas dairy products, 2015). As well as it is distributed in shopping centers and department stores in the State of Zacatecas. It offers its products on an internet platform, such as Mercado Libre. It segments its market, focusing each of its products, to a different market, such as:

1. Cheese "Pomas" type chihuahua. It is the cheese marketed in the municipality of Rio Grande, it is the chihuahua type pomas cheese, which is distributed in establishments specialized in the sale of dairy products (creameries), in presentation of bar of 1.3 kilograms. Brand that is also distributed by internet companies as a free market; and distributed in department stores and self- service as Chedraui.
2. "Carreta Menonita" type chihuahua. It is aimed at the consumer of department stores and self-services, with different product quality and at different prices, such as Chedraui, Wal-Mart, etc.
3. Quesería Derivados Lácteos Menonitas. According to the information of the portal (Derivados lácteos mennonitas, s.f.), derivados lácteos mennonitas is a producer of Mennonite origin, bring the authentic Mennonite flavor of the north of the country to the entire Mexican Republic, offering quality products that help its customers to differentiate themselves in their businesses or tables by the taste and quality of their products; Producer of dairy products that is legally constituted as a company, its products are distributed wholesale, with distribution channel also, to the final consumer.

It had its beginnings in the year of 1970, with the Dyck family that was dedicated to the elaboration of cheeses at home. Over the years and with the increase in production, Don Cornelio Dyck Thiessen decides to take charge of the business. In its beginnings the small company only produced chihuahua- type cheeses with the magical touch of the Mennonite tradition, which was simply called "La Quesería". In 1980 it was until when Don Cornelio (always with a vision of growth and drive), ventures into the great dream of forming a corporation; this is how Productos Lácteos was born. In 2009 After more than 25 years of constant struggle, for strategic reasons we changed our corporate name to Derivados Lácteos Menonitas S.A. de C.V. name that it retains to date (Derivados lácteos mennonitas, s.f.).

Derivados Lácteos Menonitas S.A de C.V., located in field 5 of Colonia la Honda, in its beginnings the production was about 500 kilos of cheese per day, today its production is (8) eight to (10) ten tons per day, in its beginnings three employees worked, while today they currently deal with eighty to (120) one hundred and twenty employees (Derivados lácteos mennonitas, s.f.).

The production is not focused on a single product, since among its range of products are the following (Derivados lácteos mennonitas, s.f.).

The raw material is not produced by the producer, as it is compared to the various Mennonite camps of the Honda. It has distribution branches in Mexico City, Monterrey, Nuevo León and Gómez Palacio, Durango. And its products are focused on customers who have food establishments, since its market is focused on the production of raw materials from restaurants or food establishments, also having brands focused on the final consumer (Derivados lácteos mennonitas, s.f.). Cheese factory the Honda. (Bergen, 2015) in its magazine History of the "Mennonites in Zacatecan lands", narrates that this cheese factory, is located in Field 10 of the Honda, was founded in 1985, where about 8 thousand tons of cheese are produced per day, and in case they have milk left without curdling, they sell it to Liconsá de Río Grande, Zacatecas. The products already have a regionally recognized brand and logo.

Distribution is wholesale, retail and final consumer. Its range of products is focused on chihuahua cheese and cream. The product is distributed in the municipalities of the northern part of the state mainly. The raw material is not produced, it is bought from milk producers in the Mennonite camps. Its elaboration process is through unsophisticated technology. The presentation of the product is in bar with an approximate weight of 0.900 kilograms to 1.2 kilograms. In its production process they employ more than ten people. El Saucito Cheese Factory. Producer of Mennonite origin, which is located in field number 16, the distribution of cheese is to wholesaler, also distributing to retailers, and final consumer. The product is distributed in the municipalities of the northern part of the state mainly. The raw material milk is purchased from the producers of the Mennonite camps, it has little technology for the cheese making process. It focuses only on chihuahua-type cheese. The presentation of the product is in bar with an approximate weight of 0.900 kilograms to 1.2 kilograms according to (Bergen, 2015).

Quesería el Saucito is a business located in the colony Ex Hacienda la Honda, Miguel Auza, Zacatecas, offers products / services such as cheese, milk, yogurt and its main activity is wholesale of milk and other dairy products. It started its activity on July 16, 2016, has around 20 employees (Las empresas México, 2021).

Cheese factory San Lorenzo. Producer of Mennonite origin that is located in Field 15, legally constituted as a company, the distribution of its products is wholesalers, retailers and final consumers. The product is distributed in the municipalities of the northern part of the state mainly. They have little technology for the processing and elaboration of Chihuahua-type cheese. Production is focused only on Chihuahua cheese. The presentation of the product is in bar with an approximate weight of 0.900 kilograms to 1.2 kilograms according to (Bergen, 2015).

With respect to the characteristics of the producers of chihuahua-type cheese in the colony La Honda, Miguel Auza, Zacatecas analyzed above, the following table of consumption conditions is obtained, see Table 3.7:

**Table 3.7** Characteristics of Chihuahua cheese producers in Colonia la Honda, Miguel Auza, Zacatecas, México

Segmentación	Pomas	Derivados lácteos menonitas	La Honda	San Lorenzo	Saucito
<b>Geographic</b>	<p><b>Number of cities:</b> Torreón, Coahuila, Durango, Ciudad Juárez, Chihuahua, Monterrey, Nuevo León, México, D.F.</p> <p><b>Regional Level:</b> North of the State of Zacatecas, Sombrerete, Miguel Auza, Juan Aldama, Francisco R. Murguía and Río Grande.</p>	<p><b>Number of cities:</b> Distribution branches in Mexico City, Monterrey, Nuevo León and Gómez Palacio, Durango.</p> <p><b>Regional Level:</b> It sells its products in self-service stores as "Alsuper" multiregional chain with presence in the state of Chihuahua, Chihuahua; Torreón, Coahuila and Gómez Palacio, Durango, counting "Alsuper" with 69 stores and 16 seats, example of the brands sold in this self-service are the brands "Los Nogales" and D'Chihuahua.</p>	<p>Presence in the states of Coahuila and Durango.</p> <p><b>Regional Level:</b> North of the State of Zacatecas, Sombrerete, Miguel Auza, Juan Aldama, Francisco R. Murguía and Río Grande.</p>	<p>Presence in the states of Coahuila and Durango.</p> <p><b>Nivel Regional:</b> Norte del Estado de Zacatecas, Sombrerete, Miguel Auza, Juan Aldama, Francisco R. Murguía y Río Grande.</p>	<p>Presence in the states of Coahuila and Durango.</p> <p><b>Regional Level:</b> North of the State of Zacatecas, Sombrerete, Miguel Auza, Juan Aldama, Francisco R. Murguía and Río Grande.</p>

<b>Demographic</b>	Household consumption Tertiary food processing sector	Household consumption Tertiary food processing sector	Household consumption	Household consumption	Household consumption
<b>Psicográfico</b>	<b>Social class:</b> High, medium and low.  They stratify their products by creating different brands for each stratum.	<b>Social class:</b> High, medium and low.  They stratify their products by creating different brands for each stratum.	<b>Social class:</b> High, medium and low	<b>Social class:</b> High, medium and low	<b>Social class:</b> High, medium and low
<b>Socioeconomic</b>	<b>Occupation:</b> Occupations in general. <b>Income:</b> Consumption is differentiated by brands for each stratum and region. <b>Education:</b> Basic onwards. <b>Socioeconomic stratum:</b> 1-7 AMAI, variety of chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.	<b>Occupation:</b> Occupations in general. <b>Income:</b> Consumption is differentiated by brands for each stratum and region. <b>Education:</b> Basic onwards. <b>Socioeconomic stratum:</b> 1-7 AMAI, variety of chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.	<b>Occupation:</b> Occupations in general. <b>Income:</b> In general, cheese is suitable for everyone's consumption. <b>Education:</b> Basic onwards. <b>Socioeconomic stratum:</b> 1-7 AMAI, variety of chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.	<b>Occupation:</b> Occupations in general. <b>Income:</b> In general, cheese is suitable for everyone's consumption. <b>Education:</b> Basic onwards. <b>Socioeconomic stratum:</b> 1-7 AMAI, variety of chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.	<b>Occupation:</b> Occupations in general. <b>Income:</b> In general, cheese is suitable for everyone's consumption. <b>Education:</b> Basic onwards. <b>Socioeconomic stratum:</b> 1-7 AMAI, variety of chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.
<b>Behavioral</b>	<b>Purported benefits:</b> satisfaction of quick cravings made with cheese and preparation of dishes. Differentiate yourself in your business or tables.	<b>Purported benefits:</b> satisfaction of quick cravings made with cheese and preparation of dishes.  Differentiate yourself in your business or tables.	<b>Beneficios Pretended:</b> satisfaction of quick cravings made with cheese and preparation of dishes.	<b>Purported benefits:</b> satisfaction of quick cravings made with cheese and preparation of dishes.	<b>Purported benefits:</b> satisfaction of quick cravings made with cheese and preparation of dishes.

*Source: Own elaboration (2021)*

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## Conclusions

Once the general analysis of the information obtained with respect to the conditions of consumption of chihuahua cheese from the perspectives of other research carried out in other cities by various authors and the contrast of analysis of the conditions of consumption in the colony La Honda, Miguel Auza, Zacatecas; as well as the study of each of the chapters of the present investigation, it can be concluded the following, see Table 3.8

**Table 3.8** Consumer behavior model of chihuahua cheese

Marketing stimuli	Consumer Box	Consumer Response
<p><b>Marketing:</b> Diversity of brands of chihuahua cheese, with different quality and prices in the market according to the social stratum, income of consumers.</p> <p><b>Product:</b> 1. The most consumed is the type and brand of cheese of the region. 2. Followed by nationally recognized brands. 3. The strata with greater purchasing power include in their diet more refined (mature) cheeses.</p> <p><b>Price:</b> 1. The price may vary depending on the social stratum and place of purchase. 2. The price may vary depending on the quality and diversity of products.</p> <p><b>Communication:</b> <b>Electronic media.</b> 1. Website. 2. Facebook. Word of mouth marketing.</p> <p><b>Distribution:</b> 1. Department or self-service stores, but cheese belongs to large companies (strata with higher income). 2. Stores specializing in the sale of dairy products. (Strata with higher income) 3. Market 4. Directly to the producer (Cheese Factories). 5. Grocery stores (the lowest strata). 6. Internet platforms: free market, Facebook.</p>	<p><b>Characteristics:</b> 1. Men and women. 2. Consumption is familiar. 3. Occupations in general. Consumed by: employees, housewives, students, professionals, teachers, activities in the field. 4. NSE 1-7 AMAI consume it, variety of cheese for each stratum. 5. Social class: high, medium and low. 6. Consumed by children, youth and adults.</p> <p><b>Decision process:</b> Purchased: 1. Flavor (flavor is given by the physicochemical profile and sensory profile of the cheese). 2. Price. 3. Recognized brand. 4. Container. 5. Availability. 6. Properties and reliability. 7. Hygiene. 8. Nutritional properties. 9. Expiration in order of importance.</p> <p>Emphasizing that women are the ones who regularly occur to make purchases for household consumption.</p>	<p><b>Product:</b> In the region the most consumed cheese is chihuahua cheese.</p> <p><b>Brand:</b> The most consumed brands are those of the region, there are about 15 brands in Río Grande.</p> <p><b>Establishment:</b> 1. Department or self-service stores, but cheese belongs to large companies (strata with higher income). 2. Stores specializing in the sale of dairy products. (Strata with higher income) 3. Market. 4. Directly to the producer (Cheese Factories). 5. Grocery stores (the lowest strata).</p> <p><b>Time of purchase:</b> It is bought: for satisfaction of quick cravings made with cheese and preparation of dishes; or it is accompanied in saucers. It is consumed with the family.</p> <p><b>Frequency of purchase:</b> Consumption is daily, twice a week, and once a week. The amount you buy is given by the frequency of purchase.</p>

The consumer of chihuahua cheese, decides: (Consumer response)

1. Consume chihuahua-type cheese from local producers (region).

Buy at:

- a) Department or self-service stores.
  - b) Stores specializing in the sale of dairy products.
  - c) Market.
  - d) Directly to the producer (Cheese Factories).
  - e) Grocery stores (the lowest strata).
2. It is bought: for satisfaction of quick cravings made with cheese and preparation of dishes; or it is accompanied in saucers. It is consumed with the family.

3. Being the daily consumption, twice a week, once a week, being conditioned
4. How much you buy by the frequency of purchase.

Due to various factors, such as: (Consumer Black Box)

1. Their characteristics, such as: age, sex, social stratum (NSE (socioeconomic level), social class, etc.
2. To your decision process which is affected by the reasons for buying chihuahua cheese, which are mentioned below, according to their order of importance such as:
  - a) Flavor (flavor is given by the physicochemical profile and sensory profile of the cheese).
  - b) Price.
  - c) Recognized brand.
  - d) Container.
  - e) Availability.
  - f) Properties and reliability.
  - g) Hygiene.
  - h) Nutritional properties.
  - i) Expiration in order of importance.

Emphasizing that women are the ones who regularly go to make purchases for household consumption, being they the potential buyers of the product.

However, despite the characteristics of the consumer, as well as the different aspects that he considered for the decision to purchase Chihuahua-type cheese, this decision is favorable or unfavorable due to: (Marketing stimuli).

1. To the marketing that is handled, the large number of varieties of product that exist, the fair price that handles, and the large existing distribution.

**Table 3.9** Variables de segmentación del consumidor del queso tipo chihuahua

Segmentation	Characteristics
Geographic	Río Grande City, Zacatecas, Mexico.
Demographic	Men and women. Household consumption Tertiary food processing sector
Psychographic	<b>Social class:</b> High, medium and low. They stratify their products by creating different brands for each stratum.
Socioeconomic	<b>Occupation:</b> Occupations in general. <b>Income:</b> Consumption is differentiated by brands for each stratum and region. In general, cheese is suitable for everyone's consumption. <b>Education:</b> Basic onwards. <b>Estrato socioeconómico:</b> 1-7 AMAI, Variety of Chihuahua cheese brands for each stratum <b>Life cycle:</b> Children, youth, adults, marital status in general.
Behavioral	<b>Purported benefits:</b> Satisfaction of quick cravings made with cheese and preparation of dishes. Differentiate yourself in your business or tables.

*Source: Own elaboration (2021)*

Each of these segmentation variables are those that must be considered in the future for the realization of marketing plans and are a large part of the conditions of consumption of chihuahua cheese.

Taking into account these considerations, it can be said that the consumer profile of chihuahua cheese is:

1. Men and women.
2. Consumed by children, youth and adults. Emphasizing that women are the ones who regularly go to make purchases for household consumption.
3. Consumption is familiar.
4. Occupations in general. Consumed by: employees, housewives, students, professionals, teachers, activities in the field.
5. NSE 1-7 AMAI They consume it, variety of cheese for each stratum.
6. Social class: high, medium and low.
7. It is bought: for satisfaction of quick cravings made with cheese and preparation of dishes; or it is accompanied in saucers.
8. Consumption is daily, twice a week, and once a week. The amount you buy is given by the frequency of purchase.
9. For the purchase of Chihuahua cheese, the following factors are taken into account, such as: Flavor (the flavor is given by the physicochemical profile and sensory profile of the cheese), price, recognized brand, packaging, availability, properties and reliability, hygiene, nutritional properties and expiration.

It should be noted that the tables of the behavior model of the Chihuahua type cheese consumer, as well as the table of segmentation variables of the Chihuahua type cheese consumer, were carried out under the approach of Kotler & Armstrong, (2013) mentioned within the frame of reference, while the consumer profile is presented based on some aspects collected in the present investigation and also that they belong to each of the aforementioned tables.

The evidence presented above shows that the producing, but above all commercial activity of chihuahua-type cheese, has many market opportunities that can be exploited in its favor, such is the case of the producers of Río Grande, Zacatecas, México since in the present study general information was obtained regarding the conditions of consumption of chihuahua-type cheese, which can be considered to direct your different efforts, not only marketing, but efforts in general.

In response to these considerations, in this study the conditions of consumption of chihuahua-type cheese were determined, since the establishment of socioeconomic variables, internal and external to the consumer, will contribute to improving the competitive position of producers of chihuahua-type cheese in Río Grande, Zacatecas, México.

By way of closing, it is highlighted that this research established the bases of the consumption analysis that exists among consumers of chihuahua-type cheese in the municipality of Río Grande, Zacatecas, México. As well as the determination of the consumer profile of chihuahua-type cheese, supporting the contribution of the rootedness of customs and traditions, since chihuahua-type cheese represents one of the traditional dairy products, most consumed in the region of Río Grande, Zacatecas, México.



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## Chapter 4 Morphological evaluation of *Dioscorea sparsiflora* and *D. alata* minitubers irradiated by gamma rays

### Capítulo 4 Evaluación morfológica de minitubérculos de *Dioscorea sparsiflora* y *D. alata* irradiados con rayos gamma

DÍAZ-GODINEZ, Laura\*, GUTIÉRREZ-MORA, Antonia, MORALES-GARCÍA, Soledad and CATAÑEDA-NAVA, José Juvencio

*Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco*

ID 1<sup>st</sup> Autor: *Laura, Díaz-Godínez* / **ORC ID:** 0000-0002-6184-6160, **CVU CONAHCT ID:** 1000970

ID 1<sup>st</sup> Co-author: *Antonia, Gutiérrez-Mora* / **ORC ID:** 0000-0001-9279-8985, **CVU CONAHCT ID:** 120691

ID 2<sup>nd</sup> Co-author: *Soledad, Morales-García* / **ORC ID:** 0000-0002-2551-2518, **CVU CONAHCT ID:** 224490

ID 3<sup>rd</sup> Co-author: *José Juvencio, Castañeda-Nava* / **ORC ID:** 0000-0002-1825-8240, **CVU CONAHCT ID:** 370014

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L. Díaz, A. Gutiérrez, S. Morales and J. Castañeda

\* [ladiaz\\_al@ciatej.edu.mx](mailto:ladiaz_al@ciatej.edu.mx)

Á. Marroquín, L. Castillo, J. Olivares and N. Olguín Cruz. (VV. AA.) CIERMMI Women in Science T-XXI Biological, Humanities and Social Sciences. Handbooks-©ECORFAN-México, Querétaro, 2023.

## Abstract

*Dioscorea* genus comprises underground tubers with economic-nutritional importance. Plagues affect its production, and as in Mexico, there is no established farming system, so its utilization is limited. Using biotechnological tools, like *in vitro* culture and genetic improvement, has led to the development of varieties with important agronomic characteristics. *In vitro* propagated plants of *D. sparsiflora* and *D. alata*, acclimatized to greenhouse conditions, were radiated with different gamma ray doses (0, 10, 20, 30, 40, 50, and 60 Gy) to improve their genetic characteristics. Minitubers were measured and weighed to identify differences between treatments. *D. sparsiflora* plants resisted all doses those with the biggest sizes were obtained when radiated with 30 Gy and higher radiation treatments. *D. alata* plants radiated with 50 and 60 Gy did not survive, and the highest number of tubers was obtained when radiating with 10 Gy. The biggest sizes were obtained when radiating with 20 Gy. These morphological changes in the minitubers could be considered variations, allowing for greater crop utilization.

### Dioscorea, Minituberization, Hill sweet potato

El género *Dioscorea* comprende tubérculos subterráneos con importancia económico-nutricional. Las plagas afectan su producción y, como en México, no existe un sistema de cultivo establecido, por lo que su aprovechamiento es limitado. El uso de herramientas biotecnológicas, como el cultivo *in vitro* y el mejoramiento genético, ha permitido el desarrollo de variedades con características agronómicas importantes. Plantas propagadas *in vitro* de *D. sparsiflora* y *D. alata*, aclimatadas a condiciones de invernadero, fueron irradiadas con diferentes dosis de rayos gamma (0, 10, 20, 30, 40, 50 y 60 Gy) para mejorar sus características genéticas. Se midieron y pesaron los minitubérculos para identificar las diferencias entre tratamientos. Las plantas de *D. sparsiflora* resistieron todas las dosis, obteniéndose los mayores tamaños cuando fueron irradiadas con 30 Gy y tratamientos de radiación superiores. Las plantas de *D. alata* irradiadas con 50 y 60 Gy no sobrevivieron, y el mayor número de tubérculos se obtuvo al irradiar con 10 Gy. Los mayores tamaños se obtuvieron al irradiar con 20 Gy. Estos cambios morfológicos en los minitubérculos podrían considerarse variaciones, permitiendo un mayor aprovechamiento del cultivo.

### Dioscorea, Minituberización, Camote De cerro

#### 4.1 Introduction

Population increases and food demand have started the search for new crops and improvement of existing products. The *Dioscorea* genus comprises species generally cultivated in developing countries, and the aim is to produce an eatable tuber rich in starch, fiber, vitamins, and minerals (Rodríguez et al., 2008; Muimba-Kankolongo, 2018).

Production systems established in some productive and commercial areas in Asia, South America, and Africa consist mainly of tuber harvest and storage until the subsequent sprouting and planting season. This can cause losses due to putrefaction and nematode infestation during the long storage periods, besides needing long periods of labor to prepare the planting field, increasing production costs (Amusa et al., 2003; Xiao et al., 2023). Of despite having defined production systems, Mexico has no *Dioscorea* or *camote de cerro* established production, even when the tuber is consumed in significant quantities, diminishing wild populations due to annual exploitation.

Implementing biotechnological tools, such as the use of *in vitro* culture techniques, represents an alternative for optimizing production and improving crop characteristics, increasing production and diminishing grow time and loss probability (Wheatley et al., 2003; Borges et al., 2004; Díaz-Godínez, 2022).

Other biotechnological techniques, such as mutagenic physical and chemical agents to help genetic variant production with improved characteristics, have been implemented (Ángeles-Espino et al., 2013). Among the physical mutagenic agents, the most used is gamma radiation, generally coming from Co60, a radioactive isotope with high ionizing capacity used in vegetable cells research and food technology to obtain resistant specimens to different types of biotic and abiotic stress, improve yield and response to disease or chemical agents and even enhance ornamental characteristics (Corrales-Lerma et al., 2019; Balvoa-Caguana et al., 2021).

Based on what has been discussed, the objective of this work was to evaluate morphologically the minitubers produced by plants radiated with gamma rays at different intensities.

## 4.2 Method

### 4.2.1 Obtaining plants

Plants of *D. alata* and *D. sparsiflora* were propagated at Centro de Investigación y Asistencia en Tecnología y Diseño del Estado de Jalisco, in *in vitro* conditions in Murashige and Skoog medium (1962) (MS) supplemented with 30 g/L sucrose, 2 mg/L kinetin (KIN) and 8 g/L agar as gelling agent. pH was adjusted to 5.8.

### 4.2.2 Plant irradiation

Radiation was carried out at Instituto Nacional de Investigaciones Nucleares. Plants of both species were placed in Petri boxes, in MS medium two days before gamma radiation. Ten *D. sparsiflora* plants and five *D. alata* plants were used. Seven treatments with three repetitions were performed at radiations: 0, 10, 20, 30, 40, 50, and 60 Gy. Plants then were transferred to MS medium with 30 g/L sucrose, 2 mg/L KIN, and 8 g/L agar added to stimulate sprouting.

### 4.2.3 Vegetable material propagation

Radiated specimens were propagated by axillary buds in MS medium supplemented with 0.5 mg/L paclobutrazol (PBZ), 30 g/L sucrose, and 8 g/L de agar as gelling agent; pH 5.8. Plants were incubated at 16 h light / 8 h darkness at  $25 \pm 2^\circ\text{C}$  until acclimatization.

### 4.2.4 Acclimatization and greenhouse conditions

Plant acclimatization for the different treatments was carried out in 50 cavities germination trays using as substrate 60 % peat and 40 % perlite. Plants were then placed in 2 L pots with the same substrate in greenhouse conditions, watering three times a week, and fertilized with Peter<sup>®</sup> (20-20-20) twice weekly. Plants were kept in these conditions until the end of the growing cycle and the complete development of the minitubers.

### 4.2.5 Harvest and minituber evaluation

Minitubers were harvested during October and November 2022; once the plant aerial part was completely dried. They were weighed individually and in groups according to treatment. Also, length and diameter were measured for each minituber on the same day they were harvested.

### 4.2.6 Statistical analysis

Statistical analysis was carried out with Statgraphics software. Data was tested for normality. ANOVA Analysis was used for normal data and the Kruskal-Wallis test for other data.

## 4.3 Results

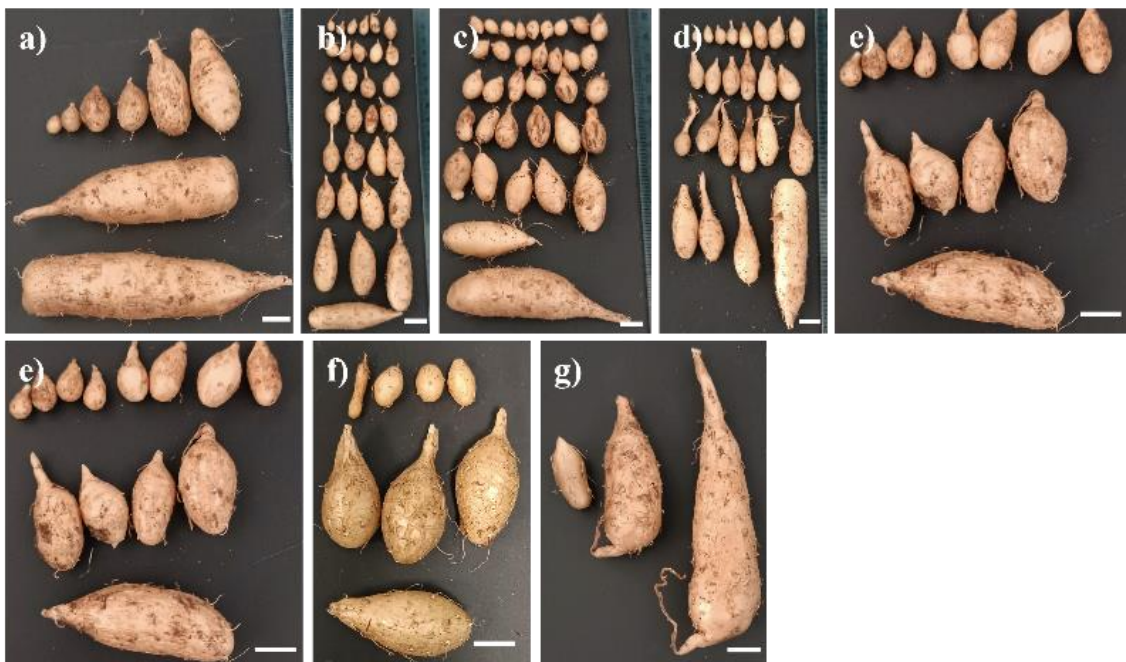
Radiation initial results showed that *D. sparsiflora* plants survived all radiation intensities. *D. alata*, plants only survived up to 40 Gy radiation (Table 4.1), contrasting with Imeh et al. (2012), who reported that 30 % of *D. alata* sprouting plants survived 80 Gy radiation and 10 % when radiated with 100 Gy. Nevertheless, they saw inhibition of plant sprouting when radiating with 100-140 Gy. All these findings differ from another work reporting a lethality percentage up to 26 % in *D. alata* plants radiated with 30 Gy (Yalindua et al., 2014).

**Table 4.1** Survival and plant counting after gamma radiating. \*Plants with low propagation percentage. Minitubers were harvested between November and December after aerial parts had wilted and dried. All minitubers were stored for the next sprouting period (Fig. 1 and 2)

Specie	Treatment	Plant survival	Total plant
<i>D. alata</i> var. Púrpura	Control	YES	80
<i>D. alata</i> var. Púrpura	10Gy	YES	103
<i>D. alata</i> var. Púrpura	20Gy	YES	64
<i>D. alata</i> var. Púrpura	30Gy	YES	53
<i>D. alata</i> var. Púrpura	40Gy	YES	111
<i>D. alata</i> var. Púrpura	50Gy	NO	-
<i>D. alata</i> var. Púrpura	60Gy	NO	-
<i>D. sparsiflora</i>	Control	YES	75
<i>D. sparsiflora</i>	10Gy	YES	81
<i>D. sparsiflora</i>	20Gy	YES	25
<i>D. sparsiflora</i>	30Gy	YES	28
<i>D. sparsiflora</i>	40Gy	YES	56
<i>D. sparsiflora</i>	50Gy	YES	10*
<i>D. sparsiflora</i>	60Gy	YES	42

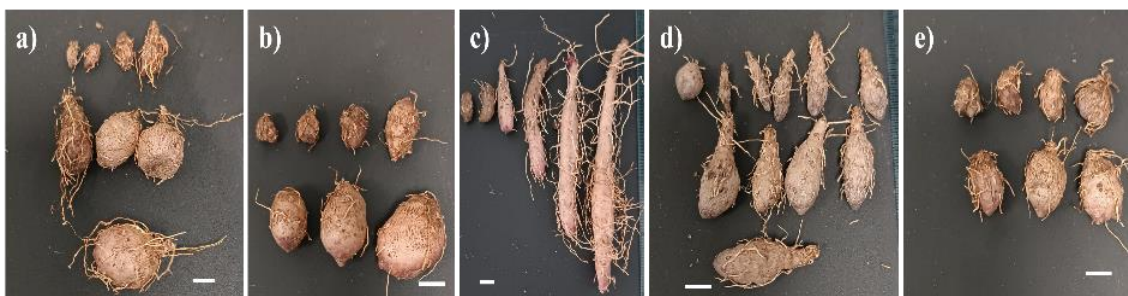
Source: Own elaboration

**Figure 4.1** Minitubers obtained from *D. sparsiflora* plants under different doses of gamma rays. a) Control; b) 10 Gy; c) 20 Gy; d) 30 Gy; e) 40 Gy; f) 50 Gy; g) 60 Gy. Bar: 1 cm.



Source: Own elaboration

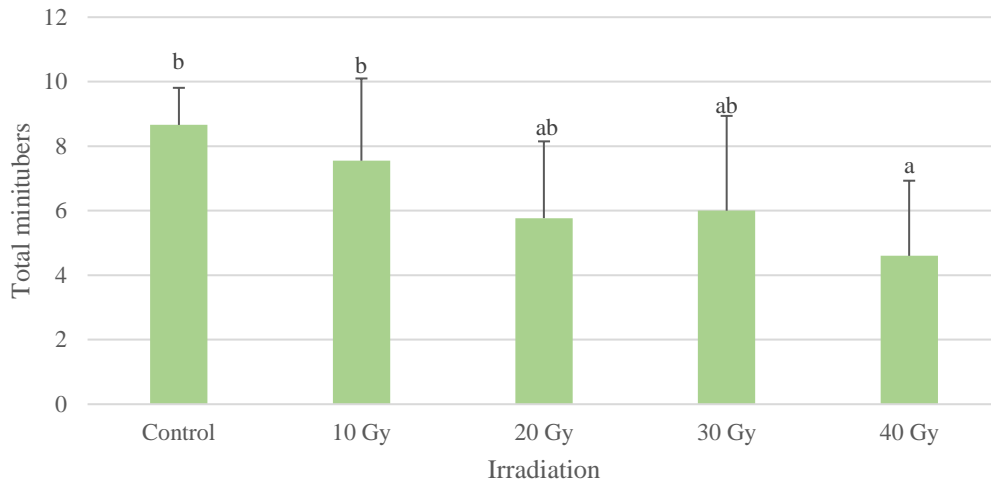
**Figure 4.2** Minitubers obtained from *D. alata* plants treated with different doses of gamma rays. a) Control; b) 10 Gy; c) 20 Gy; d) 30 Gy; e) 40 Gy. Bar: 1 cm.



Source: Own elaboration

Analysis for *D. sparsiflora* number of minitubers did not show significant differences ( $P=0.4968$ ); but for *D. alata* var. *Purpurea*, the total of minitubers showed significant differences ( $P=0.0069$ ). The total number of minitubers obtained in the control group and radiation of 10 Gy was eight minitubers on average, while for the rest of the radiations, the quantity was smaller than six (Graphic 4.1). Rodríguez (2000) mentions that early tuberization and production of more than one tuber per plant are desirable characteristics in yam plants, which could also bring an increase in yield. In potatoes, an increase of 38% has been reported in the number of microtubers produced *in vitro*, after plants were radiated with 2.5 Gy gamma rays (Al-Safadi et al., 2000).

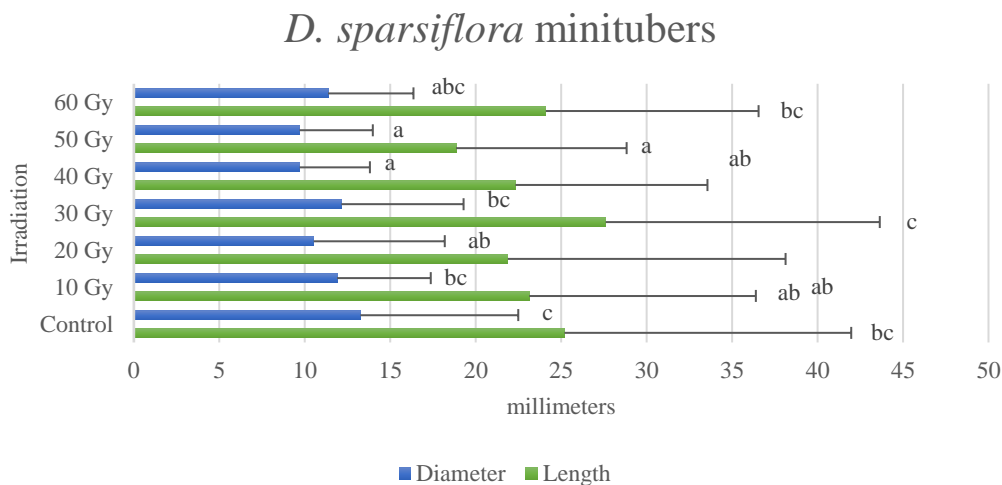
**Graphic 4.1** Total of *D. alata* minitubers obtained according to different gamma radiation intensities. Different letters mean significantly different at treatments



Source: Own elaboration

*D. sparsiflora* minitubers length showed significant differences ( $P= 0.0338$ ) and diameter ( $P=0.0007$ ) (Graphic 4.2). Minitubers of radiated plants with 30 Gy were longer, compared with other radiations, while for minitubers diameter, only slight differences between the control and the rest of the treatments were found. Nevertheless, fresh minitubers weight did not show significant differences ( $P=0.6396$ ).

**Graphic 4.2** *D. sparsiflora* minituber length and diameter under different gamma radiation doses. Different letters mean significantly different at treatments

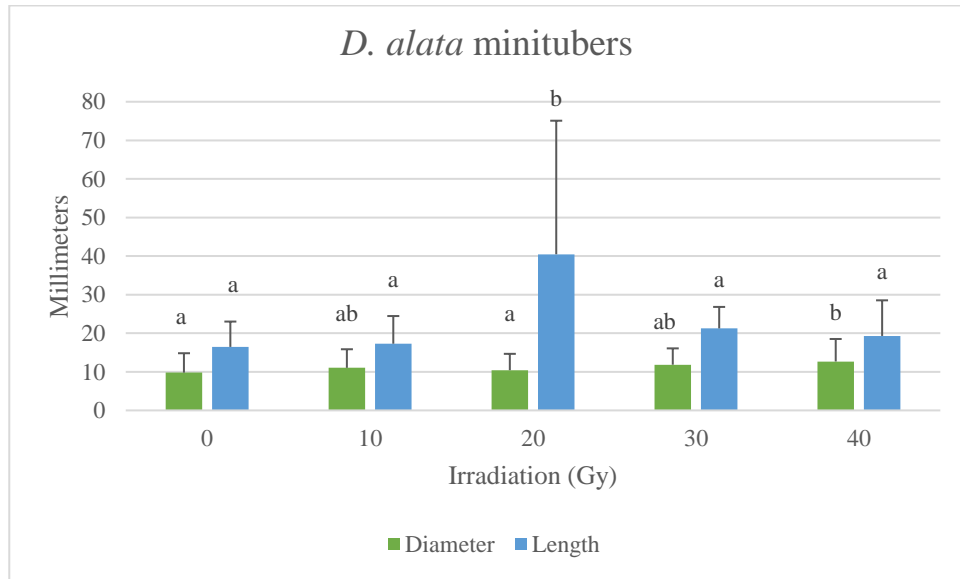


Source: Own elaboration



In the case of *D. alata*, minituber length was longer for those obtained from plants radiated with 20 Gy (Graphic 4.3), reaching up to 4 cm. Minituber diameter was lower than 1.5 cm for most minitubers, compared with other tuber roots which reach 5 cm in diameter like in *Ipomoea batata* at low level of radiation (15 Gy) for first generation; in the second generation, the majority of tuber roots reach diameters of 5 cm approximately, even in radiated plants at 75 and 90 Gy (Kalal et al., 2022).

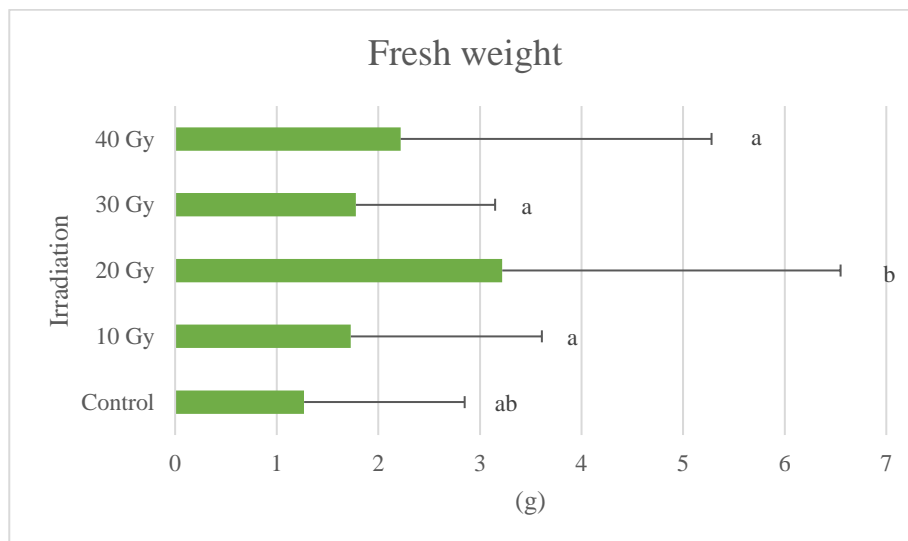
**Graphic 4.3** *D. alata* minituber length and diameter obtained after using different doses of gamma rays. Different letters mean significantly different at treatments



Source: Own elaboration

The fresh weight of minitubers from plants radiated with 20 Gy was greater (Graphic 4). Nevertheless, weight decreases in potato minitubers when incrementing gamma ray doses have been reported, where control weighted 119 mg approximately; while those obtained with greater radiation (20 Gy) did not surpass 1 mg (Bado et al., 2016). On the other hand, Mahfouze et al. (2012) reported better weights in potato microtubers radiated with 5-10 Gy doses.

**Graphic 4.** Fresh weight of *D. alata* minitubers produced from plants radiated with different gamma ray doses. Different letters mean significantly different at treatments



Source: Own elaboration

#### 4.4 Acknowledgment

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## 4.5 Financing

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## 4.6 Conclusions

Gamma ray radiation is a tool contributing to generating specific changes in different species. These genetic variations could result in new cultivars with superior characteristics than those of conventional species, incrementing tuber size and weight, increasing agronomic yield, and probably increasing important compounds in the food and pharmaceutical industries, among others. Analyzing chemical characteristics in the development of new plants is a fundamental part of studying and implementing new food sources and it is necessary to include them in future research.

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## Chapter 5 Biomass: teaching strategy for the laboratory at university level

### Capítulo 5 Biomasa: estrategias de enseñanza para el laboratorio a nivel universitario

RANGEL-RUIZ, Karelía Liliana<sup>†\*</sup>, VARGAS-SOLANO, Zaira<sup>''</sup> and GRANADOS-OLVERA, Jorge Alberto<sup>','</sup>

*†Universidad Politécnica de Cuautitlán Izcalli, Lago de Guadalupe 1, Lomas de Cuautitlán, 54720 Cuautitlán Izcalli, Méx.*

*''Universidad Tecnológica Fidel Velázquez, División Académica de Ciencias de la Sustentabilidad, Av. Emiliano Zapata S/N, El Tráfico, Nicolás Romero, Edo. de Méx. C.P.54400*

ID 1<sup>st</sup> Author: *Karelía Liliana Rangel-Ruiz* / **ORC ID:** 0000-0003-1805-0447, **Researcher ID Thomson:** GLQ-8704-2022, **CVU CONAHCYT ID:** 225798

ID 1<sup>st</sup> Co-author: *Zaira, Vargas-Solano* / **ORC ID:** 0000-0001-7404-8769, **Researcher ID Thomson:** S-5739-2018, **CVU CONAHCYT ID:** 313021

ID 2<sup>nd</sup> Co-author: *Jorge Alberto, Granados-Olvera* / **ORC ID:** 0000-0003-0546-5328, **Researcher ID Thomson:** S-57562018, **CVU CONAHCYT ID:** 946998

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K. Rangel, Z. Vargas and J. Granados

\* karelialiliana.rr@upci.edu.mx

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## Abstract

Biomass is defined as that organic product of vegetable origin that can convert the energy coming from sunlight into chemical energy contained in the vegetable carbohydrates' links. Its study is important since this contained chemical energy can be used in the form of fuels, electricity and/or chemical reagents that can be used as precursors. This paper presents a proposal for teaching biomass in the laboratory at university level. The experimental proposal exposes an easy and accessible practice to meet this objective. This methodology has been applied in two different universities where engineering courses are taught. The materials used are accessible and cheap. Once the practice was finished, a survey was applied to the students to verify the subject's understanding, obtaining favorable results. In conclusion, this proposal offers an easy and low-cost methodology for teaching the topic of Biomass in the laboratory at university level.

## Biomass, Methodology, Laboratory, University level

### Resumen

La biomasa se define como aquel producto orgánico de origen vegetal que es capaz de convertir la energía proveniente de la luz del sol en energía química contenida en los enlaces de los carbohidratos vegetales. Su estudio es importante ya que esta energía química contenida puede ser aprovechada en forma de combustibles, electricidad y/o reactivos químicos que pueden ser usados como precursores. En este trabajo se presenta una propuesta para la enseñanza de la biomasa en el laboratorio a nivel universitario. Esta propuesta experimental expone una práctica fácil y accesible para cumplir dicho objetivo. Esta metodología ha sido aplicada en dos diferentes universidades donde se enseñan carreras de corte ingenieril. Los materiales utilizados son accesibles y baratos. Una vez terminada la práctica se aplicó una encuesta a los estudiantes para verificar el grado de comprensión acerca del tema, obteniendo resultados favorables. En conclusión, esta propuesta ofrece una metodología fácil y a bajo coste para la enseñanza del tema de Biomasa en el laboratorio a nivel universitario.

## Biomasa, Metodología, Laboratorio, Nivel universitario

### 5.1 Introduction

#### 5.1.1 What is Biomass?

We can define biomass as any organic solid product formed naturally or by human action and includes all those natural components originating from land cultivation (crops and trees) as well as aquatic vegetation (algae and marine plants), which are the result of photosynthesis or animal digestion (Vassilev et al., 2010).

This biomass is produced by converting energy from sunlight through photosynthesis into chemical energy and is stored in the chemical bonds of carbohydrates such as cellulose, hemicellulose and lignin whose proportions vary depending on the type of plant (Yan et al., 2022; Odoh et al., 2023).

Biomass can be classified as follows: woody plants, herbaceous plants, aquatic plants, a mixture of biomass, biomass and animal waste (manure) contaminated, and industrial waste, the first three being the types of greatest interest in energy production, especially plants with C4 types of photosynthesis (McKendry, 2002).

It is estimated that the average amount of Biomass on Earth is  $\approx 550$  Gt C of which  $\approx 80\%$  ( $\approx 450$  Gt C) are produced by plants, mainly terrestrial ones (Barn-On et al., 2018).

#### 5.1.2 Why is it important to study biomass?

One of the most important characteristics of biomass is that it has the capacity to convert the energy stored in its bonds into usable forms for humans, each one with its own specific requirements, advantages, and disadvantages.

At least three types of energy products derived from biomass can be obtained: transport fuels, electrical energy and precursors or chemical reagents (Gallezot, P., 2012 and Srivastava et al., 2021).

Recently, interest in the use of this energy source has been renewed, some of the reasons will be listed below:

- Technological advances have increased the efficiency of the conversion of biomass into fuel and have lowered the costs of this process.
- The use of non-food crops means that the production of energy from biomass does not put the population's food security at risk.
- Biomass is considered a renewable energy source, since when crops are produced sustainably, they do not contribute to increasing CO<sub>2</sub> levels in the atmosphere, which helps against climate change.
- In the same way, biomass can be used by Indigenous peoples since it is widely available and easily accessible, and these communities have used it for a long time, which makes them familiar with its use (McKendry, 2002).
- The use of biomass known as "waste", which is part of industrial processes or from other anthropogenic activities such as livestock that produce tons of waste, contributes to reducing the amount of "garbage" residues derived from these activities (Sánchez et al., 2019).

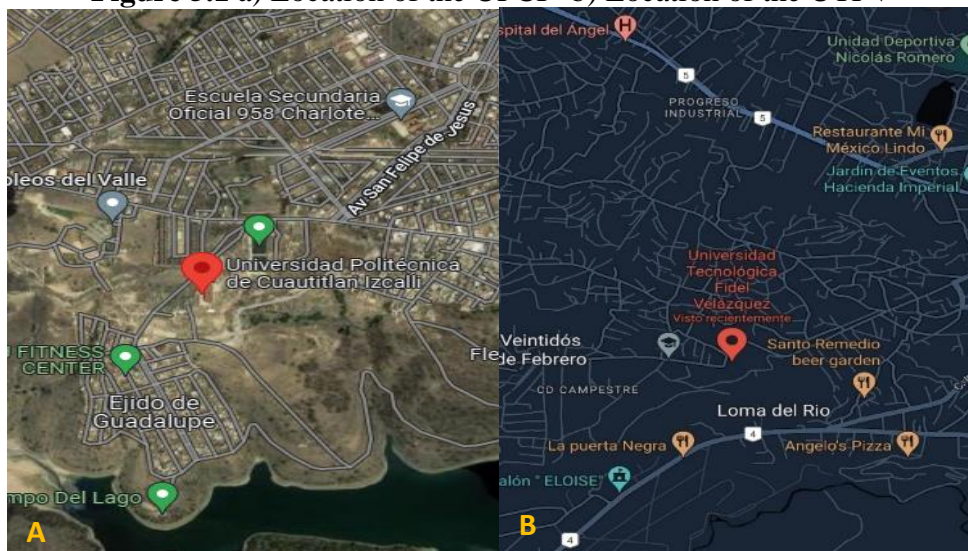
## 5.2 Methodology

In this paper, it is explained a methodology that helps the teaching of biomass in the laboratory at the university level, mainly in engineering courses such as Biotechnology Engineering (IBT), Energy Engineering (IE), Environmental Engineering (IA) and Nanotechnology Engineering (INT), to name a few.

This practice has been conducted both at the Universidad Politécnica de Cuautitlán Izcalli (UPCI) located in the municipality of Cuautitlán Izcalli and at the Universidad Tecnológica Fidel Velázquez (UTFV) (UTFV) located in the municipality of Nicolás Romero both belonging to the Estado de México State (Figure 5.1).

This methodology has been assessed on young students between the ages of 18 and 22 belonging to IBT and IE careers in the case of UPCI, as well as IA and INT in the case of UFTV.

**Figure 5.1** a) Location of the UPCI b) Location of the UTFV



Source: (Taken from Google Maps, 2023)

## Obtaining polylactic acid (pla)

### 5.2.1.1 Objective

Extract starch from the potato, to be able to synthesize lactic acid to obtain polylactic acid (PLA).

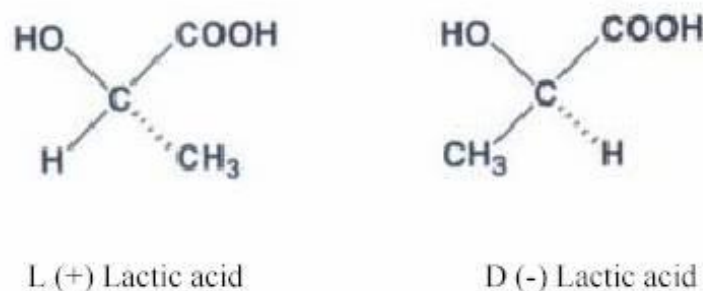
### 5.2.1.2 Introduction

Conventional plastics are a product with a great impact on the environment since they are used daily in our lives and are derived from oil. Currently, other alternatives are being sought to help us reduce this problem of plastics; an alternative is bioplastics, which are a type of plastic that characterizes by being made from organic materials and therefore can be compatible with the environment.

The advantages of bioplastics are that they reduce the carbon footprint, they save energy in production, they do not consume non-renewable raw materials and they reduce non-biodegradable waste that pollutes the environment.

Among the biodegradable materials, polylactic acid (PLA) stands out, which is a derivative of lactic acid that has characteristics similar to polyethylene terephthalate (PET) and polyethylene; it is an organic acid with three carbons, in one terminal the carbon atom is part of the carboxylic group; the other terminal carbon atom is part of a methyl; and the central carbon atom is attached to an alcohol group. There are two stereoisomers of lactic acid:

**Figure 5.2** Isomers of lactic acid



Source: (Taken from: Molina et al., 2018)

It is obtained by fermentation of a vegetable raw material with certain strains of bacteria. It presents a wide range of properties since there are different grades, with different molecular weights, stereochemistry, and morphology, ranging from the amorphous state to the semi-crystalline state. Lactic acid polymerization can be controlled to obtain different molecular weights and different degrees of crystallinity by catalyst selection.

PLA is a versatile polymer that has various applications, including in the textile industry, in the medical industry and especially in the packaging industry. Its tensile strength and modulus of elasticity is comparable to polyethylene; it is more hydrophilic than polyethylene, it has a lower density, it is stable to UV light and its flammability is too low (Serna and Albán, 2003).

### 5.2.1.3 Obtaining potato starch

#### Materials

- Three large potatoes
- 200ml of water
- Blender
- Sieve
- Muffle
- Knife

### Procedure

1. Peel the three potatoes and cut them into small cubes, to have the maximum amount of starch.
2. Blend with only 200ml of water for only three seconds (this is to avoid modifying its polymer chain).
3. Filter; use the sieve and a cloth.
4. Keep the liquid obtained at rest so that they separate.
5. Decant (remaining only with the lower part).
6. Dry for 24 hours.

#### 5.2.1.4 Synthesis of the PLA

##### Materials

- 500ml beakers
- Glass stirrer
- Granary scale
- Spatula
- Magnetic grill
- Magnetic stirrer
- Thermometer
- 250ml beakers
- Watch glass
- Molds

##### Reagents

Starch

Acetic acid

Vegetable dye

Glycerin

Lactobacilli (Yakult)

##### Procedure

1. Weigh 250g of starch on the granary scale.
2. Gradually add 250ml of water.
3. Mix and then add 30ml or 40ml of Lactobacilli.
4. Let ferment for 24 h.
5. Decant the water, leaving only the white mixture.
6. Shake on the grill, gradually adding 50ml of glycerin.
7. Leave stirring for 10 min.
8. Add 50ml of acetic acid little by little while stirring (add dye).
9. Heat the mixture until it boils, checking the viscosity with the glass stirrer.
10. Pour into the molds and put them on the stove until they have polymerized.

#### 5.2.1.5. Survey Application

Once the practice was finished, the students were asked to answer an electronic form with a check box grid with options from 1 to 5 where number 1 corresponded to the option "I don't contribute anything" and number 5 corresponded to the option "I learned something new and reaffirm the knowledge that I already had". The form consisted of five questions related to the acquisition and reaffirmation of knowledge related to biomass.



## 5.3 Results

### 5.3.1 Obtaining potato starch

For this section, the students could easily follow the instructions for obtaining potato starch, the potatoes were blended, and the mixture obtained was sifted (figure 3).

**Figure 5.3** Mixture obtained from potatoes.



*Source: Own source*

The mixture was left to rest and was decanted, discarding the supernatant, and drying the precipitate for 24h, thus obtaining a fine, whitish powder (figure 4) that was used for the next section of the practice.

**Figure 5.4** Obtaining potato starch.



*Source: Own source*

### 5.3.2 Synthesis of PLA

Once the starch was obtained, PLA was synthesized: Figure 5 shows the result of mixing hydrated starch with lactobacilli, leaving it to ferment for 24 h. Note the separation into two phases, the transparent supernatant, and the whitish precipitate.

**Figure 5.5** Result of fermentation of potato starch by lactobacilli.



*Source: Own source*

In figure 5.6 it can be seen how the PLA is added dye, at this stage the PLA has a high viscosity so that it allows the formation of small balls that do not stick to the hands when they are kneaded, once obtained this viscosity, the mixture was poured into the respective molds.

**Figure 5.6** Obtaining PLA.



*Source: Own source*

### 5.3.3 Verification survey

Once the practice was finished, the students were asked to answer a verification survey, the results obtained from this survey show that 80% of the students consider that "I learned something new and reaffirm the knowledge I already had", regarding their prior knowledge around biomass.

### 5.4 Conclusions

This paper presents a methodology that shows a laboratory practice where it is exposed that using simple and low-cost materials, basic knowledge about biomass can be taught in the laboratory. The results obtained in the survey indicate that the application of this type of methodologies helps students, not only, to better understand or reaffirm the theoretical knowledge that is explained in the specialty subjects, but also to appropriate said knowledge and realize the technological applications that such knowledge implies.

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## Chapter 6 Design of a thermal process applied to hummus (chickpea) dip

### Capítulo 6 Diseño de un proceso térmico aplicado al hummus (dip de garbanzo)

GIJÓN-ARREORTÚA, Ixchel†\*, ESPARZA-RUIZ, Adriana, PÉREZ-PADILLA, Yamile and HERRERA-ROSALES, Iloki

*Facultad de Ingeniería Química, Universidad Autónoma de Yucatán.*

ID 1<sup>st</sup> Author: *Ixchel, Gijón-Arreortúa* / **ORC ID:** 0000-0001-6011-725X, **Researcher ID Thomson:** AET-8567-2022, **PubMed** ixchel.gijon@orcid, **CVU SNI-CONAHCYT ID:** 270209

ID 1<sup>st</sup> Co-author: *Adriana Esparza-Ruiz* / **ORC ID:** 0000-0001-8046-2683, **Researcher ID Thomson:** HTP-8156-2023, **arXiv author ID:** <https://arxiv.org/a/0000-0001-8046-2683> – PubMed adriana.esparza@orcid y CVU SNI-CONACYT: 39939)

ID 2<sup>nd</sup> Co-author: *Yamile Pérez-Padilla* / **ORC ID:** 0000-0002-7560-6766, **Researcher ID Thomson:** AAR-6086-2021, **arXiv author ID:** YamilePP – **PubMed** yamile.perez@orcid, **CVU SNI-CONAHCYT ID:** 104058)

ID 3<sup>rd</sup> Co-author: *Iloki, Herrera-Rosales* / **ORC ID:** 0009-0002-2923-1788

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I. Gijón, A- Esparza, Y. Pérez and I. Herrera

\* ixchel.gijon@correo.uady.mx

Á. Marroquín, L. Castillo, J. Olivares and N. Olgúin Cruz. (VV. AA.) CIERMMI Women in Science T-XXI Biological, Humanities and Social Sciences. Handbooks-©ECORFAN-México, Querétaro, 2023.

## Abstract

A thermal process was designed in three different flavors of hummus dip using the Ball method. To guarantee commercial sterility, the sustaining times of 3 and 5 min were evaluated, a thermal death time ( $F_{proceso}$ ) greater than 2.5 min was obtained, for natural hummus (HN), with chipotle (HC) and with olives (HA), the values were 3.4, 3.4 and 2.8 min, respectively. A treatment was designed with a heating time ( $t_B$ ) using  $F_{proceso} = 2.5$  min, for *Clostridium botulinum*. The experimental  $t_B$  obtained for HN, HC and HA were 14.8, 14.1 and 13.2 min, with these values were obtained the exact times of holding in the autoclave of 1.7, 1.8 and 2.6 min in HN, HC, and HA, respectively, suitable for commercial sterility. This method allowed to know the lethality of the sterilization process for hummus dip.

**Thermal process, Ball's formula method, lethality, sterilization of foods, *Clostridium botulinum***

## Resumen

Se diseñó un proceso térmico en tres sabores diferentes de hummus usando el método de Ball. Para garantizar la esterilidad comercial, se evaluaron los tiempos de sostenimiento de 3 y 5 min, se obtuvo un tiempo de muerte térmica ( $F_{proceso}$ ) mayor a 2.5 min, para hummus natural (HN), con chipotle (HC) y con aceitunas (HA), los valores fueron 3.4, 3.4 y 2.8 min, respectivamente. Se diseñó un tratamiento con un tiempo de calentamiento ( $t_B$ ) utilizando  $F_{proceso} = 2.5$  min para *Clostridium botulinum*. Los  $t_B$  experimentales alcanzados para HN, HC y HA fueron de 14.8, 14.1 y 13.2 min, con estos valores se obtuvieron los tiempos exactos de retención en la autoclave de 1.7, 1.8 y 2.6 min en HN, HC y HA, respectivamente, aptos para esterilidad comercial. Este método permitió conocer la letalidad del proceso de esterilización del hummus de garbanzo.

**Proceso térmico, método de Ball, letalidad, esterilización de alimentos, *Clostridium botulinum***

## 6.1 Introduction

Hummus is a widely consumed dip due to its nutritional profile and consumer preferences for plant-based proteins (Ahmed et al., 2020). It is made from chickpeas and sesame seeds, which are roasted and boiled to eliminate pathogens to meet food safety requirements. It is consumed fresh or refrigerated, without any extra processing or treatment, which makes it a potential source of microbial contamination and foodborne pathogens, such as *Salmonella* spp, *Listeria monocytogenes* and *Escherichia coli* (Olaimat et al., 2018). Moreover, if packaging is required to extend its shelf life, proper thermal processing must be performed to ensure that it is free of disease-transmitting microorganisms, such as *Clostridium botulinum*, which produces a toxin responsible for botulism.

The ideal thermal processing is one that maximizes the inactivation of harmful microorganisms and enzymes, while minimizing heat damage to nutritional and sensory parameters. Therefore, it is not an easy task since foods are complex. Its design must consider an efficient sequence of operation, which generates an economic impact in energy savings and, above all, avoids lawsuits against manufacturers for marketing products with unguaranteed commercial sterility. In addition, not only quality and safety factors must be considered, but care must be taken in the calculation of process time and temperature to avoid insufficient or excessive processing (Kubo et al., 2021).

There are several methods for analyzing thermal processes in foods, such as Bigelow, Stoforos, Stumbo and Ball's method, which allow calculating the lethality of thermal processes and treatment time (Stoforos, 2010). Previous studies of the thermal analysis of natural hummus were carried out with the Stoforos method and with the Ball method, it was found that the Ball method allows optimizing this productive process, which would generate profitability in operating costs. Therefore, based on this, in this work a thermal treatment was designed by means of mathematical procedures and thermal death kinetics of the microorganism to calculate its lethality, guaranteeing the commercial sterility of the product and prolonging its shelf life. Likewise, it was ensured that the holding times used in the autoclave reached the thermal death time of the process with respect to *Clostridium botulinum*, the microorganism of reference in sterilization processes.

## 6.2 Description of the method

### 6.2.1 Materials

The three different chickpea hummus products: natural (HN), with chipotle (HC) and with olives (HA) were provided by a local supplier in the state of Yucatan.

### 6.2.2 Equipment

Extech Instruments Type K thermocouples from -30 to 300 °C were used to obtain the thermal history. The temperature data were recorded in a Datalogger, Model SDL200 (Extech Instruments). A vertical autoclave, model SM510 Yamamoto, 20 L, 105 - 123 °C and 0.18 MPa was used.

### 6.2.3 Thermal history

In order to find the optimum holding time, first tests were carried out with natural hummus at 3 and 5 min, once a thermal death time greater than 2.5 min was obtained, the experiments were carried out in triplicate for HN, HC and HA.

### 6.2.4 Heat penetration curve

It was generated by plotting the temperature difference between the autoclave and the food against the sterilization time.

### 6.2.5 Process evaluation. Ball's method

Ball's parameters were calculated. The lag time,  $t_c$ , was obtained from the thermal history. The heat treatment time to reach sterility,  $t_B$ , when starting at  $t_B=0$ , was corrected to zero, by multiplying  $t_c \times 0.58$ . The calculation  $j_h$  was with Eq. (1).

$$j_h = \frac{T_E - T_{ip}}{T_E - T_0} \quad (1)$$

The parameter  $g$  was cleared from Eq. (2).

$$t_B = f_h [\log[j_h(T_E - T_0) - \log(g)]] \quad (2)$$

The ratio  $f_h U$  was found by interpolation of the table values of  $g$ , for  $z = 10$  °C, using the values of  $g$  and  $j_h$ . This relationship was used to find out the value of  $U$ . The lethality of the process was calculated with the

$$L = 10^{\frac{T_E - T_{ref}}{z}} \quad (3)$$

The thermal death time of the process  $F_{process}$  was determined with Eq. (4).

$$U = F_{process} / L \quad (4)$$

### 6.2.6 Thermal process design

A  $F_{process} = 2.5$  min was used for *Clostridium botulinum*. The values of  $f_h$  and  $j_h$  obtained from the experimental heating curve during the process evaluation were used in the design.  $U$  was calculated with Eq. (4) using  $F_{process}$  set and the value of  $L$  from the process evaluation. With the value of  $f_h U$  and  $j_h$ , the value of  $g$  from the table was determined for  $z=10$ . The value of the heat treatment time to reach design sterility,  $t_B$ , was determined using Eq. (2). The holding time, in the autoclave for  $F_{process} = 2.5$  min for *Clostridium botulinum*, was recalculated by subtracting  $t_B$  of design from  $t_{(B\_exp)}$  of the process evaluation.

## 6.2.7 Statistical analysis

A completely randomized design was used, considering the type of hummus. From the data obtained for thermal death time, it was analyzed if there is a significant difference for the three different products when the holding time was 3 min, using a one-way ANOVA test with Excel, considering that the population means are equal. In all cases it was required to know if in the three products, with a given holding time an  $F_{\geq 2.5}$  min is reached. The independent variable was holding time, 3 and 5 min in the autoclave at 121 °C. The dependent variable was thermal death time.

## 6.3 Results

### 6.3.1 Evaluation of the process

In order to find the optimum holding time, tests were first performed with HN at 3 and 5 min. From the heat penetration curve, the parameters of Ball's method were obtained, which are shown in Table 6.1.

**Table 6.1** Parameters of Ball's method for HN at different holding times.

Time (min)	$T_E$ (°C)	$f_h$ (min)	$j_h$ (min)	$t_{B_{exp}}$ (min)	$g$ (°C)	$f_h/U$	$L$ (min)
3	120.0	5.0	0.5	10.8	0.33	1.1	0.78
5	119.9	4.9	0.6	12.6	0.15	0.8	0.76

Source: Own elaboration

For HN at different holding times of 3 and 5, the  $F_{process}$  times used were 3.4 and 4.4 min, respectively, values greater than 2.5 min, which ensures commercial sterility, so it was decided to work with 3 minutes of holding time. The recommended  $F_{process}$  value range for vegetable-based products varies from 3 to 6 min (Serment-Moreno & Welti-Chanes, 2016). Foods with a similar consistency, such as baby porridges, vegetable creams and tomato puree present  $F_{process}$  values of 3 to 4 min (Deák, 2014).

Once the holding time of 3 min was selected, tests were performed for the different hummus products. From the experimental data the initial temperature ( $t = 0$ ) of HN, HC and HA was 26.5 °C, 25.7 °C and 27.4 °C, respectively. The average sterilization temperature in the autoclave along the constant zone was 121.4, 121.0 and 121.5 °C, for each food. The delay time in the autoclave was 26.5, 30.0 and 27.5 min, and the time at the end of heating in the autoclave was 29.5, 33.0 and 30.5 min. The temperature of the food at the beginning of cooling was 119.4 °C, 119.6 °C and 119.6 °C. All these values were obtained from the thermal histories. The average sterilization temperature,  $T_E$  in the autoclave showed no significant difference ( $p > 0.05$ ), this value is to be expected since it is the same food, only the product differs with respect to its flavor. The  $f_h$  value for HC was the lowest which indicates that this product was heated faster unlike the others, in this case the heat transfer is more efficient than in solid products, because if we confront the values achieved in canned fish sterilization processes, these have reported  $f_h$  values of 27 min (Ansar Ali et al., 2006) as well as  $f_h$  values of 8.5 - 13.9 min for Chhana roll (Jat et al., 2014).

The  $j_h$  factor cannot be compared because different initial temperatures were used, but the values obtained for hummus in its different products are similar to those with the same consistency such as mashed potatoes and ketchup of 1.1 and 1.2 min, respectively (Govaris & Scholefield, 2007). Similarly,  $j_h$  values equal to 1.3 min have been reported for cream of celery and values between 0.44 and 1.17 at  $F(0)$  of 12.4, 13.2 and 14.6 min for a Kheer dairy product. Values of  $j_h$  close to 1, indicate that the product is purely convective (Jha et al., 2011). The  $t_{(B_{exp})}$  varied from 14 - 16 min, for HC and HA, but showed no significant difference ( $p > 0.05$ ).

The  $g$  value for HN and HC had no significant difference ( $p > 0.05$ ), but did for HA; which is in the range of the  $g$  values obtained by Puthanangadi, et al., in 2021 being 2.95 and 1.93 °C for an  $F_0$  of 8 and 9 min, respectively. The ratio  $f_h/U$  was different for HA with respect to HN and HC, this value decreases when the  $F_{process}$  increase, the values obtained for this parameter (Table 2) are greater than the value of  $f_h/U=0.8$  for a holding time at 5 min with which a value of  $F_{process} = 4.4$  min was obtained; a value of 1.41 is reported for  $f_h/U$  in tuna in brine (Bindu & Srinivasa Gopal, 2008). A study on heat penetration characteristics of cassava found values of  $j_h$  1.91 and  $f_h/U$  2.06, similar to those obtained in Table 2 (Dinakaran et al., 2017).

**Table 6.2** Parameters Ball's method for hummus for the evaluation of the thermal process

Paramet	HN	HC	HA
$T_E$ (°C)	$121.1 \pm 0.7^a$	$121.3 \pm 0.3^a$	$121.4 \pm 0.3^a$
$f_h$ (min)	$8.4 \pm 1.2^a$	$7.6 \pm 0.3^b$	$9.5 \pm 1.8^c$
$j_h$ (min)	$1.6 \pm 0.5^a$	$1.2 \pm 0.3^b$	$0.9 \pm 0.6^c$
$t_{B_{exp}}$ (min)	$16.0 \pm 1.6^a$	$14.4 \pm 0.3^b$	$14.4 \pm 0.4^b$
$g$ (°C)	$1.7 \pm 0.6^a$	$1.5 \pm 0.2^a$	$2.1 \pm 0.4^b$
$f_h/U$	$2.5 \pm 0.6^a$	$2.4 \pm 0.2^a$	$3.8 \pm 1.2^b$
$L$ (min)	$1.0 \pm 0.1^a$	$1.1 \pm 0.1^a$	$1.1 \pm 0.1^a$

-c Different letters in the same row represent significant difference ( $p < 0.05$ ).

Source of consultation: Own elaboration.

Since the thermal death time is the time corresponding to a theoretical thermal process, carried out at constant temperature, generates the same degree of destruction as the real process in which the temperature of the food is not usually constant, this value at a specific point of the food can be calculated by the change in the concentration of the microorganism at that point, Frequerido, or by the change in temperature at that point from the beginning to the end of the treatment, Fprocess.

The values of thermal death time, calculated by Ball's method for the different hummus products HN ( $3.4 \pm 0.2$  min), HC ( $3.4 \pm 0.2$  min) and HA ( $2.8 \pm 0.3$  min), in all cases this time was greater than 2.5 min, according to the kinetics of the microorganism ensures commercial sterility with respect to *Clostridium botulinum*. The HA showed significant differences ( $p < 0.05$ ) with respect to the natural and chipotle product, having a lower value, this result can be explained due to the fact that this product contained sliced olives, that is, the increase of solid material, which decreased the heat transfer generating a lower Frequerido value. This is due to the mechanism of heat transfer within the food, in a solid the mechanism is by molecular diffusion, while in a liquid it is by convection associated with the movement of the fluid which enhances this transport phenomenon.

### 6.3.2 Process design

In this step the heating time,  $t_B$ , was determined using the  $F_{process} = 2.5$  min, for *Clostridium botulinum*. Once the  $t_{(B_{exp})}$  was established, the exact autoclave holding time that allowed commercial sterility was known. The design parameters obtained by Ball's method are shown in Table 3. The  $g$  value, despite having significant differences ( $p < 0.05$ ), had a maximum value of 2.5 min.

With the  $t_{(B_{exp})}$  obtained in the evaluation and the  $t_B$  calculated by the design it was possible to know the specific holding for the microorganism *Clostridium botulinum* and the products HN ( $1.7 \pm 0.3$  min), HC ( $1.8 \pm 0.2$  min) and HA ( $2.6 \pm 0.4$  min). This allows efficient use of the energy used in this process, so if we take the upper standard deviation in HN and HC this would be 2 min and for HA it would remain at 3 min for *Clostridium botulinum*.

**Table 6.3** Parameters of Ball's method for the design of the thermal process.

Microorganism	Product	$U$ (min)	$f_h/U$	$g$ (°C)	$t_B$ (min)
<i>Clostridium botulinum</i>	HN	$2.3 \pm 0.04^a$	$3.1 \pm 0.9^a$	$2.1 \pm 0.4^a$	$14.8 \pm 1.7^a$
	HC	$2.4 \pm 0.15^a$	$3.2 \pm 0.3^a$	$2.1 \pm 0.1^a$	$13.2 \pm 0.5^b$
	HA	$2.4 \pm 0.15^a$	$4.1 \pm 0.9^b$	$2.3 \pm 0.2^b$	$14.1 \pm 0.6^c$

a-c Different letters between columns for each microorganism represent significant differences ( $p < 0.05$ ).

Source: Own elaboration.

### 6.4 Acknowledgements

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## 6.5 Conclusions

A thermal process applied to three hummus products: natural (HN), chipotle (HC) and with olives (HA) was designed and evaluated, using mathematical procedures and the thermal death kinetics of the microorganism, to calculate the lethality that guaranteed commercial sterility. The evaluation of the process allowed determining a holding time of 3 min, with which thermal death times higher than 2.5 min were obtained for all the hummus products studied. In particular, HA, which contained a greater amount of solid material, decreased heat transfer, generating the lowest Frequerido value. From the process design, the specific holding time for *Clostridium botulinum* and the different products was obtained, which avoids using unnecessary energy, making the process more efficient. This study demonstrated that the use of mathematical procedures and the kinetics of thermal death of microorganisms can be used to guarantee commercial sterility in the food industry.

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## **Chapter 7 Prevalence of thrombocytopenia in canine with clinical signs of ehrlichiosis in Poza Rica, Veracruz**

### **Capítulo 7 Prevalencia de trombocitopenia en caninos con signos clínicos de ehrlichiosis en Poza Rica, Veracruz**

ISLAS-PINEDA, Daniela, GARCEZ-MERCADO, Nora, ALARCÓN-ZAPATA, Marco Antonio and TABAREZ-ROJAS, Abigail\*

*Facultad de Ciencias Biológicas y Agropecuarias, Universidad Veracruzana. Tuxpan, México. Facultad de Ciencias Biológicas y Agropecuarias de la Universidad Veracruzana. Carretera Tuxpan-Tampico Km. 7.5, Colonia Universitaria, Tuxpan, Veracruz, México, CP 92895. Tel. 017838344350 ext. 46109.*

ID 1<sup>st</sup> Author: *Daniela, Islas-Pineda* / **ORC ID** 0009-0002-5699-7893

ID 1<sup>st</sup> Co-author: *Nora, Garcez-Mercado* / **ORC ID** 0000-0002-4712-4663

ID 2<sup>nd</sup> Co-author: *Marco Antonio, Alarcón-Zapata* / **ORC ID** 0000-0002-4712-6327, **CVU CONACYT ID:** 176712

ID 3<sup>rd</sup> Co-author: *Abigail, Tabarez-Rojas* / **ORC ID** 0000-0002-8766-6993, **CVU CONACYT ID:** 176667

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D. Islas, N. Garcez, M. Alarcón and A. Tabarez

\* atabarez@uv.mx

Á. Marroquín, L. Castillo, J. Olivares and N. Olgún Cruz. (VV. AA.) CIERMMI Women in Science T-XXI Biological, Humanities and Social Sciences. Handbooks-©ECORFAN-México, Querétaro, 2023

## Abstract

The objective of this study was to determine the prevalence of thrombocytopenia in dogs with clinical signs of canine Ehrlichiosis in the city of Poza Rica, Veracruz. It was used 32 blood samples from dogs with clinical signs compatible with canine ehrlichiosis. A platelet count by hematic biometry and the Uranotest Quattro test based on the immunochromatographic technique were used. To analyze the data, the Chi-square test of the statistical software SPSS 20 Windows was used. The results showed 43.8% positives samples for *E. canis* and the prevalence of thrombocytopenia was 81.3%. The statistical analysis carried out determined that the gender of the dogs was not a predisposing factor for the disease. Likewise, by age group, no statistically significant difference was shown. In addition, six animals positive for *Anaplasma* were detected, which represented 18.75% of prevalence in the sampled animals, of which five also presented thrombocytopenia and two of them were positive for both *Ehrlichia* and *Anaplasma*. Therefore, thrombocytopenia presented in canines may be due to other causes or other rickettsiae.

## Prevalence, Thrombocytopenia, *Ehrlichia canis*, *Anaplasma*

### Resumen

El objetivo de este trabajo de investigación fue determinar la prevalencia de trombocitopenia en perros con signos clínicos de Ehrlichiosis canina en la ciudad de Poza Rica, Veracruz. Se analizaron 32 muestras de sangre de caninos de diferente edad y sexo. Se utilizó un recuento de plaquetas mediante biometría hemática y la prueba URANOTEST QUATTRO basado en la técnica inmunocromatográfica. Para analizar los datos se utilizó la prueba Chi-cuadrada del paquete estadístico SPSS 20 Windows. Se detectó que el 43.8% de los caninos muestreados fueron positivos a *E. canis* y la prevalencia de trombocitopenia fue del 81.3%. El análisis estadístico realizado determinó que el género de los perros no fue un factor predisponente para la enfermedad. Así mismo, por grupo etario no se mostró una diferencia estadísticamente significativa. Además, se detectaron seis animales positivos a *Anaplasma* lo que representó el 18.75% de prevalencia en los animales muestreados, de los cuales cinco también presentaron trombocitopenia y dos de ellos resultaron positivos tanto a *Ehrlichia* como a *Anaplasma*. Por lo que, la trombocitopenia presentada en los caninos puede deberse a otras causas u otras rickettsias.

## Prevalencia, Trombocitopenia, *Ehrlichia canis*, *Anaplasma*.

### 7.1 Introduction

Canine ehrlichiosis is a tick-borne infectious disease caused by *Ehrlichia* spp. whose main etiological agent is *Ehrlichia canis*, a gram-negative, obligate intracellular, rounded or pleomorphic bacterium. This bacterium is transmitted by the tick *Rhipicephalus sanguineus*, which is distributed worldwide and its main host is the domestic dog (Hoyos, 2007; Gutiérrez et al., 2016).

The infective action of *E. canis* is produced by invading the cells of the white package that serve as transport to reach other systems and organs, *E. canis* has an enormous ability to induce severe lymphoplasmacytosis in organs that present parenchymal structure, producing bleeding in vascular endothelial cells, thus giving rise to the classic hemorrhages present and a great variety of clinical signs (Waner and Harrus, 2013; Tamez, 2015).

Ehrlichiosis is a very important disease since, by attacking the aforementioned cells, it depresses the immune system, resulting in: bone marrow aplasia, increased susceptibility to infections with other secondary agents, anemia, hemorrhage, slow kidney injury that often causes irreversible renal failure; all this affects the health and quality of life of the canine (Vadillo et al., 2002).

Thrombocytopenia is considered the most common and consistent hematologic abnormality in dogs infected with *E. canis*. Mechanisms thought to be involved in the pathogenesis of thrombocytopenia include increased platelet sequestration in the spleen and immune destruction or injury resulting in decreased platelet lifespan. In addition, studies in which radioisotopes have been used have shown that platelet survival time decreases from an average of nine to four days from two to four days after infection with *E. canis* (Harrus et al., 1999).

Therefore, the objective was to calculate the prevalence of thrombocytopenia in dogs with clinical signs of canine Ehrlichiosis in the city of Poza Rica, Veracruz and to determine if the presence of thrombocytopenia in dogs with clinical signs of canine Ehrlichiosis is a common denominator of this disease.

## 7.2 Material and Methods

Samples were collected in three veterinary clinics in the city of Poza Rica, Veracruz during the autumn-winter season. The study area is located in the northern part of the state, at coordinates 20°32'00"N 97°27'00"W. It presents a warm climate, with a mean annual temperature of 24.4 °C, with abundant rainfall in summer and early autumn. The average annual precipitation is 1,010 mm (INEGI, 2019).

The sample size was 32 dogs, which were given a clinical record with general data such as age, sex, history of having had a tick, clinical signs of *E. canis*; neurological signs, seizures, ataxia, epistaxis, hemorrhages, petechiae, ecchymosis, anorexia, uveitis, hematuria, melena, generalized skin lesions, hyperemia, edema. Blood samples were collected in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA) by aseptic puncture of the cephalic vein. Subsequently, a platelet count was performed by manual method sent to the clinical analysis laboratory Animalandia Poza Rica.

Dogs with thrombocytopenia were tested for *Ehrlichia canis* antibodies by immunochromatographic technique using the URANOTEST QUATTRO® diagnostic kit, which requires whole blood, serum or plasma. Subsequently, the sample is taken with a capillary tube and deposited in each well together with two drops of buffer, obtaining the results in 15 minutes.

### 7.2.1 Platelet count

The platelet count consists of knowing the number of platelets found in one milliliter of blood. For this it is necessary to dilute the blood using a Thoma pipette for white blood cells and a special diluent liquid that makes the platelets visible while destroying the erythrocytes. Subsequently, a known volume of the sample is examined under the microscope in the Neubauer chamber, counting the number of platelets in the reticule and by means of a calculation the number of elements per mm<sup>3</sup> is obtained (Rivadeneira, 2022).

### 7-2.2 Quattro Uranotest ® Diagnostic Kit

The URANOTEST QUATTRO diagnostic kit is based on the immunochromatographic technique and is designed for the qualitative determination of *Dirofilaria immitis* antigen and antibodies to *Ehrlichia canis*, *Anaplasma* and *Leishmania* in dog whole blood, serum or plasma. The test consists of four separate zones, one for the detection of *Ehrlichia* antibodies, one for the detection of *Anaplasma* antibodies, one for the detection of *Leishmania* antibodies and one for the detection of *Dirofilaria* antigen. Each test consists of a test strip with a zone where the sample is added and a result zone containing the T line (test line) and the C line (control line). Once the sample is applied to the rounded well and after the addition of the chromatography buffer, capillary migration along the membrane begins. If the result is negative, a single purple band will appear in the C-zone. The band in zone C always appears, as it is a control band indicating that the test has been performed correctly. If the result is positive, in addition to the C band, a purple band will appear in the test zone (T line) (URANOVet, 2022).

### 7.2.3 Reference value in platelets

In this work three different authors were taken into account, Tennant (1997) refers to a value to consider thrombocytopenia below  $150 \times 10^9/L$  platelets, Lopez and Mesa (2015) speak of values of  $175 \times 10^9 /L$  platelets. And Villiers and Blackwood (2013) of  $200 \times 10^9/L$  in this work it was considered to take the mean of these three values and resulted in a value of  $175 \times 10^9/L$  platelets.

### 7.2.4 Statistical analysis

The data were analyzed with the Chi-square statistical test using the SPSS 20 statistical package for Windows (IBM SPSS, 2011).

### 7.3 Results

The prevalence of thrombocytopenia in dogs with clinical signs of canine Ehrlichiosis was 81.3% (95%CI 67-96; Table 7.1) with a mean of  $115.03 \pm 74.92 \times 10^9/L$  platelets. However, after analyzing the variable thrombocytopenia and presence of the disease, it was determined that there is no statistical evidence to affirm that there is a relationship between thrombocytopenia and Ehrlichiosis in the sampled dogs ( $P=0.138$ ).

Table 1 Number of canines with thrombocytopenia and clinical signs of canine Ehrlichiosis

		Diagnostic to <i>E. canis</i>		Total
		Negative	Positive	
Thrombocytopenia	Negative	5	1	6
	Positive	13	13	26
Total		18	14	32

Source: Own elaboration/2023

Regarding the prevalence of canine Ehrlichiosis, it was determined that of the 32 samples processed by the rapid test for the detection of antibodies to *E. canis*, 14 were positive for this bacterium, which represents 43.8% (IC95% 26-62). In addition, it was determined through data collection that the gender of the dogs sampled was not a predisposing factor for presenting the disease ( $P=0.198$ ; Table 7.2). Likewise, when analyzing the results by age group, no statistically significant differences ( $P=0.791$ ) were observed for the age of the sampled canines (Table 7.3).

Table 7.2 Detection of antibodies to *Ehrlichia canis* according to the sex of the animals sampled

Diagnostic	Sex		Total
	Female	Male	
Negative	5	13	18
Positive	7	7	14
Total	12	20	32

Source: Own elaboration/2023

Table 7.3 Detection of antibodies to *Ehrlichia canis* according to age group

Age group	Diagnostic		Total
	Negative	Positive	
Adult	7	7	14
Puppy	4	3	7
Geronte	7	4	11
Total	18	14	32

Source: Own elaboration/2023

In the laboratory analysis using the rapid test, it was also detected that six animals were positive to Anaplasmosis, which represents 18.75% (IC95% 4-33) of prevalence of the animals sampled, of which five also presented thrombocytopenia and two of them were positive to both Ehrlichiosis and Anaplasmosis (Table 7.4).

**Table 7.4** Detection of antibodies to *Ehrlichia* and *Anaplasma platys*

Diagnostic	<i>Ehrlichia</i>	<i>Anaplasma</i>
Positive	14	6
Negative	18	26
Total	32	32

Source: Own elaboration/2023

## 7.4 Discussion

The results obtained in the laboratory analysis allowed determining that thrombocytopenia in dogs with clinical signs of canine Ehrlichiosis is not a determining factor for the diagnosis of this disease, since 81.3% (IC95% 67-96) of the animals sampled presented thrombocytopenia, but only 43.8% (IC95% 26-62) of the animals presented antibodies to *E. canis*, that is, of the 32 dogs sampled, 26 (81.3%) presented thrombocytopenia and of these, 13 animals (50%) were positive to *E. canis* and 13 (50%) animals were negative. Therefore, the thrombocytopenia presented in canines may be due to other causes, one of them being Anaplasmosis. In this study, the rapid test also detected the presence of Anaplasmosis in six animals, five of which had thrombocytopenia and four were negative for *E. canis*.

In Culiacán, Sinaloa Sosa-Gutierrez et al. (2013), evaluated 152 blood samples from dogs, with tick infestation and clinical signs of ECM, from six veterinary clinics and two shelters, 74.3% had anti-*E. canis* antibodies by Snap4Dx® ELISA from IDEXX Laboratory. In addition, 40.1% had morulae characteristic of *E. canis* in their blood smears. Regarding clinical manifestations, there was fever (91.2%), anorexia (86.7%), depression (85.0%), lethargy (72.6%) and petechiae (72.6%). Similarly, those positive for *E. canis*, 87.6% had thrombocytopenia.

Diaz et al. (2016), evaluated a population of 200 domestic dogs from Molas, Yucatan, Mexico, 70% were infested with 1,116 *R. sanguineus* ticks. In addition, a prevalence of *E. canis* of 71% was calculated. However, no association of gender, age, body condition, bleeding, thrombocytopenia and tick infestation with *E. canis* infection was found.

In the province of Maynas-Iquitos, Peru, Villaverde (2017), evaluated a group of dogs with clinical suspicion of Ehrlichiosis and thrombocytopenia and determined the presence of antibodies to *Ehrlichia* spp in 60% of the canines. It was also reported that the canines with thrombocytopenia had a median age of 18 months (inter-quartile range (IQR): 12 to 24 months), 16 (53%) were males, 22 (73%) were mongrels and 12 (40%) came from the Iquitos district. In the clinical examination, 13 (43%) canines had ticks, 23 (77%) had a history of having had ticks and 13 (43%) had fever. The mean platelet count was 33,000 platelets/ $\mu$ L (IQR: 23,000 to 69,000). Application of the IDEXX SNAP4DX diagnostic kit detected antibodies to *Ehrlichia* in 18 (60%) of the canines evaluated. Of the 18 canines with positive antibodies, 13 were mongrels, nine were males. On clinical examination, 14 had a history of having had ticks, seven had evidence of ticks and five had fever. Canines with positive antibodies to *Ehrlichia* spp had a median age of 24 months compared to 12 months for canines without infection, however, there was no statistically significant difference ( $p = 0.06$ ). No statistically significant association was found between the presence of *Ehrlichia* antibodies and breed ( $p = 0.6$ ), sex ( $p = 0.4$ ), history of ticks ( $p = 0.5$ ), presence of ticks at the time of evaluation ( $p = 0.4$ ) and fever ( $p = 0.06$ ). These results coincide with those reported in this study where no association was found between the variables sex, age and race. The platelet count was 28,500/ $\mu$ L in canines with positive antibodies to *Ehrlichia* compared to 37,000/ $\mu$ L in canines without infection, in our study it was found with a mean of  $115.03 \pm 74.92 \times 10^9/L$  platelets.

Flores(2020), determined that the prevalence of canine Anaplasmosis in dogs with thrombocytopenia in the province of Maynas, Iquitos, obtained antigenic evidence confirming the presence of *Anaplasma* spp. in the study sector of an overall prevalence of 20.10%  $\pm$ 0.02 with a confidence level of 95%, constituting a report of its presence in this study area. The studied area presents risk factors associated with Anaplasmosis. Due to the high rate of cases found in this research, this is considered of scientific connotation since it is a triggering factor of a large enzootic disease.

Harvey (1978), cited by Arraga-Alvarado et al. (2003) indicated that *Anaplasma platys* is the causal agent of clinical infectious thrombocytopenia. This gram-negative, obligate intracellular bacterium has an affinity for canine platelets and causes cyclic thrombocytopenia that can last between seven and 14 days (Ettinger, 1992). This thrombocytopenia is apparently of a regenerative type, due to the megakaryocytic hyperplasia found in the bone marrow of experimentally infected dogs (Gaunt et al., 1990).

Román (2021), comments that another disease causing thrombocytopenia is canine hepatozoonosis; it is caused by a protozoan called *Hepatozoon canis* which is found inside neutrophils and monocytes of vertebrates in the form of microgametes and macrogametes. It is transmitted by *Rhipicephalus sanguineus* which ingests the microorganism by feeding on the blood of an infected canine. Platelets originate from megakaryocytes present in bone marrow and to a lesser extent in the lung. The causes of decreased platelet production may be due to alterations in these precursor cells, but are usually disorders that also affect other hematopoietic cell lines (Weiss et al., 2010). Among the most frequent disorders we can mention bone marrow hypoplasia or aplasia, drug myelotoxicity, necrosis, myelofibrosis, sclerosis, myeloptosis, myelodysplasia, increased cell sequestration or destruction, immune-mediated alterations, sepsis, disseminated intravascular coagulation, hemangiosarcoma and splenomegaly (Nelson and Couto, 2010).

Recently, Christodoulou et al. (2023), by comparing the clinical and clinicopathological features of ehrlichiosis and primary immune thrombocytopenia in dogs diagnosed with these partially overlapping diseases, determined that dogs with ehrlichiosis had a lower albumin concentration, therefore, it may be a useful discriminator between the two diseases in the clinical setting.

Although the URANOTEST QUATTRO diagnostic kit has a high sensitivity and specificity, a small incidence of false positive or negative results cannot be ruled out. As with any other laboratory procedure, a definitive clinical diagnosis should not be based on the performance of a test alone, but should be the result of a series of clinical and laboratory findings. In case of doubt, repeat the test and/or contrast with other diagnostic methods (URANOvet, 2022).

Regarding susceptibility by gender, it was determined by statistical analysis to the antibody response against *E. canis* that there is no effect, this coincides with Franco-Zetina et al. (2022) in the city of Merida, Yucatan, who also indicates that the sex of the animals did not present differences, so it was not a factor associated with the antibody response against *E. canis*. Similarly, Requejo (2018), in the district la Victoria, Peru, in his research indicates that in females there is a prevalence of 65.85% (27/41) and in males of 70.18% (40/57). However, the number of male canines evaluated was higher than the number of females; therefore, it cannot be affirmed that canine ehrlichiosis is more prevalent in males than in females.

## 7.5 Conclusion

According to the results obtained it is concluded that 81.3% (CI95% 67-96) of the dogs sampled with clinical signs of canine Ehrlichiosis presented thrombocytopenia and only 43.8% (CI95% 26-62) were positive for *E. canis*, which determines that it is not a common denominator for the disease, since thrombocytopenia does not ensure a positive diagnosis of *E. canis* because it can be due to other causes. Likewise, 18.75% of the 32 dogs were positive for *Anaplasma*.

According to the statistical results of the chi-square test, the age and sex of the canines sampled were not predisposing factors for the presentation of canine ehrlichiosis.

## 7.6 Acknowledgements

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## Chapter 8 Financial management in SMEs of two municipalities in the north zone of the State of Mexico

### Capítulo 8 Gestión financiera en PyMes de dos municipios de la zona norte del Estado de México

ESCAMILLA-SALAZAR, Zugaide´\*, BADA-CARBAJAL, Lila Margarita´´ and BECERRIL-GARCIA, Analady´

´Centro Universitario Atlacomulco, Universidad Autónoma del Estado de México, México.

´´Tecnológico Nacional de México, Instituto Tecnológico Superior de Álamo Temapache, México.

ID 1<sup>st</sup> Author: *Zugaide, Escamilla-Salazar* / **ORC ID:** 0000-0002-3666-4760

ID 1<sup>st</sup> Co-author: *Lila Margarita, Bada-Carbajal* / **ORC ID:** 0000-0001-7757-5601

ID 2<sup>nd</sup> Co-author: *Analady, Becerril-García* / **ORC ID:** 0009-0000-6994-936X

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Z. Escamilla, L. Bada and A. Becerril

\* zescamillas@uamemex.mx

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## **Abstract**

Financial management is vital to the growth and development of small and medium-sized enterprises (SMEs). Therefore, an adequate implementation supports financial decision-making, allowing opportunities for monetary sustainability. Therefore, the present work aims to analyze some financial management variables in SMEs in two municipalities in the north of the State of Mexico to detect those financing alternatives they use for development and consolidation. The main results show that SMEs in Atlacomulco and Ixtlahuaca opted for external financing, leaving internal financing in the background.

## **Financial Management, Financing and SMEs**

### **Resumen**

La gestión financiera es clave para el crecimiento y desarrollo de las pequeñas y medianas empresas (pymes), por ello, una adecuada implementación apoya la toma de decisiones financieras, permitiendo oportunidades de sostenibilidad monetaria. Por tanto, el presente trabajo tiene como objetivo analizar algunas variables de la gestión financiera en las pymes de dos municipios de la zona norte del Estado de México a fin de detectar aquellas alternativas de financiamiento que utilizan para el desarrollo y consolidación. Los principales resultados muestran que las PyMes tanto en Atlacomulco como en Ixtlahuaca apuestan por el uso del financiamiento externo, dejando en un segundo plano al interno.

## **Gestión Financiera, Financiamiento y Pymes**

### **8.1 Introduction**

Delve into financial management emphasizes the studies done to formulate approaches or theories that seek to explain and enrich it and to understand the importance of developing and consolidating small and medium-sized companies, which are essential in the world economy. However, despite all the contributions that SMEs make in economic terms, they also face different challenges that limit their development and consolidation in the market where they have a presence, which is often due to poor financial management on the part of the owners or administrators, as they are the people in charge of making decisions in them. In this sense, the present work aims to analyze some variables of financial management in SMEs in two municipalities in the north of the State of Mexico to detect those financing alternatives that they use for development and consolidation.

Therefore, the central hypothesis raised is that financial management analysis (financing, use of accounting and financial information and characteristics of the company) in the SMEs of two municipalities in the northern area of the State of Mexico allows the detection of financing alternatives. That they used for development and consolidation.

The research is structured by a brief literature review on the importance of creating companies immediately after the financing characteristics and problems for SMEs are presented, followed by the importance of financial management in SMEs, management theories and models. Financial, as well as the theoretical model selected for this work, and finally, the results and conclusions are shown.

#### **8.1.1 Business creation**

Small and Medium-sized Enterprises (SMEs) have growing importance, especially in developing countries, due to their contribution to employment and economic well-being. Also, because these types of companies are part of the productive apparatus, as part of the value chain, which contributes to diversification and revitalization of the economy, which translates into the creation of more employment and promotion of wealth (Cardoso et al., 2012), in this sense, Saavedra and Hernández (2008) refer that small and medium-sized companies are more than necessary; they are indispensable because they are the main generators of employment and income among the population and regions; they are essential in the existence of large companies and represent a factor of social cohesion.

While Oropeza and García (2013) point out that SMEs worldwide are the most significant number of economic units, they also employ the most personnel; therefore, their relevance and need to strengthen their performance through the improvement of internal processes. Díaz et al. (2005), for their part, express that the creation of companies directly affects the solution of three problems: economic growth, employment generation and business innovation.

According to Chagerben et al. (2017), financing is critical for the development and growth of SMEs since, through it, they can continue with daily operations and even increase productive capacity, allowing them to generate more income and increase revenues—financial performances. However, according to León and Saavedra (2018), access to financing is limited due to high-interest rates, the lack of access to long-term credit, and difficulties establishing guarantees. Regarding the above, it is unfortunate that different aspects limit financing even though this is key in the creation, continuity and growth of companies by everything that can be done or achieved by obtaining it.

As mentioned in previous paragraphs, even though SMEs are the engine of development and economic growth in countries, they present multiple problems that prevent them from remaining in the market for a long time due to their high mortality rates. Hence, eighty per cent of these companies fail to exceed two years (Raufflet et al., 2021, as cited in Laitón and López, 2018). The preceding because they start their operations without sufficient capital, for which reason they cannot ensure their evolution; additionally, given their nature, obtaining resources is difficult, mainly when it comes to financing because the characteristics of the company such as age, size, sector, among others directly condition this. Intriago et al. (2021) confirm that there are limitations in the development of small and medium-sized companies, one of which is access to financing since many of these are created, developed and even positioned informally in the market. , which means they do not have accounting and financial information when applying for a loan. Therefore, the lack of financing for their subsistence constitutes one of the main problems faced by these types of companies; in addition, some entrepreneurs do not go to the existing sources of financing, either due to ignorance or lack of interest. , although it can also be because they do not meet the requirements: credit history, economic solvency or sufficient guarantees (Guercio et al., 2015).

Recapitulating what has already been mentioned, obtaining financing can sometimes be a problem that SMEs have to face repeatedly, but also the ignorance of its existence is a problem since, despite being excellent businesses, they cannot grow; it is that is, to go from small to medium and from medium to large companies.

### **8.1.2 Importance of financial management in SMEs**

Cabrera et al. (2017) postulate that one of the significant challenges that companies face is to be able to administer and manage the financial resources they have efficiently; this is because any activity carried out within a company must inevitably be expressed in monetary terms. For their part, Rodríguez (2016) and Terrazas (2009) argue that financial management is essential in SMEs since it helps the administration, planning and control of financial resources that give sustainability to it. Likewise, financial management is vital because it is related to the control of operations, the search for new sources of financing, operational effectiveness and efficiency, the reliability of financial information, and compliance with applicable laws and regulations. However, it is closely related to the size and composition of the assets, financial level and structure and focuses on profit maximization and wealth (Córdoba, 2012). For this reason, financial management plays an essential role in SMEs, since when these types of companies put it into practice, they achieve an efficient administration of the necessary resources for their continuity, the above by maximizing profit and wealth, given their relationship with operations, financing and information.

### **8.1.3 Classification of financing sources**

According to Van Horne and Wachowicz (2002, as cited in Bohórquez, López and González, 2013), financing sources are essential tools that help companies ensure financial sustainability. Hence the proper selection of this type of financing is crucial, since it will influence the company's ability to generate the necessary funds to recover or pay as appropriate since financing can be both internal (own resources) and external (third-party resources).

In this order of ideas, Bohórquez et al. (2018) mention that internal financing is constituted by the company's resources, obtained through contributions from the owners or shareholders, using the daily flows, or from the profits generated during a given period. Regarding external financing Levy (2008), cited in Torres et al. (2017), is one in which third parties are involved because their resources are insufficient for the normal development of the company.

#### **8.1.4 Financial management characteristics**

In this context, the financial function is integrated by all those tasks related to the achievement, use and control of resources, they also mention that financial management is related to decision-making related to the size and composition of assets, financial structure and dividend policy, so each company must define its strategic objectives from its particular perspectives. It has needed (Cabrera et al., 2017). Given the above, it is essential that each company analyzes its particular situation, that is, its characteristics, and based on these, determine the necessary activities that allow it to fulfil its strategic objectives through the use and control of its resources.

#### **8.1.5 Theories or models on financial management**

According to Gómez, García and Marín (2008), since the 1960s, theories have been developed to explain the factors that influence the financing preferences of companies. However, they are mainly focused on large companies. Therefore, of these is the Hierarchy Theory, Pecking order Theory (POT) Order of preference theory, which says that when companies need financing, they first resort to internal financing through retained earnings. However, when they require external financing, they first acquire bank debt, followed by long-term obligations and finally, the issuance of shares. Along the same lines, the Financial Cycle Theory states that information asymmetry is among the problems companies must face when they request external financing (Berger and Udell, 1998). In this context, several theories or models exist regarding financial management. However, these are focused on financing since they state entrepreneurs' preferences before the options that exist, as well as the problems that revolve around them are around this.

#### **8.1.6 Recent Financial Management Research**

Regarding the research related to the study of financial management, some works were detected; in Europe, there was the work of Intriago et al. (2021), while in America, works such as that of Paredes et al. (2019) were found, and in Ecuador found Marcotrigiano (2013), while in Venezuela those of Martínez et al., (2017), while in Colombia there is Lopera et al., (2014). On the other hand, in Mexico, there are the works of Oropeza and Garcia (2013), Saavedra, Tapia and Aguilar (2016), Esparza et al. (2010), Gómez, Garcia and Marín (2008) and Saavedra and Milla (2017) in the states of Tabasco, Mexico City, Quintana Roo, Puebla and Querétaro respectively. Although there are few investigations carried out recently, thanks to these, it has been possible to shed light on knowledge. Therefore, it has been possible to formulate new theories and models which could have modified existing ones.

#### **8.1.7 Generalities of the financial system**

The financial system is a subject investigated since it promotes transferring economic resources or money to those who require it. Hence it is essential because it offers different benefits, among which are the growth of the economy through the promotion of economic activities and minimising economic crises. However, the financial system has challenges, which, once overcome, would lead to more significant benefits than those it already offers to individuals and companies since it would make it possible to change the panorama in this context.

Regarding the concept, López and Lara (2017) state that the financial system comprises financial intermediaries or entities that promote the transfer of resources through specialized instruments to comment on financial inclusion. In the same way, Morales (2017) mentions that the financial system can also be known as the financial sector and that this is made up of institutions authorized by the State that are in charge of capturing, managing and investing the money of natural or legal persons and National and international. For his part, Méndez (1999) argues that the financial system comprises the stock and capital market, the set of institutions that provide credit to finance different economic activities. Going back to what the authors pointed out, the financial system can be conceptualized as those institutions and entities authorized by the State that act as intermediaries that offer credit to people or companies that need it through stock or capital markets.

Regarding the importance, the financial system is fundamental in the structure of a country because it promotes the economy's growth by promoting certain economic activities that lead to development. Therefore, financial stability is considered a fundamental pillar in the construction of the financial system to avoid and minimize the consequences of economic crises and their spread to other economies (Terceño and Guercio, 2010). In a word, the financial system is essential under all the benefits people and companies can obtain thanks to it, given that it promotes those who need it and improves the economic context, which translates into stability.

Regarding the challenges of the financial system, Quintana (2014) establishes that financial systems have been unable to promote the development and growth of the economy since how it operates only benefits large companies, promoting inequality and weakening the system. Democratic mainly in Latin America. Therefore, there is the challenge of transforming the framework that regulates the financial system at the international level because the effects of the crises would be minimal if this change were to take place. Uribe (2013), in turn, points out that another challenge is to create an adequate regulation for the different activities carried out by institutions in the financial markets, thus limiting the creation of financial instruments or operations outside of the regulation.

### **8.1.8 Some recent research on the financial system**

According to Terceño and Guercio (2010), in the economic literature, concern about the effects of the financial system on economic growth is visible, given that these two variables are interrelated. Likewise, they show the correlation that exists between indicators of the financial system. and the GDP; they also verify that in the Latin American economies, the banking sector presents a more significant link with the growth of the economy, regardless of the financial structure that the country has. For his part, Uribe (2013) analyzes the structure, the conglomerates, the operation and the leading indicators of the Colombian financial system to delimit its behaviour. Meanwhile, De Jesús et al. (2021) consider that the emergence of collective funding was a solution to the shortcomings that financing presents in Mexico, both for investors and for funders, as it is a watershed in the risk relationship. performance of the offer of the Mexican financial system, being a technological finance sector with a formal, reliable and transparent scheme. Although there are few recent investigations on the financial system, they have contributed in one way or another to formulating new theories and models or modifying existing ones, which means a contribution to knowledge.

### **8.1.9 Research carried out with the variables under study.**

Next, the variables included in this work are presented as a group, extracted from a brief review of the literature on financial management. In this sense, SMEs are an economic unit that produces goods and services, at a small size, with a limited number of workers and market coverage, usually directed by the owner or an external person (Cardozo et al., 2012 ), generating employment and economic well-being that they generate in the areas where they have a presence. To which, for the present investigation, it is divided into three variables, the first is financing, the second is the use of accounting and financial information, and the third is the characteristics of the company.

Regarding financing, according to Bohórquez et al. (2018) explain that financing can come from two sources: the use of own resources and access to external sources. In this sense, Treviño et al. (2012), based on a study carried out with exporting SMEs from Nuevo León (Mexico), distinguish that bank financing generates a significant increase in sales and, in turn, López and Lara (2017) specify After an investigation of the banking institutions that provide credit in Mexico, Banco Santander is the best option for this type of company. However, Esparza et al. (2010) show that companies established in emerging economies do not resort to bank financing due to the costs to access it, the little development of the Mexican banking system, and above all, for fear of default. Therefore, they prefer using their resources as the primary source of financing.

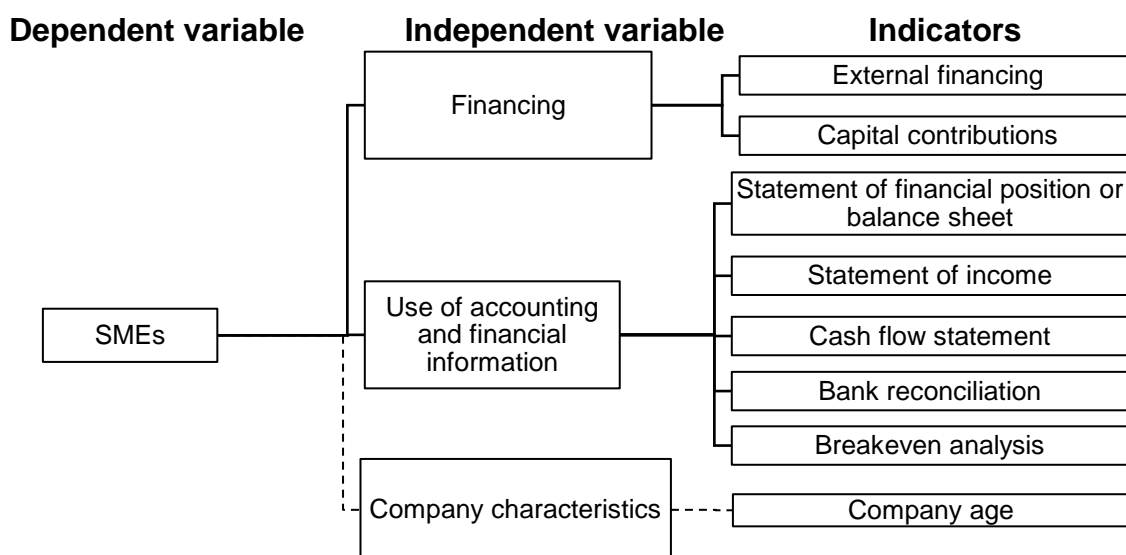
Regarding the use of accounting and financial information, Martínez et al. (2017) mention that lacking this is one of the main obstacles SMEs present when seeking financing. Oropeza and Garcia (2013), in turn, postulate that this depends on the size of the companies since, as their size increases, so does their application level. For their part, Saavedra-García, Tapia-Sánchez, and Aguilar-Anaya (2016) mention that even though SMEs have an accounting information system, they do not dimension what this means since their concern is focused on complying with their tax obligations and not having problems with Despite the Treasury, thanks to this, decisions on investments and credits could be made, the financial resources of the business (origin and characteristics) evaluated, as well as the financial management that is carried out (Sandoval, 2008). However, according to the investigation of Saavedra, Tapia and Aguilar (2015), with a sample of 300 SMEs from the Federal District, found that there is a relationship between business studies and the adoption of accounting and financial information in the same way, that the preparation of financial forecasts and analyzes is scarce. In this sense, Collin and Jarvis (2002), through a study carried out on 385 SMEs in the United Kingdom, identify that this type of company uses formal methods for planning and control.

Regarding the characteristics of the company (age of the company), León and Saavedra (2018) specify that for external financing (bank credit), they usually require SMEs to be between 2 and 3 years old. In addition, Oropeza and Garcia (2013) verify a positive relationship between bank financing and the company's age, since as age increases, the use of financing also increases, although it is almost null.

### 8.1.10 Choice of theoretical model

The present work has as a solid base the research carried out by Esparza et al. (2010); said research includes two fundamental variables within financial management: financing and the use of accounting and financial information (see Figure 8.1).

**Figure 8.1** Diagram of study variables



Source: Self made.



## 8.2 Methodology

### 8.2.1 Study population

In this context and for the present investigation, the small and medium-sized companies of the State of Mexico are considered as population, according to data provided by the Institute of Statistical and Geographic Information (IEEG) of the year 2018, in the State of Mexico there are a total of 3,955 companies, of these companies 3,169 correspond to small and 786 to medium-sized companies.

### 8.2.2 Sample selection

To calculate the sample size, a probabilistic sample was used under the formula calculating a margin of error of 12.15%, a confidence level of 95%, with a population size of 3,955 and 50% heterogeneity, obtained a sample size of 65 SMEs (see Table 8.1).

**Table 8.1** Research data sheet

<b>Geographic scope</b>	The municipalities of Atlacomulco and Ixtlahuaca.
<b>Universe population</b>	Small and medium businesses
<b>Size of the universe</b>	3,955
<b>Application mode</b>	Survey through structured questionnaire
<b>Sampling method</b>	Probability sampling
<b>Sample size</b>	65
<b>Mistake</b>	12.15%
<b>Confidence level</b>	95%

*Source: Self made.*

### 8.2.3 Measuring instrument design

The measurement instrument to obtain data used in this research is the questionnaire used by Esparza et al. (2010), at the convenience of this work. The instrument is made up of 11 statements in 3 blocks, block one referring to company characteristics, is made up of four multiple-choice questions; block two, referring to financing, is made up of 2 statements with a Likert scale of five positions, where 1 is little use and 5 a lot of use and block three of accounting and financial information for 5 approaches with a Likert scale of five positions, where 1 is no use and 5 much use.

It should be noted that this instrument will be applied electronically through a form in Google Forms and will be addressed directly to company managers, considering the structural characteristics of small and medium-sized companies and the lack of economic databases in Mexico where the data obtained were analyzed using Cronbach's  $\alpha$ .

### 8.2.4 Coding of the variable skills and perceived abilities

To carry out the data processing, an exhaustive review of the information collected from the questionnaires applied during October and November 2021 to the owners or administrators of small and medium-sized companies in the municipalities of Atlacomulco and Ixtlahuaca respectively, was carried out without. However, it should be noted that responses were also obtained from business people belonging to other municipalities located in the northern zone of the State of Mexico, obtaining a total of 64 responses.

The review process consisted of the following:

- 1) Check that the answers to the questionnaires belonged to entrepreneurs of small or medium-sized companies.
- 2) Check that the place where the small and medium-sized companies surveyed belong to municipalities located in the northern zone of the State of Mexico (Atlacomulco and Ixtlahuaca).
- 3) Verify that the questions contained in the questionnaire were answered.

Once the review process was completed, the data obtained was recorded in an Excel spreadsheet, which was transferred to the statistical program SPSS version 20.0 for the social sciences, performing the analysis and coding of the data (see Table 8.2).

**Table 8.2** Coding of perceived skills and abilities

<b>CODING</b>	<b>ITEM</b>	<b>DESCRIPTION</b>
Company characteristics BUED	Company age	Variable of scalar type corresponding to the age of the company. 1) 0 to 2 years 2) 3 to 5 years 3) 6 to 8 years 9 to 11 years
Use of accounting and financial information BTBG	Statement of financial position or balance sheet	Likert scale that identifies the degree of use of the statement of financial position or balance sheet as a source of accounting and financial information. (1= Little use, 5= Much use).
BTER	Income statement (sales, costs, expenses)	Likert scale that identifies the degree of use of the income statement (sales, costs, expenses) as a source of accounting and financial information. (1= Little use, 5= Much use).
BTFE	Cash flow statement (cash flow or cash flow)	Likert scale that identifies the degree of use of the cash flow statement (cash flow or cash flow ) as a source of accounting and financial information. (1= Little use, 5= Much use).
BTCB	Bank reconciliation	Likert scale that identifies the degree of use of bank reconciliation as a source of accounting and financial information. (1= Little use, 5= Much use).
BTPE	Analysis of the break-even point (sales-expenses and costs)	Likert scale that identifies the degree of use of the break-even analysis (sales-expenses and costs) as a source of accounting and financial information. (1= Little use, 5= Much use).
Financing BDFI	external financing	Likert scale in which the degree of use of external financing in companies is identified. (1= Little use, 5= Much use).
BDAC	Capital contributions (shareholders or owners)	Likert scale in which the degree of use of capital contributions in companies is identified. (1= Little use, 5= High use)

*Source: Self made.*

### 8.3 Results

Significant correlation results were obtained between the company group (BUEF) and independent variables (BDFI, BDAC, BTBG, BTER, BTFE, BTCB and BTPE); however, in a second statistic considering all the variables, more significant results were obtained (an additional correlation). In this sense, according to the results obtained, the following significant correlations are recorded (see Table 8.3):

**Table 8.3** Correlation of the financing dimension and accounting and financial information

		BUED	BUSE	BUEF	BUUG	BDFI	BDAC	BTBG	BFER	BTFE	BTCB	BTPE
<b>BUED</b>	Pearson correlation	1										
	Next (bilateral)											
	No.	65										
<b>BUSE</b>	Pearson correlation	-0.097	1									
	Next (bilateral)	0.44										
	No.	65	65									
<b>BUEF</b>	Pearson correlation	0.122	0.028	1								
	Next (bilateral)	0.334	0.824									
	No.	65	65	65								
<b>BUUG</b>	Pearson correlation	0.115	-0.151	0.016	1							
	Next (bilateral)	0.364	0.231	0.899								
	No.	65	65	65	65							
<b>BDFI</b>	Pearson correlation	-0.111	-0.002	-0.08	-0.003	1						
	Next (bilateral)	0.378	0.985	0.528	0.978							
	No.	65	65	65	65	65						
<b>BDAC</b>	Pearson correlation	-0.172	.312*	0.035	0.093	0.116	1					
	Next (bilateral)	0.171	0.011	0.784	0.459	0.357						
	No.	65	65	65	65	65	65					
<b>BTBG</b>	Pearson correlation	-0.088	-0.077	0.213	-0.003	0.103	-0.004	1				
	Next (bilateral)	0.488	0.544	0.089	0.98	0.416	0.976					
	No.	65	65	65	65	65	65	65				
<b>BFER</b>	Pearson correlation	-0.028	-0.111	0.026	0.032	-.289*	0.024	.350**	1			
	Next (bilateral)	0.828	0.377	0.837	0.803	0.02	0.851	0.004				
	No.	65	65	65	65	65	65	65	65			
<b>BTFE</b>	Pearson correlation	0.078	-0.111	0.027	-0.026	0.032	0.119	.507**	.510**	1		
	Next (bilateral)	0.534	0.38	0.83	0.835	0.8	0.344	0	0			
	No.	65	65	65	65	65	65	65	65	65		
<b>BTCB</b>	Pearson correlation	-0.134	0.143	-0.202	-0.135	-0.077	.279*	.342**	.524**	.422**	1	
	Next (bilateral)	0.288	0.257	0.106	0.284	0.54	0.025	0.005	0	0		
	No.	65	65	65	65	65	65	65	65	65	65	
<b>BTPE</b>	Pearson correlation	-0.104	-0.072	-0.179	0.118	-0.115	.329**	0.205	.455**	.395**	.304*	1
	Next (bilateral)	0.41	0.569	0.155	0.349	0.361	0.007	0.101	0	0.001	0.014	
	No.	65	65	65	65	65	65	65	65	65	65	65

Age (BUED); economic sector (BUSE); family business (BUEF); geographic location (BUUG); external financing (BDFI); capital contributions (shareholders or owners) (BDAC); statement of financial position or balance sheet (BTBG); income statement (sales, costs, expenses) (BTER); cash flow (cash flow) (BTFE); bank reconciliation (BTCB) and break-even point (sales-expenses and costs) (BTPE).

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

Source: Self made.

There is a weak positive correlation between the economic sector of the company (BUSE) and capital contributions (shareholders or owners) (BUAC) ( $r=0.312$ ), indicating a relationship of 9.73% ( $r^2=0.097344$ ).

The correlation between external financing (BDFI) and the income statement (BTER) is weak negative ( $r=-0.289$ ), therefore, there is a relationship of 8.35% ( $r^2=0.083521$ ).

On the other hand, capital contributions (shareholders or owners) (BUAC) and bank reconciliation (BTCB) show a weak positive correlation ( $r=0.279$ ), for which the coefficient of determination indicates a relationship of 7.78% ( $r^2=0.077841$ ).

Capital contributions (shareholders or owners) (BUAC) with the break-even point (BTPE) are weakly positively correlated ( $r=0.329$ ) by 10.825 according to the coefficient of determination ( $r^2=0.108241$ ).

While there is a weak positive relationship between the balance sheet (BTBG) and the income statement (BTER) ( $r=0.350$ ), representing a 12.25% relationship between both ( $r^2=0.1225$ ), that is, for the performance of the balance sheet small and medium-sized companies rely on the income statement.

Similarly, there is an average positive correlation between the balance sheet (BTBG) and the cash flow (BTFE) ( $r=.507$ ) where they are related by 25.70% according to the coefficient of determination ( $r^2=0.257049$ ).

In the same way, the general balance (BTBG) is positively weakly correlated with the bank reconciliation (BTCB) ( $r=.342$ ), indicating the coefficient of determination ( $r^2=0.116964$ ) that there is a relationship of 11.69%.

Likewise, there is an average positive relationship between the income statement (BTER) and the cash flow (BTFE) ( $r=0.510$ ), for which the coefficient of determination ( $r^2=0.2601$ ) indicates that there is a relationship between the items of 26.01%.

Likewise, the items income statement (BTER) and bank reconciliation (BTCB) are positively correlated on average ( $r=0.524$ ), with a relationship of 27.45% ( $r^2=0.274576$ ).

Between the income statement (BTER) and the break-even point (BTPE) there was a weak positive correlation ( $r=0.455$ ), for which the coefficient of determination ( $r^2=0.20705$ ) indicated that there is a relationship of 20.70%.

Similarly, there is a weak positive correlation between cash flow (BTFE) with bank reconciliation (BTCB) ( $r=.422$ ), relating to 17.80% based on the coefficient of determination ( $r^2=0.178084$ ).

The cash flow (BTFE) and the break-even point (BTPE) are related in a weak positive way ( $r=0.395$ ) indicating a relationship of 15.60% ( $r^2=0.156025$ ).

And between the bank reconciliation (BTCB) with the break-even point (BTPE) there is a weak positive relationship ( $r=0.304$ ), so there is a relationship of 9.24% ( $r^2=0.092416$ ).

#### **8.4 Gratitude**

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#### **8.5 Conclusions**

Based on the general objective stated at the beginning of the investigation, which consists of analyzing some variables of financial management in SMEs of two municipalities in the northern zone of the State of Mexico in order to detect those financing alternatives that they use for development and consolidation, carried out from the adaptation of an instrument constituted by Esparza et al. (2010), which measures financial management in companies through financing and the use of accounting and financial information, the following is found:

Concerning financing, small and medium-sized companies in both Atlacomulco and Ixtlahuaca make greater use of external financing, leaving internal financing in the background, which is contrary to what the Hierarchy Theory, Pecking order Theory (POT) or Preference Order Theory, and research such as that of Cemil et al. (2006) with 851 companies in 18 cities in the Black Sea region of Turkey, Esparza, et al. (2010) with 122 tourism MSMEs from the State of Quintana Roo (Mexico) and Eldomiaty et al. (2017) in their empirical evaluation, since these reflect that companies follow an order of preference and are financed in the first instance with their resources. The aforementioned is probably due to the international economic situation due to the Covid-19 pandemic, which has limited small and medium-sized companies' ability to subsist on their own, that is, through their resources.

Meanwhile, the use of accounting and financial information by managers for decision-making is mainly supported by two financial statements: a statement of financial position or balance sheet and an income statement; in this sense, Collin and Jarvis (2002), Through a study carried out with 385 SMEs in the United Kingdom, they identify those small and medium-sized companies use formal methods for planning and control. Additionally, Saavedra, Tapia and Aguilar (2016), in their research with 300 SMEs in the Federal District (Mexico), mention that even though these companies have an accounting information system, their concern is focused on complying with their tax obligations and not having problems with the Treasury, which demonstrates its frequent use in this type of company.

However, the other financial statements: cash flow statement, bank reconciliation and analysis of the break-even point, have a significant presence but less than the previous ones, by which the most significant ones are created; that is, they provide the information that allows its creation and therefore its delivery, although they may be omitted on occasions.

Continuing with this order of ideas, it is concluded that financial management in small and medium-sized companies in the Municipalities of Atlacomulco and Ixtlahuaca, State of Mexico, is based on the use of external financing, which changes the theoretical approach that exists in this regard, and that in terms of accounting and financial information, they only use two financial statements to a large extent: statement of financial position or balance sheet and income statement, which have the particularity that they must be delivered to the Tax Administration System (SAT).

In summary, it is possible to specify that the hypothesis raised at the beginning of this investigation is accepted, since through the analysis of financial management (financing, use of accounting and financial information and characteristics of the company) in SMEs in the area north of the State of Mexico (Atlacomulco and Ixtlahuaca) it was possible to detect the financing alternative that they use for their development and consolidation, this being external financing, which is a contribution in the light of knowledge since it contradicts what the theory affirms to the regard. However, this is likely due to the situation in the State of Mexico and even more specifically in the northern zone, given that a solid economic crisis has broken the possibility of internal financing, which was the most used until recently.

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## Chapter 9 Bioinformatic practical applications in biotechnology, medicine, environmental and agricultural sciences

### Capítulo 9 Aplicaciones prácticas de la bioinformática en biotecnología, medicina y ciencias medioambientales y agropecuarias

RAGGI, Luciana†\*<sup>1</sup>, GODOY-LOZANO, Elizabeth Ernestina<sup>2</sup>, JIMENEZ-JACINTO, Verónica<sup>3</sup> and ESCOBAR-ZEPEDA, Alejandra<sup>4</sup>

<sup>1</sup> *CONAHCYT - Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo*

<sup>2</sup> *Centro de Investigación Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública, Cuernavaca, Morelos, Mexico*

<sup>3</sup> *Unidad Universitaria de Secuenciación Masiva y Bioinformática, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Morelos, Mexico.*

<sup>4</sup> *Microbial Informatics Team, European Bioinformatics Institute (EMBL-EBI), Cambridge, UK*

ID 1<sup>er</sup> Author: *Luciana, Raggi* / **ORC ID:** 0000-0001-8502-4834

ID 1<sup>er</sup> Co-author: *E. Ernestina, Godoy-Lozano* / **ORC ID:** 0000-0001-6927-9132

ID 2<sup>do</sup> Co-author: *Verónica, Jiménez-Jacinto* / **ORC ID:** 0000-0001-6742-1537

ID 3<sup>rd</sup> Co-author: *Alejandra, Escobar-Zepeda* / **ORC ID:** 0000-0003-3549-9115

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L. Raggi, E. Godoy, V. Jimenez and A. Escobar

\* luciana.raggi@umich.mx

Á. Marroquín, L. Castillo, J. Olivares and N. Olguín Cruz. (VV. AA.) CIERMMI Women in Science T-XXI Biological, Humanities and Social Sciences. Handbooks-©ECORFAN-México, Querétaro, 2023.

## Abstract

The new massive sequencing technologies of nucleic acids (DNA and RNA) have allowed a great advance in health, biology, environmental, agricultural, and biotechnology sciences. However, the gigantic amount of data (big data) obtained from each experiment requires increasingly demanding computational power and also experimented computer scientists. Therefore, the field of bioinformatics, or the use of computing applied to the understanding of biological systems, requires specialized analysts who have an understanding of both biology and computational systems.

In this chapter, we set down examples of bioinformatics around the study of a) microorganisms, b) food science, c) health studies concerning the immunological repertoire, and d) studies in agricultural sciences.

## Metagenomics, Transcriptomics, Metaprofiling, Microbiomics, Omics Data Science

### Resumen

Las nuevas tecnologías de secuenciación masiva de ácidos nucleicos (ADN y ARN) han permitido un gran avance en las ciencias de la salud, la biología, el medio ambiente, la agricultura y la biotecnología. Sin embargo, la gigantesca cantidad de datos (big data) que se obtiene de cada experimento requiere una potencia de cálculo cada vez más exigente o, tal vez, unos informáticos cada vez más experimentados. Por lo tanto, el campo de la bioinformática, o el uso de la informática aplicada a la comprensión de los sistemas biológicos, requiere analistas especializados que comprendan tanto la biología como los sistemas computacionales.

En este capítulo, exponemos ejemplos de bioinformática en torno al estudio de a) los microorganismos, b) la ciencia de los alimentos, c) los estudios de salud relativos al repertorio inmunológico, y d) los estudios en ciencias agropecuarias.

## Metagenómica, Transcriptómica, Perfiles metagenómicos, Microbiómica, Ciencia de datos ómicos

### 9.1 Introduction

Computer science or informatics is a young science that has had an accelerated development, that is, in less than a human generation, more than 20 generations of computers or ways of programming have been developed. Thus, computing has quickly permeated all areas of knowledge.

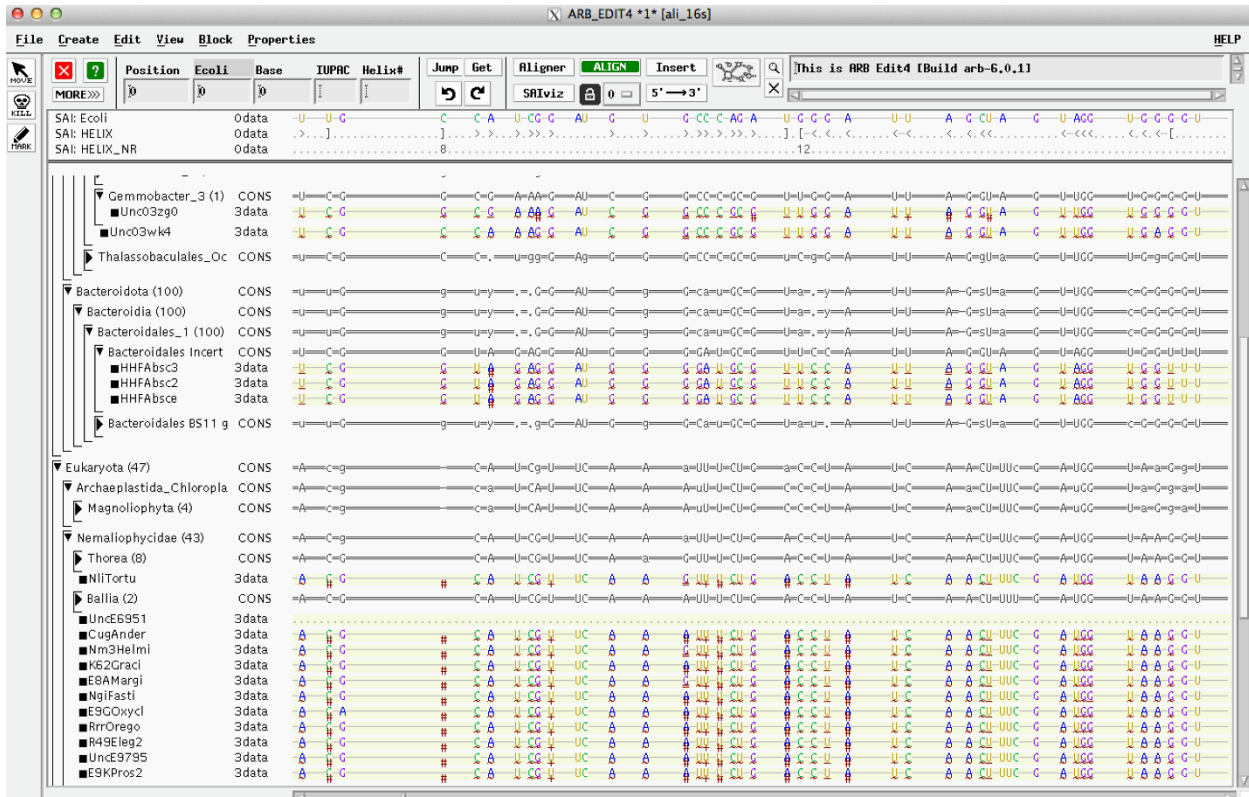
Bioinformatics is the use of computing applied to the understanding of biological issues, and it arose in the mid-1960s when computers began to reach the hands of scientists in the area of life sciences, such as Margaret O. Dayhoff, a pioneer in the area and who published the first atlas of protein sequences and their structures (Dayhoff, 1979). In this chapter we show our research, as scientists in the area of life sciences, using bioinformatics oriented to omics sciences.

With the development of molecular methods, particularly those used for the manipulation, characterization, and sequencing of DNA and RNA, it was possible to establish molecular protocols aiming to characterize biological species, particularly microbial communities associated with complex environments, and on the other hand, they are used to study the immunological repertoire of humans and other beings with a similar defense system. It was in 1988 that the term metagenomics, coined by Jo Handelsman (Handelsman, 2004), became widely used to refer to the study of environmental DNA associated with a biome.

Studies of metagenomic DNA for the scrutiny of specific metabolic functions began by preparing metagenomic libraries in plasmids and transforming them into competent strains (microorganisms that are easy to modify genetically, such as *Escherichia coli* or *Saccharomyces cerevisiae*) for the subsequent scrutiny of enzymatic functions of interest.

At the same time, sequencing using DNA chain termination inhibitors, now called Sanger sequencing (Sanger et al., 1977), was developed, which was a spark that started an explosion of sequencing of all model organisms within laboratories, and afterward all living organisms. Dendrograms derived from this sequencing, and from the alignment of these sequences (Fig. 9.1) currently show the connection of all living organisms in an evolutionary context (Fig. 9.2).

**Figure 9.1** 16S/18S sequences alignment in ARB software utilizing SILVA/ARB database



The taxonomy or classification of microorganisms is currently based on the study of ribosomal genes that Carl Woese worked on in the 70s (Woese, 1966; Woese & Fox, 1977). Phylogenetic studies are based on the alignment of the sequences of highly conserved genes, particularly those that code for ribosomal RNA (16S in prokaryotes or 18S in eukaryotes). All living organisms have these genes in their genome, in such a way that their sequence can be aligned and then a dendrogram or phylogenetic tree of all living organisms currently shows that the largest quantity of living beings (biodiversity) are microscopic, that is, they are microorganisms (Fig. 9.2).

Massive sequencing of DNA came later with new generation sequencing (NGS) technologies being 454 the first, followed by Torrent and several others until reaching the sequencing by synthesis of the Illumina® company, which is the most relevant by volume of data generated and therefore the most used in today (Table 1). New sequencing technologies are currently trying to overcome the limitation of sequencing by short pieces of DNA, Oxford Nanopore technology has generated very long reads with a polymerase-independent chemistry, however, it has still a high error rate, and the second one is the PacBio Hifi that has managed to generate long reads even with a low error rate.

Myriad computer programs can currently analyze millions of sequences efficiently and allow the design of workflows with different capacities; finally, the DNA and RNA sequences are 4-letter (GCAT nucleotides) sequences and their analysis becomes mathematically relevant with the combinatorics and statistics that a computer can particularly analyze.

**Figure 9.2** Dendrogram based on the alignment of 16S (prokaryotes) and 18S (eukaryotes) sequences in which the red circle surrounds living organisms with macroscopic dimensions (macrobiota) and all the other groups that dominate in quantity belong to the microscopic world (microbiota).



## 9.2 Bioinformatics in the study of microorganisms

The microbiota is the large number of microorganisms associated with a certain habitat, whether it is a terrestrial or aquatic environmental ecosystem or a more complex organism, such as a fungus, plant, or animal, that makes a habitat for microorganisms. The rules of host-microorganisms interaction are not yet fully known, however, some of the niches provided by a complex organism are densely colonized by endogenous microbiota (Medzhitov, 2007). It is calculated that the density of microorganisms, for example in an intestine, can become of the order of  $10^{10}$  per  $\text{cm}^3$ . One of the favorite sites for microbial colonization is the digestive tract or alimentary canal of both vertebrates and invertebrates. At present, there are extensive studies on the characterization of the intestinal microbiota of various hosts, which are carried out thanks to technological advances. "Microbiomics" studies, as they are called, are based on omics analysis of the microbiota: through the development of DNA, RNA, and protein sequencing techniques, and thus trying to elucidate the function of the microbiota on organisms: digestion, energy homeostasis, synthesis of vitamins, amino acids and fatty acids, and direct interaction with the immune system.

It is well known that an immune system depends on an interrelationship with microorganisms and their molecules, consequently, the paradigm of growing animals with a strengthened immune system is reinforced with the idea of continuous exposure to microorganisms, contrary to keeping them in a sterile environment with a depressed immune system; thus, experiments are going in that direction.

The microbiota is becoming a "proxy" or biomarker, in both environmental and health studies, that is, patterns and communities of healthy or altered intestinal microbiota are analyzed, contaminated or healthy soils or aquatic bodies, and the health of other several habitats is beginning to be microbiologically categorized. Microbiota will indicate the state of each environment, showing for example a possible dysbiosis in organisms, caused by stress or contamination of the system (Perry et al., 2020). In nutritional science, many studies have been carried out in various species, including humans, with prebiotic and/or probiotic supplements added to diets to observe their influence on the microbiota and host (namely the holobiont, that is made of these two components), and the health effects.

Currently, holo-studies recognize the symbiotic and therefore natural and essential association of an organism with its endogenous microbiota and its interaction with the environmental microbiota (Limborg et al., 2018). Organisms are exposed to a great diversity of pathogenic bacteria and viruses and their microbiota is seen as a defense barrier against these pathogens (Chiu et al., 2017).

### **9.3 Metagenomics of agricultural and aquaculture systems**

At present, global climate change has an impact on agricultural systems. Microorganisms contribute to climate change, the clearest example of how microbial life contributes to atmospheric changes was the oxygenation of our atmosphere in the early days of Earth's geological history. Today, microorganisms continue to be the main players in atmospheric changes at all levels, including terrestrial, oceanic, and urban areas. From gases produced by cows' rumens to melting soils in permafrost regions, symbiotic coral systems in oceans, and carbon waste from our cities, microbial metabolism produces and absorbs gases that can affect the environment and climate (Tiedje et al., 2022).

At the level of food production systems, it is important to take into account the microorganisms of the system and observe them as holo-systems or holobiomes (Gutiérrez-Pérez et al., 2022). Integrated agro-aquaculture systems encompass the integration of aquaculture production with agriculture, where waste from one system is passed on to the other, and this recirculation of nutrients is attainable owing to the microorganisms that usually accumulate and proliferate in biofilters to leverage their metabolism. Microbiology is an indispensable component of these nutrient recirculation and water purification systems, and other environmental services (e.g., bioremediation and alternative energy), thanks to its biogeochemical characteristics, metabolic diversity, resilience capacity, rapid adaptation, and evolution. The integration of aquaculture into terrestrial systems provides an additional source of protein and high-quality fatty acids (e.g. DHA) that are essential for food security. Fish are born and perish in an aquatic environment that is densely populated with microorganisms ( $\sim 10^6$  bacteria and  $10^9$  viruses per mL of water, Whitman et al., 1998), in contrast to the terrestrial/aerial environment; and even though environmental conditions tend to be more constant in the aquatic environment, the microbial load and diversity are also high. Consequently, the immune system of aquatic organisms co-develops with this high density of microorganisms, thus establishing an interdependent relationship between the host and its microbiota (Kogut et al., 2020), which, in fact, co-regulate and co-evolve.

There are numerous microbiological occurrences in the agricultural systems that can be capitalized on, and through understanding them, we may be able to regulate and modify the systems to make them ecologically more efficient and therefore ecologically sustainable.

### **9.4 Metagenomics of fermented food**

#### **9.4.1 Fermented foods are cultural markers**

Fermented foods hold a significant place in the cultural practices of all societies, intricately woven into their historical and societal fabric, and closely intertwined with the establishment of settled communities.

As humans evolved, the process of cultivation led to the deliberate selection of the most nutritious plant varieties, optimizing harvest yields and fostering specialization among communities in the production and processing of essential grains and vegetables, forming the cornerstone of their dietary patterns. In contemporary times, corn, wheat, and rice have emerged as the most extensively cultivated cereals globally (Erenstein et al., 2021). Just as agriculture spurred progress, the transition to sedentary lifestyles facilitated the domestication and controlled breeding of animals for human consumption. Moreover, the confluence of diverse cultural traditions through cultural exchanges and syncretism has enriched the spectrum of consumable goods by amalgamating raw materials and techniques from disparate cultural heritages.

In tandem with the advancements in food production, preservation technologies have evolved, encompassing a spectrum that spans from rudimentary methods like drying and salting to more intricate techniques such as smoking, pickling, and fermentation. These techniques, adept at bestowing the desired attributes upon the end product, have endured through the establishment of standardized manufacturing processes and the continual introduction of fresh batches of raw materials. Coincidentally, this very mechanism has inadvertently catalyzed the natural selection of microorganisms, perpetuating their prevalence and influence.

#### **9.4.2 The role of microbial communities in fermented foods**

Intricate matrices rich in concentrated nutrients are found in fermented foods, serving as substrates that provide sustenance to microorganisms either naturally present or deliberately introduced during processing.

Throughout the fermentation process, the dynamic enzymatic activities of these microorganisms orchestrate a remarkable transformation of the food matrix. This metamorphosis encompasses alterations in critical physicochemical attributes, such as pH and redox potential, alongside enhancements in microbiological attributes. This microbial-driven evolution fosters the emergence of robust strains that adeptly navigate the novel environmental milieu and adeptly withstand the presence of antimicrobial agents.

In parallel, the microbial metabolic endeavors not only reshape the fundamental sensory properties of the food but also bestow upon it a spectrum of secondary metabolites and release degradation byproducts from its elemental building blocks—carbohydrates, proteins, and lipids. This intricate interplay generates a multifaceted medley of compounds intricately woven into a complex tapestry of aroma and flavor profiles (Bamforth, 2005).

The environment serves as a pivotal wellspring of fungi and bacteria that enrich fermented food processing. The diverse array of microorganisms found in the environment defies replication within aseptic confines or in settings apart from their indigenous origins. This notion forms a cornerstone in comprehending the innate uniqueness of traditional fermented foods, a uniqueness that, in select instances, finds safeguarding under the mantle of Designation of Origin (Reinders et al., 2019).

Exemplifying this concept are several traditional non-distilled fermented beverages deeply rooted in Mexican heritage. These include maguey mead pulque, pineapple tepache, coconut palm mead tuba, red prickly pear colonche, and corn-derived tegüino and pozol, among others. While these treasures possess rich tradition, an exceptional nutritional value, and embody the artistry of local artisans, it's noteworthy that none of these legacies enjoys the shield of designation of origin.

#### **9.4.3 Biotechnological applications of microorganisms associated with fermented foods**

Traditional fermented foods have garnered substantial interest in industrial realms due to their status as a secure reservoir of microorganisms, thoughtfully culled through the annals of tradition, and suitable for human consumption. The daily incorporation of these time-honored original communities creations has been empirically linked to a wealth of health advantages for consumers (Cuamatzin-García et al., 2022).

In the quest for elucidating the mechanisms underpinning these benefits, scrutiny has turned towards identifying the presence of probiotic strains (Soemarie et al., 2021) and the extraction of prebiotic compounds, envisioning their integration as additives in diverse comestibles. This strategic action aims to foster the proliferation of beneficial human gut bacteria (Christensen et al., 2022), thereby enhancing the intestinal microbiota's desirable equilibrium.

Concurrently, probing into the microbial profiles of foods serves as a guiding compass in crafting starter cultures, steering the preparation of authentic 'type' foods, and exploring novel product frontiers within the industry. This endeavor is not merely confined to creating delectable offerings but underscores paramount considerations of safety and transformative efficiency (Hansen, 2002).

Moreover, it is a fact that the orchestration of physicochemical changes during fermentation and maturation ushers in a selective process to the microorganisms primed for a substrate battle. In this enthralling contest, the victors often bear the mantle of producing antimicrobial compounds or harbor steadfast defense mechanisms. These strategic attributes confer a distinct competitive edge. Leveraging this insight, the realms of food and biomedicine converge in their fervent exploration of harnessing nature's arsenal, such as the remarkable bacteriocins. Embodied by nisin as the pioneering industrial example, the application of such natural preservatives beckons as an avenue of great interest (Lahiri et al., 2022).

## 9.5 Application of Bioinformatics in Immune Repertoire Studies

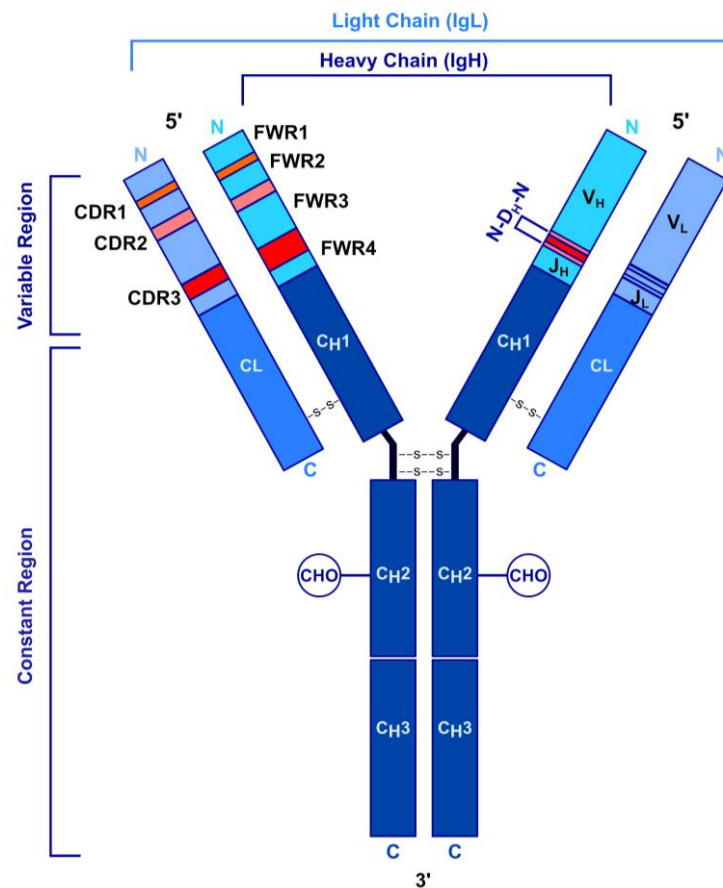
### 9.5.1 Introduction to the Immune Repertoire

The human immune system shows an astonishing ability to produce a diverse array of antibodies capable of recognizing an extensive range of antigenic structures. The immune repertoire encompasses all B cell receptors (BCRs or antibodies) and T cell receptors (TCRs) within an individual. This assembly of receptors and cells emerges through various processes, including, but not limited to, combinatorial diversity, somatic recombination, class switching, and somatic hypermutation. These receptors display remarkable variability, and the advent of high-throughput sequencing has revolutionized our capacity to scrutinize them in greater detail (Calis & Rosenberg, 2014).

Currently, our understanding of the human immune response to vaccinations, cancer, and viral infections relies heavily on advanced "omics" technologies. These innovative methodologies facilitate the quantification of genetic behavior, mRNA (single-cell transcriptomics), proteins (proteomics), metabolites (metabolomics), cells (mass cytometry), and epigenetic modifications (ATAC-seq). In conjunction with computational approaches, bioinformatics plays an indispensable role in the exploration of these domains. (Pulendran & Davis, 2020).

When focusing on the diversity of antigen receptors, specifically TCRs and BCRs, it is of paramount importance to thoroughly explore the mechanisms that foster such diversity. This process begins by estimating the theoretical diversity, which indicates the potential of germline segments to combine. The maximal amino acid diversity of immune repertoires is estimated to be approximately  $10^{140}$ , calculated as  $20^{110} \times 2$ . This calculation considers the 20 amino acids, the 110-amino-acid variable region of immune receptors, and the two variable regions constituting each receptor (IGVL-IGVH for B lymphocytes or TCRV $\alpha$ -TCRV $\beta$  for T lymphocytes). However, it is important to note that this vast diversity in humans is confined by initial segments of V, D, and J genes, resulting in a conceivable diversity range between  $10^{13}$  y  $10^{18}$ . At any given moment, only a fraction of this potential diversity can manifest in an individual due to limitations in the number of circulating B and T cells (humanos:  $10^{11-12}$ ) and the count of distinct clones, as defined by clonotypes, which approximates  $10^9$  in humans and  $10^{6-7}$  in mice (Miho et al., 2018).

**Figure 9.3 BCR Structure.** The soluble form of the B-cell receptor (BCR) is the immunoglobulin, consisting of two identical heavy chains (IgH) and two light chains (IgL) connected by disulfide (S-S) bonds. The variable region of the heavy chain comprises the VDJ segment, while the light chain's variable region comprises only the VJ segments. The junction of the V(D)J segments for IgH and VJ segments for IgL forms the CDR3 region. The variable region of the antibody is composed of three complementarity-determining regions (CDRs) and four framework regions (FRs) of both IgH and IgL. The constant region consists of two or three constant domains from the heavy chains, depending on the antibody's class



## 9.5.2 Structure of Immune Receptors

### 9.5.2.1 B cell receptor (BCR)

The B cell receptor, known as BCR, is composed of two identical heavy chains (IgH) and two identical light chains (IgL) that are linked by noncovalent interactions and disulfide bonds. The IgL chains consist of two types, lambda (Ig $\lambda$ ) and kappa (Ig $\kappa$ ), and their relative proportion varies among species, with an average ratio of 2:1 in humans (Schroeder & Cavacini, 2010) (Fig. 3).

The BCR plays a crucial role in recognizing and responding to antigens, inducing a cascade of events that leads to cell proliferation and differentiation into plasma B cells. Subsequently, plasma cells produce immunoglobulins, commonly known as antibodies, which represent the secreted form of the BCR and effectively neutralize pathogens.

Antibodies can be classified into two functional regions: the variable region, located at the amino terminus, responsible for recognizing and binding antigens, and the constant region, determining the antibody's isotype and effector functions. BCRs on lymphocyte surfaces lack effector functions due to the constant region being embedded in the cell membrane. The heavy chain isotype type determines the antibody's functional properties, and five major types are recognized: IgM, IgD, IgG, IgA, and IgE (Schroeder & Cavacini, 2010). Within the antibody's variable region, there are three complementarity-determining regions (CDRs) and four IgH and IgL Frameworks (FRs). The CDRs, mainly CDR3, exhibit the most variability and are crucial for pathogen recognition. The constant region comprises two or three constant domains from both heavy chains, and the number varies based on the antibody's class.



### 9.5.2.2 T Cell Receptor (TCR)

The T cell receptor (TCR) is composed of two chains that bear resemblance to immunoglobulins. However, a notable distinction exists: TCRs are not secreted; instead, they remain consistently associated with the cell membrane. Consequently, they traverse a segment in both chains that spans the lipid bilayer of the membrane, encompassing a small intracellular portion. These two chains are identified as TCR $\alpha$  and TCR $\beta$  and placed next to each other through disulfide bonds. CD3, CD4, and CD8 represent specific molecules present on the surface of T cells that stabilize both TCR-mediated interactions and intracellular communication.

### 9.5.3 Library Preparation and Sequencing Platforms for Repertoire Studies

The analysis of immune repertoires can involve the selection of DNA or RNA as source material. The integration of antigen receptor sequences into sequencing libraries is typically achieved through targeted PCR amplification. However, amplifying the variable region sequences of highly diverse BCRs and TCRs presents a significant challenge. Designing PCR strategies that allow unbiased and complete amplification of these exceptionally variable receptors is particularly demanding. This challenge becomes critical in experiments that necessitate high-throughput massive sequencing for quantifying receptors and lymphocyte clones, as imbalances in amplification efficiencies can introduce biased clonal frequency measurements. To tackle these difficulties, diverse amplification approaches have been utilized. These methods encompass multiplex PCR with intricate combinations of direct primers, multi-step PCR, and utilization of multiple primer mixes.

Alternatively, PCR initiated from engineered adapter sites has also been employed, particularly for RNA input. This technique involves introducing a consistent adapter sequence into the variable regions, followed by PCR amplification using a single forward primer and reverse primers that target J segments or constant regions. This innovative approach effectively mitigates amplification bias and generates a library encompassing the complete variable region (ORF), suitable for functional assessments of TCRs or antibodies. Given the unique characteristics of the amplicons to be sequenced, the selection of a suitable sequencing technology becomes crucial. The landscape of sequencing methodologies continually advances in terms of depth and precision. Consequently, depending on the sequencing technology employed, the intended application and its implementation can vary (see Table 9.1).

**Table 9.1** Common Platforms Used for Sequencing the Immune Repertoire (Modify of Chaudhary & Wesemann, 2018).

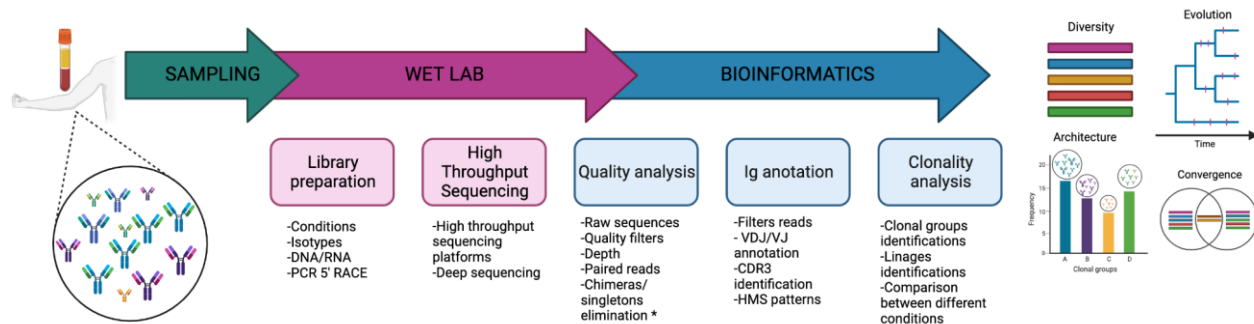
High Sequencing platform	Method	Read size	Depth sequencing	Reads per run	Covered Region	Accuracy and error rate (%)	Error
Roche's 454 GS FLX	Pyrosequencing	700 pb	450 Mb	~1 million	FR1- Constant region	1	Indel
Illumina MiSeq	Dye terminator sequencing	300 pb x 2	13.2-15 Gb	~ 3 million	FR1- Constant region	~0.1	Substitution
Illumina HiSeq	Dye terminator sequencing	250 pb x 2	500-1000 Gb	~ 2 billion	FR1- Constant region	~0.1	Substitution
Ion torrent	Synthesis (detects H <sup>+</sup> ions)	> 100 - 200 pb	30 Mb - 2 Gb	~60 -80 million	FR3- Constant region	~1	Indel
PacBio	Synthesis (fluorescence tag attached to phosphate chain)	860 - 1100 pb	5-10 GB	~0.01 million	Amplification of Linked Heavy and Light Chains	~13	Indel

Note: Each platform has its own strengths and limitations, and the choice of platform depends on the specific research goals and characteristics of the immunological repertoire being studied

### 9.5.4 Immune Repertoire Analysis

The immunological repertoire is characterized by its diversity, architecture, evolution, and convergence (Fig. 9.9.4). The wide-ranging diversity of immune repertoires is a crucial attribute that facilitates the recognition of a broad spectrum of antigens. To measure the diversity of a repertoire we can use alpha diversity descriptors such as clonotype richness and clonotype abundance. Accurate diversity assessment hinges on the accurate annotation of sequencing reads. The annotation process follows these key steps: (i) identification of the V, D, and J segments, (ii) identification of the positions that define the frameworks and CDRs, (iii) identification of nucleotides inserted and deleted in the binding region, and (iv) quantification of somatic hypermutation (in the context of antibodies). (Miho et al., 2018).

**Figure 9.4** Immune repertoire analysis process. In the context of repertoire analysis, sample selection is a crucial step in experimental design. This selection must accurately reflect the study conditions and populations being compared, including any enrichment of B or T cells that may be performed. Typically, the immune repertoire is accessed through peripheral blood samples. The subsequent library preparation step involves the isolation and amplification of genetic material fragments, such as genomic DNA or mRNA (in cases where mRNA is used, an additional step is required to convert RNA to DNA). Following this, the region of interest - typically the variable region - is amplified through PCR 5'RACE. The desired sequencing type is chosen and the necessary steps to add adapters for massive sequencing and barcodes corresponding to each individual/condition are taken. Once raw sequences are obtained, a quality control process is implemented to filter out sequences with a quality  $Q > 20$ , and eliminate chimeras and singletons. Subsequently, sequenced regions are annotated. VDJ segments for the heavy chain and VJ segments for the light chain are identified, CDR3 is determined, and patterns of somatic hypermutation are compared with the germ line. In clonality analysis, sequences are grouped based on their characteristics into clonal groups and lineages, and subsequently, comparisons are made between different study conditions. With these components, diversity, evolution, architecture, and convergence of the immune repertoire can be studied. Image created with BioRender.com.



The full spectrum of similarity relationships among immune receptor sequences is referred to as the similarity architecture within an immune repertoire. Consequently, the diversity of the immune repertoire, derived from frequency profiles of clonotypes, differs from the sequence similarity architecture, which is based on similarity ratios of clonotypes, regardless of their frequency. The degree of similarity among immune receptors significantly influences the breadth of antigen recognition; as receptor differences increase, the coverage of the antigen space expands. Network theories provide a framework to delve into this subject. Clonal networks are established by defining clonotypes as network nodes, and connections (edges) between clonotypes are established based on specific similarity conditions, measured by a distance, resulting in undirected Boolean networks.

To deduce ancestral evolutionary relationships among individual B cells, lineage trees are constructed from sets of sequences belonging to the same clonotype. A clonal lineage encompasses receptor sequences originating from the same recombination event. When creating lineage trees, a common preliminary step involves pooling sequences with identical V and J genes, as well as the same CDR3 length. In the realm of antibody repertoire phylogenetics, no consensus has been reached regarding the optimal method for inferring lineage evolution. Techniques such as LD, Neighbor-Joining (NJ), Maximum Parsimony (MP), Maximum Likelihood (ML), and Bayesian Inference (BEAST) are employed.

The reconstruction of phylogenetic trees for antibodies necessitates a profound understanding of the physical and temporal dynamics of somatic hypermutation, a process integral to antigen-driven antibody sequence evolution. Mutation statistics can be leveraged to infer the probability of mutation, which is unevenly distributed across the VDJ region of the antibody.

Convergence of immune repertoires refers to the exchange of identical or similar immune receptor sequences between two or more individuals. Sequence convergence can be indicated by the interchange of clonotypes (public clonotypes, full clonal sequence, or pool of clonotypes) or motifs (substrings of sequences). Numerous researchers in this field have endeavored to quantify the degree of convergence of the naive repertoire and the antigen-modified repertoire, employing a wide range of computational approaches that determine sequence similarity between individuals.

### 9.5.5 Application of Machine Learning in Immune Repertoires

Machine Learning (ML) has become an indispensable subset of Artificial Intelligence (AI) that has gained substantial recognition in recent times due to its application in various research domains. Notably, DeepMind's Alpha Fold tool (Jumper et al., 2021), which is founded on AA, has achieved a significant breakthrough in structural biology. The tool has the capacity to predict the three-dimensional structure of proteins with high precision, which is arguably the most noteworthy contribution of AI to the advancement of scientific knowledge. Moreover, AA has been utilized to forecast susceptibility to ailments such as cancer, disease recurrence, and life expectancy.

In the field of immunology, AA has been employed to scrutinize the response of the adaptive immune system to vaccines and infections and to recognize molecules with potential therapeutic benefits, such as monoclonal antibodies. In this sense, AA has enabled the identification of new monoclonal antibodies *in silico* based on extensive sequencing data of the variable regions repertoire (Greiff et al., 2020). Additionally, AA has facilitated the optimization of binding to the target molecule and comprehension of the biophysical properties of antigen-antibody interaction.

Recently, AA has been utilized to identify potential candidate monoclonal antibodies to treat COVID-19 based on the CDR3 sequence, as well as to identify biomarkers that correlate with disease severity (Magar et al., 2021).

Antibody therapeutics have become highly effective biotherapeutics, securing four of the top ten therapeutics in terms of sales in 2021. Furthermore, antibody-based biotherapeutics, comprising antibody-drug conjugates and bispecific antibodies, have exhibited potential as therapeutic modalities. Traditionally, experimental approaches, such as phage or yeast display, and animal immunization have propelled the discovery and development of antibodies. However, these methods are time-consuming and labor-intensive and have various limitations, including challenges in specifying antibody binding sites (epitopes) and manufacturing antibodies at scale. Despite the various reported strategies to optimize the experimental workflow, significant challenges remain. In recent years, computational and AI-based methodologies have gained importance at various stages of the antibody development workflow. This is analogous to small molecule drug development, where computational methods have made significant progress. Specifically, the prediction of interactions between drugs and therapeutic targets has considerably benefited from the marked improvement in the performance of computational methods.

## 9.6 Methods

There is an abundance of techniques and computer software currently available, and the challenge in sequence analysis is selecting the most suitable one. The databases selected, particularly for sequence annotation (i.e., aligning new sequences with existing ones from repositories), will always serve as a crucial element in the analysis (Escobar-Zepeda et al. 2018). Among the most meticulously curated collections and repositories of ribosomal subunits (including 16S, 18S, 23S, and 28S rRNA gene sequences) is the SILVA-ARB database, which also offers a local sequence manipulation application known as ARB (Quast et al., 2013; Yilmaz et al., 2014). Both the database and the program were utilized in this study to construct the 16S and 18S dendrogram (Fig. 2).

With the advent of RNAseq high throughput sequencing technologies (transcriptomics), the power of genomics data analysis and the global transcription of an organism's collection of mRNAs have increased. However, such analyses require new capabilities in computing infrastructure and large data handling skills on the part of the data analyst.

A typical workflow in RNAseq data analysis commences with the extraction of genetic material from the messenger RNAs and their transport to a massive sequencing center. This center generates text files in the fastq format, much like the case of DNA analysis. The fastq format often employs four lines to describe each sequence, with the first line containing the name of the sequence, the second line containing the actual nucleotide sequence (ATCG), the third line starting with a "+" symbol, which may repeat the sequence's name or display the "+" symbol, and the fourth line containing the sequence's qualities in PREHD 33 format.

When a reference genome is present, the next step is to align the fastq files against the reference genome using software designed for this purpose. Over 100 software are available for this step, but the most commonly used or referenced in articles are bowtie (Langmead & Salzberg, 2012) and BWA (Li & Durbin, 2009), with their selection closely linked to the genetic material used and the size of the sequences in the fastq files. An analysis of the advantages and disadvantages of five highly popular aligners is available at this site: <https://www.ecseq.com/support/benchmark>.

In the absence of a reference genome, the recommended approach is to perform a *de novo* assembly of the genome or transcript using specialized software. Trinity is a popular software for this purpose, and a highly detailed execution protocol can be found in the protocol published in nature protocols (Haas et al., 2013).

In the third step, a table of transcript abundances is generated whether one has aligned against a known reference genome or with the transcript assembly. This table consists of a transcript in each line and a column for each library that has been sequenced. It is crucial to sequence a basal or "Wild type" condition and a condition against which one wants to contrast, be it an experimental condition (due to a specific environmental condition, a drug, a disease, a tissue, a strain), etc., a mutant or variant. Moreover, it is imperative to consider biological replicates. Multiple publications have shown that the greater the number of replicates, the greater the understanding of the experiment under study (Schurch et al., 2016; Lamarre et al., 2018). Although the methodologies for generating replicates can be expensive or complicated, it is recommended that there should never be less than 3 replicates per condition. It is also vital to avoid the "batch" effect, which is generated when samples of the same condition are prepared at different times, by different people, or in different situations. The greatest variability occurs when the genetic material is extracted, so it is imperative to reduce the variables that could generate greater dispersion (e.g. time, environmental conditions, temperature, sex, size, etc.).

The fourth step involves the differential expression analysis (DEA). There are numerous R-language software packages available for this step, but proficiency in the language is required. Fortunately, we have developed a website for this last step. The DEA analysis begins with the abundance table described in step 3 and defines the value of LFC (logfoldChange or value of change) that is considered necessary, a relevant p-value, and a CPM (counts per million). These three values are set by default in  $LFC \geq 1$ ,  $pvalue \leq 0.5$ , and  $CPM = 1$ , which are common.

Integrative Analysis of Differential Expression for Multiple Experiments (IDEAMEX) (Jiménez-Jacinto et al., 2019) performs differential expression analysis using four Bioconductor packages: DESeq2, EdgeR, NOIseq, and Limma. It also includes a module for integrating results that reports the coincidences in the sets of differentially expressed genes using Venn Diagrams, plain text lists, and heatmaps. All of this is presented in an easy and user-friendly visualization environment, enabling analysis to be carried out without any prior experience in handling the R language.

The condition of samples used in the study of immune repertoires holds paramount importance for ensuring the validity and relevance of research findings. These samples need to accurately reflect the specific conditions under investigation, such as infections, vaccinations, or autoimmune responses, to provide insights applicable to real-world scenarios. Comparable sample conditions are essential for accurate comparisons between populations or experimental conditions, preventing biases and confounding variables. Using representative samples also avoids introducing artifacts, supports data reproducibility, and aids in the development of clinically relevant therapies, vaccines, and diagnostics. Moreover, maintaining consistent sample conditions before and after enrichment processes is crucial to attribute observed changes to the experimental manipulations rather than to technical variations. In essence, the accuracy of antibody repertoire analysis hinges on the fidelity of sample conditions, influencing the reliability, applicability, and clinical significance of the research outcomes. To access a sample of the immune repertoire for study, a multi-step process is undertaken. Initial sample collection involves obtaining biological material rich in immune cells, often from peripheral blood or other relevant tissues. The isolated immune cells are then processed to extract their genetic material, which encodes the antibodies of interest. This genetic material is transformed into sequencing libraries through fragmentation, amplification, and tagging steps. High-throughput sequencing technologies are employed to generate vast amounts of sequence data.

Within the field of immune repertoires, it is important to consider certain limitations, including the depth and quality of sequencing. In cases where sequencing depth is limited or quality is poor, segment allocation is restricted, thereby limiting the characterization of sample diversity. Moreover, while some studies have focused solely on heavy chain clonotypes, few have examined full clonotypes. Finally, the databases used in such studies often fail to reflect the variability of alleles across populations.

Given the vast amount of data generated by High-throughput sequencing technologies in the study of immune repertoires, the application of bioinformatics tools is critical. Currently, a variety of software programs exist that provide the necessary tools for such studies (as outlined in Table 2). Notably, the majority of these software have been developed with a focus on the antibody repertoire, given its high degree of variability.

**Table 9.2** Specialized Software for Immunological Repertoire Analysis

Software	Description	Repertoire type	Reference
ImmunediveRsity	Tool based mainly on R language for comprehensive data analysis of the B cell repertoire. Performs clonal and lineage grouping.	BCR	(Cortina-Ceballos et al., 2015)
IMGT/High V-Quest	Web tool that allows rapid identification of the germ line (allele assignment), determination of the structure of TCR and BCR.	BCR, TCR	(Li et al., 2013)
VDJFasta	Tool that uses Hidden Markov Models to determine all CDRs, performs frequency analysis.	BCR	(Glanville et al., 2009)
ImmuneDB	Tool that identifies genes, determines clones, builds lineages and provides information such as selection pressure and mutation analysis.	BCR	(Rosenfeld et al., 2017)
immunarch	An R package designed to analyze TCR and BCR repertoires, designed primarily for medical scientists and bioinformaticians.	BCR, TCR	(Samokhina et al., 2022)
Immcantation	Tool that provides an end-to-end analytical environment for high-throughput AIRR-seq data sets. From raw reads, Python and R packages are provided for preprocessing, population structure determination, and repertoire analysis.	BCR	(Gupta et al., 2015)
IgBLAST	Tool identifies germline gene matches, assesses rearrangements, and delineates IG V domain regions, supporting both nucleotide and protein sequences with parallel database searches for comprehensive insights.	BCR, TCR	(Ye et al., 2013)
IGGalaxy	A web application using the Galaxy GUI, and can be used on a single computer and on a server.	BCR	(Moorhouse et al., 2014)
SONAR	Tool is specifically designed to analyze the development of antibody lineages over time.	BCR	(Schramm et al., 2016)

Bioinformatics analysis follows, encompassing quality control, alignment, and annotation of the sequences to identify key regions like antibody variable domains. Clonality analysis groups similar sequences to reveal immune cell population dynamics, while comparative analysis provides insights across different conditions. Several steps can be identified for the analysis of the immune repertoire. The initial step involves data pre-processing, whereby the primary objective is to rectify sequencing errors and eliminate noise from the raw sequences. This step bears much resemblance to procedures in other types of studies. Subsequently, germline annotation of the crude sequences is carried out, which is deemed one of the most crucial steps as it involves the inference of the correct germline alleles that recombined to produce each TCR/BCR/antibody. Following this, clonal assignment, which is mostly accomplished by CDR3 sequence homology at either the amino acid or nucleotide level, is conducted. In essence, it can be stated that a sequence that originates from the same V and J segment and has the same CDR3 can be classified as belonging to the same clonal group. Within the clonal group, different lineages that give shape to that clonal group can be grouped at the complete sequence level. Lastly, the characteristics of the repertoire can be described in terms of diversity, architecture, evolution, and convergence in the various study conditions.

## **9.7 Results and Discussion**

### **9.7.1 Application of metagenomics of microorganisms in environmental sciences**

The consideration of microbial contributions to carbon fluxes to and from the atmosphere is imperative in all climate change models. The microbial realm has the potential to become a crucial accomplice in endeavors to mitigate the outcomes of human greenhouse gas emissions, as it may be feasible to encourage alterations in microbial activities in various environments to consume more and generate fewer gases that contribute to global warming. To address intricate issues, more interdisciplinary research is required to probe into the relationships between microorganisms, climate change, and human well-being. Microorganisms' adaptation to a warming world may directly affect human well-being through modified patterns of host-microbe interactions, microbial biogeography, and altered terrestrial, aquatic, and urban microbiology. Consequently, omics studies of environmental microbiology play a pivotal role in continuing to comprehend the microbial world and its vast diversity that surpasses that of the macroscopic world (Fig. 2).

### **9.7.2 Our understanding of fermented foods through metagenomic studies**

The investigation of microbial communities has been enhanced by the emergence of new techniques for the molecular characterization of microorganisms. The employment of laboratory isolation and culture methods renders the restoration of the original microbial community unattainable, as community members often survive solely as a consortium, which cannot be isolated. Additionally, the enumeration of species richness present in the sample is skewed due to the inability of only certain species to grow under laboratory culture conditions, leading to the serious issue of pathogenic species not being detected (Fakruddin et al., 2013).

The characterization of microbiotas through the reconstruction of the taxonomic profile by high throughput massive sequencing of metagenomic DNA has highlighted that cultivable organisms are not necessarily the dominant ones (Escobar-Zepeda et al., 2016). These studies, in combination with other omics, have also demonstrated the complexity of the microbiota of fermented foods and the interactions between the populations of fungi, yeasts, and bacteria (Saak et al., 2023). Therefore, the development of starter cultures and simulation of processing conditions for large-scale production must be replaced with efforts to preserve traditional processing techniques.

The implementation of high-performance sequencing technologies in the field of food has provided evidence of the safety of fermented foods produced by traditional methods. The abundance of beneficial bacteria and a low proportion of opportunistic pathogens and antibiotic resistance genes, as compared to non-fermented foods, serve as indicators of high microbiological quality, especially in fermented foods of dairy origin (Xu et al., 2022).

Similarly, the clarification of the functional profile of the microbial communities associated with fermented foods has demonstrated the role of specific species in the degradation of compounds and the production of bacteriocins and other antimicrobials (Yasir et al., 2022; You et al., 2022).

### 9.7.3 Application of the study of immune repertoires in health

The examination of immune repertoires has been employed to investigate various aspects of health, disease, and vaccination conditions. The primary objective of such studies is to explore a particular signature of the repertoire under specific circumstances, while also providing insight into the immunological processes that take place.

The investigation of the human B cell response to dengue virus (DENV) infection is crucial in understanding serotype-specific protection and cross-reactive subneutralizing response. While serotype-specific protection is advantageous and represents the main objective of vaccination, cross-reactive subneutralizing response has been linked to the development of severe disease, occurring in a small but significant fraction of DENV secondary infections. Primary and secondary infections are associated with the production of polyreactive and cross-reactive IgG antibodies. Studies of the antibody repertoire in DENV indicate that during the acute phase of the disease, there is an increase in the diversity of IgG B cells, and changes in the relative use of the IGHV1-2, IGHV1-18, and IGHV1-69 segments are observed (Godoy-Lozano et al., 2016). Convergent patterns in the antibody repertoire that are specific to DENV have been identified and serve to define prevalent and specific indicators of DENV infection. These immune signatures have the potential to be useful in the development of protein- or nucleic acid-based diagnostic tools designed to detect acute dengue, as well as to evaluate and monitor DENV exposure in endemic communities (Parameswaran et al., 2013).

A primary goal in developing vaccines against swiftly mutating viruses like influenza or HIV is to generate antibodies with the capacity to neutralize a broad spectrum of variants. Nevertheless, it is important to acknowledge that B cell precursors, capable of evolving into broadly neutralizing antibodies (bNAbs), are usually rare in the immune system.

In the specific case of HIV-1 vaccine development, emphasis has been placed on activating B cell receptors to generate naïve antibodies or bNAb precursors, followed by expansion and maturation of intermediate B cell lineages, which ultimately results in the production of bNAbs with high affinity. The conserved regions of the HIV-1 coat glycoprotein trimer, commonly referred to as Env, are the intended targets of bNAbs (Spencer et al., 2021). When present during viral exposure, these antibodies can block infection. The potential therapeutic application of bNAbs is very promising, and efforts are currently underway to facilitate their development for broad clinical use. Studies of immunological repertoires have described these antibodies (Caskey, 2020).

Recently with the COVID-19 pandemic, investigating the modulation of the immune repertoire in this disease has become one of the priorities of the scientific community. Through investigating the antibody repertoire in infected patients, particular patterns of the use of segments were found. These segments include IGHV3-30, IGHV3-53, IGHV3-23, and IGHV3-9 of the heavy chain, and IGKV1-39, IGKV1-33, IGLV3-21, IGLV3-25, and IGLV6-57 of the light chain (Nielsen et al., 2020; Robbani et al., 2020; Zost et al., 2020). The low percentage of somatic hypermutation at the beginning of the pandemic suggests a primary infection. However, with the advance of the pandemic, highly neutralizing antibodies have been described with a higher somatic hypermutation.

### 9.7.4 Application of transcriptomics in agronomy

At the National Center for Disciplinary Research in Animal Health and Safety, National Institute of Forestry, Agriculture, and Livestock Research (Mexico), mechanisms of resistance to ivermectin of the parasite *Haemonchus contortus*, which affects small ruminants, were investigated to develop new control and diagnostic strategies. Samples of the nematodes were obtained, and total RNA was extracted and purified with chloroform and isopropanol, then precipitated with 75% ethanol. The RNA concentration was estimated at 3 µg using spectrophotometry. RNA purity and integrity were evaluated through 1% agarose gel electrophoresis, stained with ethidium bromide, and assessed by fluorometry with a Bioanalyzer 2100 following the manufacturer's instructions (Agilent, Santa Clara, CA, EE. UU.). This process aimed to achieve an RNA Integrity Number (RIN)  $\geq 6$ , which was deemed sufficient for the assay

The *de novo* assembly was conducted using all RNAseq sequencing and samples of the two *H. contortus* strains, IVMs, and IVMr. The nucleotide sequence data is available in GenBank, under BioProject PRJNA877658. The bioinformatics analysis was carried out using the computational cluster of the Massive Sequencing and Bioinformatics University Unit at the Institute of Biotechnology/UNAM. First, the quality of the sequences was analyzed using the FastQC v0.11.8 software, which indicated no presence of adapters and confirmed that the qualities were on average above one Q30. The sequences were then aligned against the reference assembly genome GCA\_000469685 using the Smalt v0.74 software, but the alignment percentage was very low (56.11%). Consequently, the transcript was assembled *de novo* using Trinity software v 3.0. Subsequently, the RSEM software was utilized to generate the table of abundances. The data was then analyzed with four methods from the EdgeR Bioconductor package, NOISeq, limma, and DESeq2, and the results provided by DESeq2 were the most consistent with the other methods. Finally, the annotation was conducted with the Trinotate software (Griffith et al., 2015).

The novel information presented in this study indicates the presence of significant genetic diversity in various populations of *H. contortus*, which could be a result of several factors, including anthelmintic drug pressure, the prevalence of nematodes in their environments, and geographic regions. This diversity may also be a consequence of different factors related to host-parasite interactions. The species *H. contortus* has evolved genetic properties that enable them to withstand anthelmintic drugs and evade the host's immune response through specific up/down-regulated genes.

## 9.8 Conclusions

The limitations of the repertoire sequencing, metagenomic, and transcriptomic analyses are numerous, yet it is crucial to consider sequencing depth, sequencing quality, the use of population-specific databases, and appropriate computational methods for sample type. These biases constrain the accurate characterization of immunological and microbial diversity.

For many scientists, data analysis is a challenging task, as most available tools are implemented in a UNIX-based environment and require programming languages like R, Python, or Perl, which are intended for students with at least a basic understanding of these languages and coding skills.

However, alternative tools on the web such as Galaxy (The Galaxy Community et al., 2022), and servers like MGnify (Richardson et al., 2023) provide both public and private repositories of data analysis that are accessible upon request. Additionally, these resources offer a collection of Jupyter Notebooks that are easily comprehensible and navigable (<http://notebooks.mgnify.org>).

Differential expression analysis and RNA-Seq have become popular and useful methods to evaluate gene expression changes in any organism. The IDEAMEX web server was developed to address the above-mentioned difficulties (Jiménez-Jacinto et al., 2019). IDEAMEX requires a raw headcount table for as many replicates and conditions as required, enabling the user to select which conditions are compared. The whole process consists of three main steps: (i) Data analysis, which provides a preliminary quality control analysis based on the data distribution per sample using various types of graphs; (ii) Differential expression, which performs differential expression analysis with or without error for a batch effect and generates reports for each method, using the Bioconductor, NOISeq, limma-Voom, DESeq2, and edgeR packages; (iii) Integration of results, which reports the integrated results using different graphical outputs, including correlograms, heatmaps, Venn diagrams, and gene lists in text files. IDEAMEX provides easy interaction during the analysis process, error tracing, and debugging by generating output log files. The server is currently accessible on <http://www.uusmb.unam.mx/ideamex/>, where documentation and example input files are provided, and can help researchers with no prior bioinformatics background perform differential expression analysis of RNAseq data easily.



Bioinformatics has brought about a paradigm shift across diverse domains, wielding transformative influence in biotechnology, medicine, environmental science, and agriculture. In biotechnology, its progress is evident in the accelerated design of genetically modified organisms, fostering innovations in enzymes, biofuels, and biomaterials with far-reaching implications for sustainable practices. Meanwhile, in the medical area, bioinformatics stands as a guiding tool, shaping personalized healthcare through in-depth genomics, nutrigenomics, pharmacogenomics, clinical data analysis, and immune repertoire analysis. By pinpointing disease biomarkers, it facilitates early diagnosis and tailored treatments, and its role in drug and vaccine discovery expedites the possibilities to improve the treatment of diseases.

The reach of bioinformatics extends to environmental science, where its analytical power might uncover intricate ecological interactions. Metagenomics and transcriptomics, enabled by bioinformatics, shed light on microbial communities in ecosystems, enabling comprehension of their roles in nutrient cycling and ecosystem stability. This knowledge, in turn, informs strategies for pollution control, bioremediation, and sustainable resource management. In agriculture, bioinformatics has heralded a new era by contributing to the development of genetically enhanced crop varieties, displaying augmented yields, disease resistance, and improved nutrition. Additionally, precision agricultural techniques, harnessed through bioinformatics, optimize resource allocation, fostering resource-efficient practices and bolstering global food security.

As technology and knowledge continue to evolve, the interdisciplinary partnership between life sciences and computational analysis is poised to reshape these fields even further, propelling us into a future characterized by sustainable solutions, personalized interventions, and profound scientific insights.

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## **Chapter 10 Recruitment of personnel in companies using the 4.0 technique in times of COVID-19**

### **Capítulo 10 Reclutamiento del personal en empresas utilizando la técnica 4.0 en tiempos de COVID-19**

VÁZQUEZ-VÁZQUEZ, Erika†\*, CRUZ-VÁSQUEZ, Jimena and MONTES DE OCA-ESTRADA, Anabel Regina

*Tecnológico de Estudios Superiores de Villa Guerrero, Mpexico.*

ID 1<sup>st</sup> Author: *Erika, Vázquez-Vázquez* / **ORC ID:** 0009-0009-7835-6545

ID 1<sup>st</sup> Co-author: *Jimena, Cruz-Vásquez* / **ORC ID:** 0009-0002-8344-5939

ID 2<sup>nd</sup> Co-author: *Anabel Regina, Montes de Oca-Estrada* / **ORC ID:** 0000-0003-1586-6440

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E. Vázquez, J. Cruz and A. Montes de Oca

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## **Abstract**

Recruitment is part of staffing in the area of human resources to integrate the right people into organizations. However, technological platforms have been implemented in this type of process thanks to industry 4.0. That is why this research of non-experimental design, retrospective research, qualitative method aims to analyze the results obtained in recruitment 4.0 as a result of its implementation by the COVID-19 pandemic in Mexico. Considering for this articles and theses that talk about this.

## **Recruitment 4.0, Human Resources, Staffing, COVID-19**

### **Resumen**

El reclutamiento forma parte de la dotación de personal en el área de recursos humanos para integrar a las personas adecuadas a las organizaciones. Sin embargo, las plataformas tecnológicas se han implementado en este tipo de procesos gracias a la industria 4.0. Es por ello, que esta investigación de diseño no experimental, investigación retrospectiva, método cualitativo pretende analizar los resultados obtenidos en el reclutamiento 4.0 a raíz de su implementación por la pandemia por COVID-19 en México. Considerando para ello artículos y tesis que hablen sobre esto.

## **Reclutamiento 4.0, Recursos humanos, Dotación de personal, COVID-19**

### **10 Introduction**

Society is constantly undergoing changes, facing challenges (social, cultural, political, economic, among others), which force organizations to respond immediately (Alfaro, 2012). Similarly, important changes in the personnel area occurred in Mexican companies due to the COVID-19 pandemic,

In relation to the field of human resources management (HRM), it is clearly reflected that it had to comply with its purpose, which is to efficiently and effectively use the resources in any social organization (Alfaro, 2012). Transformations had to be made in the processes developed, such as in the integration of personnel, specifically in recruitment. Although many companies use their own intranet, as well as their websites and other public websites of job offers to recruit candidates (Snell & Bohlander, 2015, p. 181).

To better understand, Human Resource Management "Is the area of management concerned with all aspects of an organization's personnel: determining staffing needs, recruiting, selecting, selecting, developing, mentoring and rewarding employees; liaising with unions and handling other welfare issues" (Byars and Rue cited by Alfaro, 2012). That is why, every function and activity that this department develops becomes relevant, since people are required in every department, in order to achieve organizational objectives. In addition, it is not easy to achieve effective recruitment. First, some recruitment methods are better than others, depending on the position. Secondly, recruitment depends on extraneous issues such as pay scales. Third, labor laws determine what the employer can do (Dessler, 2015, p. 95).

Added to this, despite the fact that recruitment has evolved, little is known about the 4.0 technique that has been practiced in companies, hence this research has been given the task of analyzing and explaining what has been done in practice with respect to this sub-process belonging to staffing, so that other companies can continue to apply it.

Thus, this research contains the following sections: literature review, methodology, results, conclusions and references.



## 10.2 Literature Review

### *Human Resources Management*

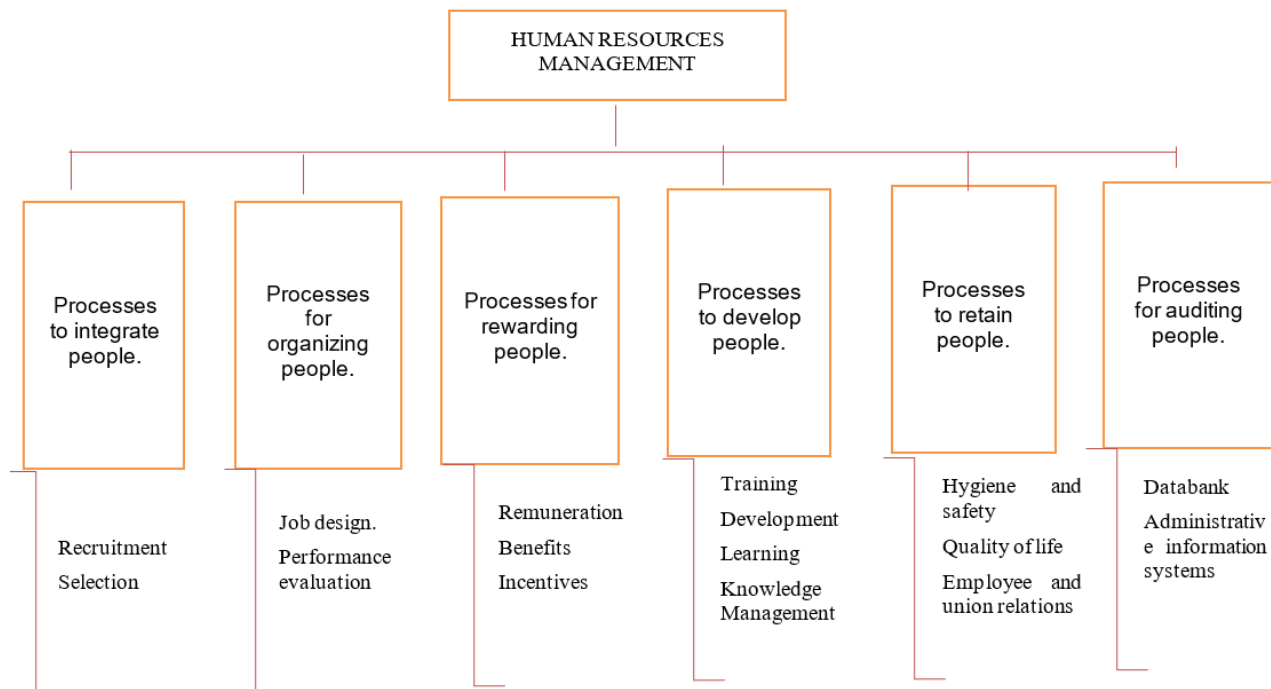
Human resources administration has the enormous responsibility of the entire human element within a company, since it not only deals with hiring and firing, but also with the management and precise strategy so that each of the collaborators can make their potential grow (COFIDE, 2022). It is also the discipline in charge of planning, developing and managing aspects related to work teams in an organization. It focuses on maximizing the potential of people to achieve the objectives of the organization (Castelan, 2023).

In addition, its radius of action contemplates aspects such as recruitment and selection, compensation, social benefits, occupational health and safety, organizational development, personnel training and development, labor relations, database and information systems, and auditing (Armijos Mayon, Bermudez Burgos, & Mora Sanchez, 2019). It includes some important processes that it develops in its functions (See Figure 10.1).

Since people ceased to be perceived only as a resource for the company, they subsequently became the human capital invested in to achieve organizational objectives, and became the talent to be identified in the market, to be integrated into organizations.

This is done by attracting these talents through recruitment.

**Figure 10.1** Human Resources Management Processes



Source: *Human Resources Administration (Chiavenato, 2011).*

### *Staffing*

In the field of labor relations, the process of human talent management is called staffing. This includes all activities developed to recruit, hire, orient, retain and dismiss employees, which must follow a logical step-by-step process (Jimenez, 2021).

Staffing can be internal or external, depending on the unit in charge of performing the tasks. Internal staffing implies that these activities are executed with people from the human resources area, while for external staffing consists of activities executed by people outside the company (Jimenez, 2021), these types of staffing contribute to the formation and continuous updating of the talent pool that will serve as a source for future recruitments.

Furthermore, according to Rodriguez (2007) that staffing includes the processes of recruitment, selection, hiring and induction, to acquire the necessary personnel to be incorporated into the company as vacancies open up (p. 145).

### *Recruitment*

#### *Definition*

A very basic definition of recruitment says that it is finding and attracting candidates for the employer's vacant jobs (Dessler, 2015). However, it is further considered as the process of gathering applications from individuals, identifying and attracting skilled and suitable prospective employees for the fulfillment of organizational objectives, once the distinctive elements that the candidate should possess can be established one can decide on the sources of recruitment as ideal (Ortega, 2016) Also, in 2015, Sanchez (Guerra, 2022) considered it as the process of identifying the best qualified candidates, qualified to take a defined position in a period of time, its purpose is to attract selectively through different techniques to candidates who meet the predisposed requirements that the position so requests. Recruitment not only involves seeking talent pools of employees, but making the effort to understand what they want and the establishment of the company as an employer of choice so that people will want to work for it (Snell & Bohlander, 2015, p. 180). Recruitment is the process of identifying qualified candidates to fill the organization's vacancies (Werther & Davis, 2014).

#### *Types of recruitment*

When an organization needs personnel to fill any vacancy, it must say whether it will carry out internal recruitment, referring to that which takes place among the personnel of a company; or external recruitment, with people available in the external labor market (Werther & Davis, 2014). Internal recruitment occurs when the company tries to fill a given vacancy by rearranging its employees, with promotions, transfers, transfer with promotion, which also involves personnel development programs and career plans for staff (Chiavenato, 2011, p. 133). External recruitment impacts actual or potential candidates, available or employed in other organizations, through techniques that the organization uses to disclose the existence of a job opportunity (Chiavenato, 2011, p. 136).

#### *Recruitment Techniques*

Some of the advances in the way of recruiting have been the following:

**Recruitment 1.0** In this, candidates saw job offers published in newspapers and went with the resume in hand to the company to apply for the job. Companies collected the resumes and stored them in a multitude of folders depending on the selection process they were applying for (DocuSign, 2021). Therefore, 1.0 was also underestimated for the quality and management of this technique, it is the one that can be considered the least developed, since its role is totally transactional, the structure that human resources have within an organization is small (Molinero, 2020). Recruitment was done only from personal databases of each company, which were difficult to maintain. Then, recruitment processes were more complicated and had limitations for both employers and prospective employees (Dempsey, 2016).

**Recruitment 2.0** Includes offers that were disseminated through the World Wide Web and made it easier for companies to access candidates and attract talent. Now more people arrive as ideal candidates to fill the position, in different recruitment portals through different social networks (Arranz, 2022). Both the 2.0 technique no longer has the same function as the 1.0 version, this leads to the structure is not handled as a unit, but the functions are dispersed, with many responsible for each subsystem, generating horizontal organizational structures; in terms of technology, it gives a big step for the use of software to improve the experience of both the employee and the person who manages human resources, as observed also facilitated the work of the recruiter was already a little easier and simpler to perform there we can realize that they were evolving gradually.

Recruiting 3.0 or Mobile Recruiting refers to the search for talent through mobile devices that allows reaching a greater number of candidates through different media such as websites, social networks, job boards and more. The aim is to make the professional want to join the organization (Grupo Gestal, 2023). This arises in 2011 with the increased use of the Internet, using various networks, such as LinkedIn that begins to gain strength and more professionalized job portals appear (Dempsey, 2016). In addition, with recruitment 3.0 it is sought that applications and the internet not only allow you to apply for the vacancies you need to fill, but also allows you to get all the information you need to make an informed decision. 88% of companies acknowledge reviewing the identity or digital footprint of their candidates on the Internet and their social networks (HR Management, n.d.).

Recruitment 4.0 This type of recruitment is focused on the possibilities offered by social networks to find the best prepared candidates with the necessary characteristics to join the companies, and with the sufficient and necessary talent that each department of the organization needs. The digital transformation has made the use of these platforms important for companies not only to attract, retain talent and obtain a good digital reputation, but also for the candidates themselves. Currently the 4.0 technique is the most used since the methods changed, it could be said that both facilitates the work is carried a better control. The resume is not something theoretical, but you can prove your worth with projects and case studies in an updated way (Bizneo, 2022).

AI benefits in the recruitment, hiring, identification and attraction of talent to perform a given job and produces an increase in impartiality, thus decreasing the risk of biased hiring (Garcia, 2021).

A notable difference in recruitment 4.0 is: brand image. Not only for companies, but also for candidates: the so-called and more than well-known personal brand (Estébanez, 2019). In this sense, the advantages of this type of recruitment are: a) Access to a greater number of candidates, b) Optimization of time and money, c) Greater segmentation, d) Skills and competencies in sight and in real time, e) Ease of networking (virtual events), and f) Access to new methods of personnel selection (Gamification, Inbound recruiting, Nanotechnology).

On the other hand, the digitalization of recruitment is no longer limited only to the publication and dissemination of a vacancy on the Internet, CompuTrabajo highlights that technological advances today allow to centralize the applications received from various sources, filter them and even evaluate the interviews. According to the platform, recruitment 4.0 can reduce the hiring process by up to 30% and increase agility by 80% (Hernández G. , 2022).

In the COVID-19 event, the most viable was the use of the 4.0 technique, emphasizing online communication, discovering and executing the new selection management, since nowadays it is considered efficient, giving positive results and better control of the selection. As part of the challenges that organizations face as a result of the pandemic by COVID-19, the tasks of recruitment and selection of personnel are an essential point that needs an adaptation to changes and trends (Cortés, 2022).

### **10.3 Methodology to be developed**

The present study is of non-experimental design due to the fact that what is done in this type of design is to observe phenomena as they occur in their natural context, in order to subsequently analyze them (Hernández, Fernández, & Baptista, 2014). Qualitative type, research that is oriented to qualify and describe the social phenomenon from determining features, as perceived by the elements themselves that are within the situation studied (cited by Bonilla and Rodriguez, 2000, in Bernal, 2010).it is also a longitudinal research, which refers to comparing data obtained at different times or moments of the same population, with the purpose of evaluating changes (Bernal, 2010) retrospectively, since it studies past events. This is because the articles and theses used to analyze the information are from the period of the COVID-19 pandemic in Mexico (2020-2022). On the other hand, this research was only applied to analyze recruitment 4.0, because thanks to new technologies help to obtain a fast, agile and dynamic activity; this means that, with the set of tools offered by this modality, it is possible to overcome and change the old methods of the selection process making them more efficient (Rodríguez-Altamirano, Higinio-Meléndez, & Ovalle-Paulino, 2021).

The search was done through open access search engines such as Google Scholar and Scielo, identifying four theses and four articles that were directly related between recruitment 4.0 and its use in the wake of the pandemic by COVID-19 (2020-2022).

#### 10.4 Results

A synthetic-analysis of the documents obtained (four theses and four articles) was carried out, to subsequently perform the content analysis of these.

**Table 10.1** Analysis of Theses

THESIS			
TITLE	YEAR	SUMMARY	CONCLUSION
HR 4.0 La transformación digital de RRHH (Simón, 2021)	2021	Based on the experience of leading companies in the Argentine market that went through and continue to evolve in this challenge, we will study how they implemented the transformation, their most advanced practices, how the role of HR was modified, as well as the main obstacles they faced and the lessons they were able to incorporate. In addition, we will explore the state of the art of HR digital transformation, including how it is affected by the current global pandemic context.	HR teams undoubtedly face an enormous challenge and must be able to keep up with the demands of the 4.0 revolution. Their own transformation and subsequent management towards the rest of the organization will be what will allow them to remain competitive in the VUCA environment in which we are immersed. For HR, this is an opportunity to rethink their structures, incorporate agile methodologies, redesign their processes with a focus on the customer, reduce time on tasks of little added value to focus on defining strategy and lead the upskilling of the organization. It is essential to convey to the team the enormous advantages that this entails, ranging from broadening their profile and developing new skills to taking the employee experience to another level. Companies are demanding a new HR adaptation, they need to be strategic allies and ambassadors of the cultural change that the digital transformation (not only of HR, but of business in general) requires. It is a historic moment where we are invited to transform ourselves from the inside out, to create the culture and mindset that enables collaboration, innovation and digitization of our processes and products. HR professionals are not alone, there are many who have already embarked on the challenge and, based on trial and error, are helping us to build this new framework. Let's continue to generate spaces for exchange to enrich our vision, understanding that nothing is static and we are all evolving together, and it is precisely in diversity where we will find new opportunities.
La industria 4.0 en la gestión de los recursos humanos (García Rodríguez, 2021)	2021	According to the methodology followed, a literature review on Industry 4.0 and HR management has been conducted, with the main objective of showing the existing relationship between both terms; in addition to the exposition of a series of cases in which several companies have used different technologies in their HR processes.	We have been able to verify that depending on the human resources management task (recruitment, selection, training, evaluation...) to be automated, a company will have to decide to implement one technology rather than another. Furthermore, we can say that there are technologies that are used on a greater average and others that are used less, due to the fact that the latter are more aimed at performing other types of tasks that are not related to the human resources area of the company.

Reclutamiento 4.0 (Pastor, 2020).	2020	This work collects a study on Human Resources Management in organizations, with emphasis on human resources recruitment, more specifically on recruitment 4.0. I have chosen this topic because I consider this section as the most important of Human Resources, since it aims to find the best people for a specific job, resulting in increased productivity. I also wanted to analyze Recruitment 4.0 because I believe that companies should rely on ICT to improve human resources management. Also, this work contains practical examples of Recruitment 4.0. The first objective of this final thesis is to demonstrate the growing influence of new technologies in recruitment.	It should be noted that one of the events that has prompted companies to automate some of their processes (not only those of human resources) has been the health crisis of the HIV/AIDS pandemic, with the main objective of reducing the number of infections.
El reclutamiento 4.0 en la era digital (Hernández Martínez, 2020)	2020	In recent years, the recruitment department has advanced along with new technologies. The current market has been digitalized due to the technological revolution, as a consequence, it is now more competitive and open. The new business success lies in attracting talent by deploying online strategies for HR experts to attract and retain potential through marketing. New applied technologies, digital recruitment and staff loyalty are some of the challenges facing recruiters in the 21st century. This paper begins by analyzing the concepts of recruitment, its digital transformations, the application of digital marketing and the key events for the HR area that will predict the future of recruitment and the main trends in attracting talent.	In terms of recruitment, more emphasis must be placed on the use of the new technologies that today's world offers us, since it has many benefits for the company, already mentioned above, such as a better image for the company's brand, lower costs, speed in the whole process, greater access to information, etc.

*Source: Own adaptation*

Table 10.2 Article Analysis

ARTICLES			
TITLE	YEAR	ABSTRACT	CONCLUSION
Modelo de proceso de selección de personal utilizando las técnicas de Reclutamiento 4.0 y Plataformas Digitales en tiempos de COVID-19 (Rodríguez-Altamirano, Higinio-Meléndez, & Ovalle-Paulino, 2021).	2021	This scientific article presented a proposed model for the selection process using novel techniques, such as recruitment 4.0 together with digital platforms. It guarantees the realization of the process adapted to the new recruitment trends and to the measures opted by the Government to stop the advance of COVID-19; all this allowed to obtain as a result an efficient and effective model of selection process that guaranteed the decrease of time and costs.	Likewise, the proposed model is adapted to the measures that must be taken due to the pandemic that we are currently experiencing, making it applicable and very generic for its application in companies. Finally, the proposed model, counts with the techniques of recruitment 4.0 and the use of digital platforms that are adapted to the new normality that will be experienced after and during the duration of the pandemic.
La industria 4.0 y las nuevas formas de trabajar: una perspectiva desde el caso mexicano en tiempos del COVID-19 (López, 2020).	2020	The objective of this paper is to analyze the new ways of working and their social protection as a result of Industry 4.0 from a Mexican perspective with emphasis on COVID-19 times.	The pandemic generated by the COVID-19 has shown that certain work activities can be performed without the need to travel to the workplace thanks to the use of ICT's, however, it has also shown that some societies (as in Mexico) are not able to perform it, either by the lack of skills and digital skills of people or otherwise, by the lack of inputs to perform it.
Reclutamiento y selección virtual por competencias (Valeriano Ortiz, 2021)	2021	Technologies have changed the way of conceiving and developing the lives of people where work is not alien to it, these technologies have allowed to perform new ways of working that previously had not been implemented and currently have gained strength by the pandemic generated by the virus COVID-19.	Companies have their development agenda reflected in their strategic and tactical plans to incorporate new employees. To achieve leadership and competitiveness, companies have to bet on change and need to hire people with creativity and innovation.
El Enfoque RR.HH. 4.0. ¿Está Cambiando Finalmente La Función Recursos Humanos? (Cardozo, 2021).	2021	The sustained advance of the digital economy is finally impacting HR management in organizations. A change that is in full swing and that highlights the inertia that the function has had for decades compared to the rest of the basic organizational functions. On this point, there is international consensus that the area has been the least evolved in terms of hierarchy, structure and management, modifying over time only operational issues. However, the technological impact and the emergence of proposals such as HR 4.0, e-HRM or Agile HR with digitalization as a common pattern would seem to finally evidence the necessary adaptation to the context and the enrichment of the function. The paper discusses the already historical demand for changes in the area, the necessary bases for such transformation, the characteristics of its eventual implementation, and a preview of the state of affairs in a sample of local companies.	Finally, it should be mentioned that during the development of this work, the COVID 19 pandemic emerged, with some consequences already known, others in development and others still unknown at all levels of human life. From a business perspective, some recent works show results on how companies that had been developing agile management were able to adapt much more quickly to this uncertain context with measurable evidence from practice. This adaptation involved increasing the speed of decision making, while improving productivity, using technology and big data. The combination of technologies, big data, reinvention of processes and new collaboration tools generated the so-called post-pandemic rapid organization.

<p>Los efectos de la pandemia de COVID-19 en la industria de la construcción del Reino Unido y proceso de negocio aprueba de fututo. (Stride, 2023)</p>	<p>2023</p>	<p>COVID-19 was officially declared a global pandemic by the World Health Organization (WHO) on March 11, 2020, before the UK was confined on March 23, 2020. Organizations had to reconsider their policies and procedures to allow their business to continue. This paper focuses on the effects of COVID-19 that the UK construction sector has had to undertake to enable businesses, while employees had to comply with the COVID-19 confinement rules. In addition, how the sector can continue positively once normality has returned within the industry. In doing so, this paper understands the historical problems within the construction sector and have had an effect during COVID-19.</p>	<p>COVID-19 has changed the way we work and the way companies operate and therefore studies on how companies have had to adapt have been critical to help protect against radical change against future pandemics and organizational crisis. There is a culmination of issues that the construction industry is struggling with due to the coronavirus pandemic, but this research also takes into account the historical issues that have been inflated due to the pandemic within the sector that allows for a collaborative approach to help move forward and progress collectively. Therefore, by completing a literature review of current research and interviewing construction workers who provided real evidence of the issues and adaptations within their organizations, this study has reviewed the barriers of Covid-19, organizational crisis, prefabrication, Industry 4.0, skills shortages and job termination, the 2008 UK recession, mental health and wellbeing, health and safety, cost inflation and the UK housing shortage. Subsequently, this research has completed its objective of understanding the historical issues within the sector, the impact that COVID-19 is having on businesses and employees and concentrates on the changes that the construction sector has had to undertake to enable businesses and employees to adhere to the COVID-19 confinement rules and how the sector can continue positively once normality has returned within the industry.</p> <p>Once all the information was reviewed and analyzed, the data collection was completed, allowing a comprehensive perspective of the industry to be concluded. The research found that there are serious problems within the industry, and the research suggests that a more diverse workforce could have enormous benefits in producing new technologies, reducing skills shortages and building more homes. However, it was suggested that the industry needs to provide better working conditions for both current workers and attract people to join the sector by offering remote and flexible conditions, better health and safety environments and upskilling the current workforce. This will enable program reductions, which will cause fewer delays and save costs in the long run.</p>
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			<p>The information also showed that organizations should invest in greater use of technology, as respondents felt that this would have a positive impact while working remotely, but also</p> <p>AAM: Innovation in Construction: Information, Process, Management</p> <p>explaining that greater use of prefabrication would reduce schedules and create safer working environments as materials would be manufactured in factories and improve social distancing on site.</p> <p>It was understood that Industry 4.0 could also have a positive impact on mental health and well-being, as greater use of technology would aid colleague interaction with the use of initiatives such as virtual "coffee mornings." In addition to this, respondents state that organizations need to invest more to help combat mental health and wellbeing issues within the workplace and that it benefits employees and improves morale within companies.</p>
<p>El proceso de transformación digital en el sector de la pequeña y mediana empresa (PYME) en la era de la pandemia COVID-19: un estudio de Polonia y Croacia (Kuczevska, 2023)</p>	<p>2023</p>	<p>This paper aims to explore the extent to which the pandemic has forced SMEs to accelerate their digital transformation efforts to remain competitive and adapt to the changing business landscape. The focus is on three key research questions: how did digitalization manifest itself in SMEs prior to the pandemic outbreak? How did the digital transformation of SMEs evolve during the pandemic? And what business challenges SMEs face in the post-COVID period due to the acceleration of digital transformation? For the empirical research, qualitative research methods such as in-depth interviews in 10 SMEs in Poland (six) and Croatia (four) are used. The main results emphasize three main findings. First, even before the pandemic, SMEs recognized and used several digital transformation technologies to improve their business processes and performance. Second, the development of digitization processes in SMEs observed during the pandemic was driven primarily by the need for remote working, e-commerce, virtual events, and automation of business relationships among all stakeholders. Third, the post-pandemic world confirms the need for SMEs to invest in cybersecurity, talent acquisition, infrastructure, customer engagement and data privacy to remain competitive in the digital economy.</p>	<p>To investigate the progress of digital transformation processes in Polish and Croatian SMEs before, during and after the pandemic, ten companies were studied in the form of interviews. Interviews with Polish and Croatian companies show that the pandemic outbreak was an accelerating factor for this transformation. It contributed to the digitization of more operational areas of companies that were previously non-digital or only to a limited extent, such as sales or marketing. The growth prospects of companies are closely linked to the increasing global digitization of societies, which affects the volatility of GVCs. On the other hand, the adoption of digital solutions promotes adaptation to an unpredictable external environment. Companies in the post-pandemic period see the need to conduct some of their business in a hybrid way, such as contacting potential customers.</p> <p>This study contributes twice to the discussion and research on digitization processes in SMEs. First, it contributes to the literature on digital transformation and the links between pandemic crises. Second, it shows that digitization processes are not temporary and help to improve the operational performance and competitiveness of firms, especially in times of global turmoil.</p>



			<p>Overall, the research contributes to the literature on knowledge about digital transformation processes and the extent of pandemic digital change, including its lasting nature for business operations. The relatively small number of interviews allows extending the present study to other countries and companies. In addition, it is worth noting that the research focuses on a specific group of companies, e.g., the ICT industry, that the digital organization of the company may affect. The potential development of further research in this area, as the global economy is still in the post-COVID-19 era, and the topic of digital transformation should be further explored.</p>
<p>Evaluación de actividades y participación con respecto a un programa piloto de vacunación contra el COVID-19 en el lugar de trabajo en el sur de Alemania considerando la perspectiva de la salud ocupacional: un estudio de métodos mixtos. (Wagner, 2023)</p>	<p>2023</p>	<p>This mixed-methods study retrospectively assessed the attitudes and participation of employees, occupational health staff and key personnel regarding the deployment of a pilot COVID-19 workplace vaccination program in five German companies in May/June 2021 in Baden-Württemberg (southern Germany) by combining survey data and qualitative interviews. A total of 652 employees completed a standardized questionnaire and we conducted ten interviews with occupational health staff and key personnel with other professional backgrounds organizing the workplace vaccination pilot program. The survey data were analyzed descriptively and the interviews were audio-recorded, transcribed verbatim, and analyzed using qualitative content analysis. Employees participated extensively in COVID-19 vaccinations at their workplaces, and the majority of employees (n = 608; 93.8%) had full COVID-19 immunization at the time of the survey. The main advantages of the COVID-19 workplace immunization pilot program were seen in the flexible and time-saving immunization offer, as well as the trust and long-standing relationship with occupational health physicians. The main disadvantage of the pilot vaccination offering was the increased workload for occupational health staff, especially during the launch phase of the program. The COVID-19 workplace vaccination pilot program was predominantly evaluated positively, and the important role of occupational health services in the management of the COVID-19 pandemic was highlighted. The main criticisms of the COVID-19 workplace vaccination program were related to the high organizational and administrative burden. The findings of our study may support the development of future programs for the administration of the generally recommended workplace vaccination in Germany.</p>	<p>We conducted a comprehensive mixed-methods study to retrospectively evaluate the COVID-19 workplace vaccination pilot program in different companies initiated by the Ministry of Social Affairs, Health and Integration in Baden-Württemberg, Germany. In summary, we identified a predominantly positive evaluation and considerable participation in the COVID-19 workplace vaccination pilot program in five companies in southern Germany (Baden-Württemberg). We found that adding the workplace setting and including occupational health physicians could actively support the launch of the COVID-19 vaccination campaign in Germany. One explanation here is the long-standing and trusting relationship that employees have with occupational health physicians, and the important role that occupational health physicians incorporate in the delivery of medical care and prevention in the workplace. However, we also identified challenging aspects, including low vaccination rates in some companies and a generally high workload for occupational health services, especially during the overall implementation phase of the COVID-19 workplace vaccination pilot program. Therefore, we need to learn more about the hesitancy in workplace vaccination and determine the extent to which occupational health services can help increase vaccination rates across the workforce.</p>

			<p>From our perspective, there is a crucial need for a systematic review and evaluation of the administration of generally recommended vaccines in the workplace to assess and compare their quality and derive recommendations regarding the development and implementation of future vaccination programs. In addition, we detected employee recognition of the important role played by occupational health physicians in the fight against the COVID-19 pandemic.</p> <p>The occupational setting was shown to present an additional opportunity beyond general practices to provide easily accessible, flexible, and time-saving preventive medical care and infection prevention during the COVID-19 pandemic. The current results of the COVID-19 workplace vaccination pilot program and the important role of occupational health services (e.g., provision of vaccination services, timeliness of consultation, and occupational health expertise) should be further considered in future vaccination campaigns in and outside the workplace.</p>
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*Source: Own adaptation*

According to all the data obtained from the analysis of each of the articles and theses, the results show that the evolution should be frequent and using digital media because at the time it was necessary to have knowledge of it, since it is, how to apply it and how the organization is benefited in the recruitment of an effective staff.

In addition, it was noticed that the theses and articles that were analyzed talk about the importance of having a good human talent through digital media, at the beginning it was complicated since it was unknown the subject, so today it is already common to use the 4.0 technique.

Due to the drastic changes that are occurring in society, companies must be attentive to meet the needs of consumption and the company itself. That is why the recruitment and selection process should not be taken lightly. Therefore, we must move on from the traditional view that considered recruitment and selection processes as specific acts within the company and integrate these processes as a fundamental part of the life and growth of the company.

If the personnel selection process is not done correctly and a worker is hired who is not suitable for the position, it can cause the company low productivity. It is useless to develop a company philosophy if it does not have the administrative structure to support it.

## **10.5 Gratitude**

We thank our parents who have been the driving force behind our dreams and hopes, who were always by our side supporting us during the days and nights of study. We would like to thank our tutor, Anabel Regina Montes de Oca Estrada, for her knowledge, skills, advice and virtues, which helped us to complete this work. And finally we want to thank the Tecnológico de Estudios Superiores de Villa Guerrero for forming us as professionals today, for opening its doors and filling us with knowledge, and also thank the teachers who taught us classes because thanks to them we can continue to share the experiences and advice they gave us.

## 10.6 Conclusions

The research aimed to analyze Recruitment which is the process by which the organization identifies and attracts future skilled and suitable employees for the fulfillment of organizational objectives (Snell & Bohlander, 2015). In particular of recruitment 4.0, which emerged as an implicit need as a result of the pandemic by COVID-19, although the technology was already booming, it had a more accelerated pace in the processes and functions also of the Human Resources area, thus giving the acceleration of the application of industry 4.0.

According to the various authors, it was concluded that the 4.0 tools were of great help during the COVID-19 period, so that companies had to make changes, which benefited the recruitment of personnel, giving way to new technologies and thus facilitating the work and selection of Human Resources. Due to the same conditions of health and distance, organizations opted for technological applications to recruit personnel, and even more, to continue with subsequent processes such as the selection and hiring of personnel.

In addition, Garcia (2021) talks about the consistency in the application of digital marketing techniques in the recruitment process of the organization's personnel, using mass media. This research has shown that the evolution of the techniques was favored after living a complicated situation for the recruitment of personnel, however, there is still much room for improvement because it was also found that the technological skills required to carry out the processes through digital platforms are not so present in the staff, in addition to the lack of tools that allow them to properly carry out their functions. In addition, many platforms require an additional payment to have more functions to make the activities more efficient, which should also be considered for the budget of the Human Resources area, which should be part of the organizational objectives.

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## Abstract

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Indicate (3-5) keywords in Times New Roman and Bold No.12.

## 1 Introduction

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Explanation of the topic in general and explain why it is important.

What is its added value with respect to other techniques?

Focus clearly on each of its characteristics.

Clearly explain the problem to be solved and the central hypothesis.

Explanation of the sections of the Chapter.

Development of Sections and Sections of the Chapter with subsequent numbering.

**[Title in Times New Roman No.12, single space and Bold].**

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## Inclusion of Graphs, Figures and Tables-Editables

In the content of the Chapter, all graphs, tables and figures must be editable in formats that allow modifying size, type and number of letters, for editing purposes, these must be in high quality, not pixelated and must be noticeable even if the image is reduced to scale.

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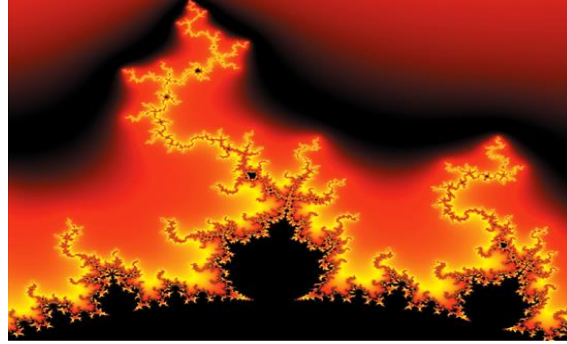
**Table 1.1 Title**

Variable	Description	Value
P <sub>1</sub>	Partition 1	481.00
P <sub>2</sub>	Partition 2	487.00
P <sub>3</sub>	Partition 3	484.00
P <sub>4</sub>	Partition 4	483.50
P <sub>5</sub>	Partition 5	484.00
P <sub>6</sub>	Partition 6	490.79
P <sub>7</sub>	Partition 7	491.61

*Source:*

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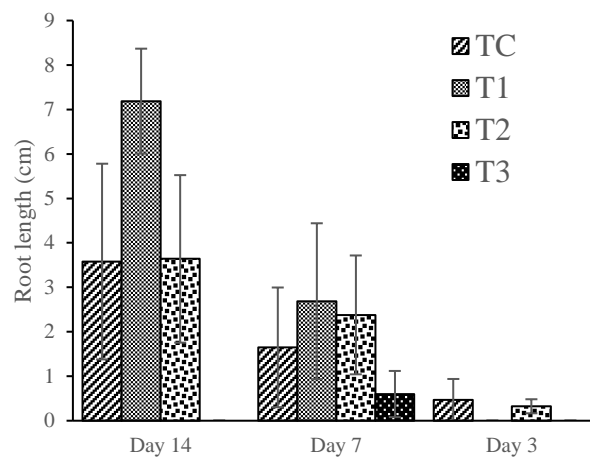
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### Methodology to be developed

Give the meaning of the variables in linear wording and it is important to compare the criteria used.

### Results

The results should be per section of the Chapter.

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Tables and appropriate sources.



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## **Conclusions**

Clearly explain the results obtained and the possibilities for improvement.

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3. *Analysis based on demand curve regression*
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