Handbook T-XIV
CIERMMI Women in Science
Biology, Chemistry and Life Sciences

MARROQUÍN-DE JESÚS, Ángel
OLIVARES-RAMÍREZ, Juan Manuel
DECTOR-ESPINOZA, Andrés
CRUZ-CARPIO, Luis Eduardo

Coordinators

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ECORFAN CIERMMI Women in Science

Volume XIV

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 Biology, Chemistry and Life 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 Biology, Chemistry and Life Sciences.

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MARROQUÍN-DE JESÚS, Ángel. PhD
OLIVARES-RAMÍREZ, Juan Manuel. PhD
DECTOR-ESPINOZA, Andrés. PhD
CRUZ-CARPIO, Luis Eduardo. BsC

Coordinators

CIERMMI Women in Science T-XIV
Biology, Chemistry and Life Sciences
Handbooks

Colegio de Ingenieros en Energías Renovables de Querétaro A.C. – Mexico. October, 2021

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Prologue

Whenever you think of a scientist you imagine a white man in a lab coat looking at a flask, this is one of the paradigms that has been given in society despite being one of the most prominent areas of a woman. We have endless examples at national and international level of women in science and technology, who have won the most outstanding awards in this niche of society. However, there is still a great bias of women recognized in science and technology, which is why this book recognizes the work and career of women who stand out for their great work in science, which focuses on the knowledge of the human body and which impacts on improving the living conditions of each human being.

Living beings require oxidation-reduction processes to obtain cellular energy, but due to several factors an excess of oxidation can be generated and oxidative stress appears, generating a series of free radicals that in low concentrations help the body to adapt to its physical activity, But they are also attributed to skeletal muscle wear in sedentary conditions and to avoid this type of wear have sought various improvements such as the intake of functional foods that prevent skeletal muscle fatigue, is one of the issues addressed in a chapter in this book, as the consumption of *Stevia rebaudiana* Bertoni as a natural and artificial sweetener on fatigue and oxidative stress of skeletal muscle. A widely studied alternative for oxidative stress are antioxidants, which have a wide benefit for the human body not only for skeletal muscle, which is why several studies focus on their chemical activity and the combination of various food sources to obtain better results. Focused on this topic we find the obtaining of a natural fiber with antioxidant effect from cashew nut bagasse which is promising for the effects of lipoinflammation. We can also find several natural products of chemical compounds that help humans to perform various daily activities such as cleaning, disinfection as it is with alcohol, in one of the chapters you can learn how to obtain an ethanol extract from the leaves of *Tradescantia spathacea* and how this product is characterized to be able to give the proper use. It is sought that the obtaining of this type of products comes from natural sources because they should not present a major problem of intake for humans, however, this is not necessarily correct, which is why both the source and the derivatives obtained must be evaluated. In this book you will find how important is the source of proteins, whether animal or vegetable, which are associated to the reduction of health problems of the metabolic syndrome and its comorbidities. Metabolic syndrome is a set of health problems related to diabetes, coronary heart disease and cerebrovascular disease. It is not only essential to know the origin of each of the chemical compounds, you should also take into account the interactions they have with other elements, compounds or living beings, because they generate reactions which are of vital importance at the biochemical level. In another chapter you will learn about the biogeochemical cycle of manganese, which is important to know how our planet and the living beings that inhabit it have developed. In recent years, we have learned how changing natural conditions can develop new organisms and how we can generate defenses for those that are not so beneficial to humans, thanks to SARS-CoV-2 we can understand the importance of personal, home and, therefore, hospital sanitation. Here you can find information on physical control methods for pathogenic microorganisms in hospital areas. In addition to cleaning, which is fundamental for good practices in medical and hospital care services, it is necessary to maintain instruments and biological products. One of the alternatives presented to preserve the latter is the use of ultrasound, supercritical fluids and membrane technology, described in one of the chapters.

CRUZ-RAMÍREZ, Marisela. PhD

Introduction

The Colegio de Ingenieros en Energías Renovables de Querétaro, A.C. (CIER-QUERÉTARO), and its chapters of Renewable Energy, Industrial Maintenance, Mechatronics and Computer Science, technical sponsors of the International Interdisciplinary Congress on Renewable Energy, Maintenance, Mechatronics and Computer Science, CIERMMI 2021 has as general objective to establish a space for discussion and reflection on issues related to the areas of: renewable energy, industrial maintenance, mechatronics and computer science with the participation of students, professors, researchers and national and international speakers, promoting the formation and consolidation of research networks. Contributing to provide a space for dissemination and discussion of the presentations of students, graduates, academics and researchers, representatives of various higher education institutions, research centers in our country, as well as educational institutions beyond our borders. Promoting the formation of research networks between different institutions. Offering a space for undergraduate, master's, doctoral and postdoctoral students, in which they can present the progress of the research they carry out in their different educational centers. Providing a space in which study groups and members of academic bodies, linked to the curricular program of renewable energy, industrial maintenance, mechatronics and computer science careers, can present the research work developed within their institution and in collaboration with other national or international educational institutions. Establishing a training space for the attendees, through the development of specific lectures and conferences.

This volume, Women in Science T-XIV-2021 contains 7 refereed chapters dealing with these issues, chosen from among the contributions, we gathered some researchers and graduate students from the 32 states of our country. We thank the reviewers for their feedback that contributed greatly in improving the book chapters for publication in these proceedings by reviewing the manuscripts that were submitted.

As first chapter, *Martínez, Bravo, Sánchez and Montoya* present Effect of the consumption of Stevia rebaudiana Bertoni as a natural and artificial sweetener on fatigue and oxidative stress of skeletal muscle, as second chapter, *Hernández, Ramírez, Chávez and Oliart*, will talk about Cashew bagasse (Anacardium occidentale L.) as a source of fiber-antioxidant and its possible use in lipoinflammation models as the third chapter, *Marcos, Ramirez, Oliart, and Guadarrama* present The relevance of the source of animal or vegetable proteins on the metabolic syndrome and its comorbidities, as the fourth chapter, *Damián, Rivera, Lizárraga and Vázquez*. propose Wanderings of a magic element: the biogeochemical cycle of manganese, as the fifth chapter, *Sánchez, Paniagua, Temiche and Alexander*, perform Methods of physical control of pathogenic microorganisms in hospital areas, as the sixth chapter, *Paniagua, Sánchez, Corro and Alexander* develop Use of power ultrasound, supercritical fluids and membrane technology to obtain and/or preserve biological products for clinical use, and as the last chapter, *Estrada, Figueroa, Sierra and Aguilar*, focus on Obtaining and characterization of the ethanolic extract of the leaves of the Tradescantia Spathacea SW

MARROQUÍN-DE JESÚS, Ángel
OLIVARES-RAMÍREZ, Juan Manuel
DECTOR-ESPINOZA, Andrés
CRUZ-CARPIO, Luis Eduardo

Coordinators

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Capítulo 1 Efecto del consumo de la *Stevia rebaudiana* Bertoni como edulcorante natural y artificial sobre la fatiga y el estrés oxidante del músculo esquelético

MARTÍNEZ-MARTÍNEZ, Cielo Maritza´†, BRAVO-SÁNCHEZ, Estefanía´, SÁNCHEZ-DUARTE, Elizabeth´´ and MONTOYA-PÉREZ, Rocío´*

´Universidad Michoacana de San Nicolás de Hidalgo, Instituto de Investigaciones Químico-Biológicas, Mexico.

´´Universidad de Guanajuato, Campus León, Department of Applied Labor Sciences, Mexico.

ID 1st Author: *Cielo Maritza, Martínez.Martínez* / **ORC ID:** 0000-0002-7781-0090

ID 1st Co-author: *Estefanía, Bravo-Sánchez* / **ORC ID:** 0000-0002-8012-6126

ID 2nd Co-author: *Elizabeth, Sánchez-Duarte* / **ORC ID:** 0000-0002-7089-4373

ID 3rd Co-author: *Rocío, Montoya-Pérez* / **ORC ID:** 0000-0002-7569-1358

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* Correspondence author

A. Marroquín, J. Olivares, A. Dector L. Cruz. (Coord.) CIERMMI Women in Science TXIV Biology, Chemistry and Life Sciences. Handbooks-©ECORFAN-México, Querétaro, 2021.

Abstract

Stevia rebaudiana Bertoni has non-caloric sweetening properties and its use has been linked to therapeutic effects. However, Stevia sp is sold as a substitute for sugar commercially, not only includes steviosides but is also combined with other high-intensity artificial sweeteners, which questions its safety. Our objective was to evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on fatigue and oxidative stress of skeletal muscle. Twenty-four male Wistar rats were divided into: (C) rats receiving water, (S); rats receiving 41.2 g/L sucrose solution (SRA); rats receiving solution with commercial sweetener (*Svetia*) 6 g/L; (SRN); rats receiving solution with the dried and powdered leaf of the *Stevia* sp. plant. 4.4 g/L. Eight weeks after the treatment, muscle tension recording, and measurement of oxidative stress markers were performed: levels of reactive oxygen species (ROS) and catalase activity. Additionally, body weight, postprandial glucose, and food intake were recorded throughout the experiment. The SRA caused an increase in body weight and a significant reduction in the resistance time to muscle fatigue and the maximum and total muscle tension force. Treatment with SRN caused significant improvements in the parameters studied ($p < 0.05$). We conclude that natural *S. rebaudiana* is an essential alternative for weight control and the development of antioxidant defense against muscle fatigue but not in synergy with artificial sweeteners.

***Stevia rebaudiana* Bertoni, Muscle fatigue, antioxidant**

Resumen

Stevia rebaudiana Bertoni tiene propiedades edulcorantes no calóricas y su uso se ha relacionado con efectos terapéuticos. Sin embargo, la Stevia sp se vende como sustituto del azúcar comercialmente, no sólo incluye esteviósidos sino que también se combina con otros edulcorantes artificiales de alta intensidad, lo que pone en duda su seguridad. Nuestro objetivo fue evaluar el efecto de la Stevia rebaudiana Bertoni natural y artificial sobre la fatiga y el estrés oxidativo del músculo esquelético. Veinticuatro ratas Wistar macho fueron divididas en: (C) ratas que recibieron agua, (S); ratas que recibieron una solución de sacarosa de 41,2 g/L (SRA); ratas que recibieron una solución con edulcorante comercial (*Svetia*) de 6 g/L; (SRN); ratas que recibieron una solución con la hoja seca y pulverizada de la planta *Stevia* sp. 4,4 g/L. Ocho semanas después del tratamiento, se realizó el registro de la tensión muscular y la medición de los marcadores de estrés oxidativo: niveles de especies reactivas del oxígeno (ROS) y actividad de la catalasa. Además, se registraron el peso corporal, la glucosa postprandial y la ingesta de alimentos durante todo el experimento. El SRA provocó un aumento del peso corporal y una reducción significativa del tiempo de resistencia a la fatiga muscular y de la fuerza de tensión muscular máxima y total. El tratamiento con SRN provocó mejoras significativas en los parámetros estudiados ($p < 0,05$). Concluimos que la *S. rebaudiana* natural es una alternativa esencial para el control del peso y el desarrollo de la defensa antioxidante contra la fatiga muscular, pero no en sinergia con los edulcorantes artificiales.

Stevia rebaudiana Bertoni, Fatiga muscular, Antioxidante

1.1 Introduction

Skeletal muscle myopathy is a common clinical condition and a much less studied complication in chronic diseases; these muscle alterations lead to a reduced ability to withstand fatigue (Fernández et al. 2009).

The consumption of sugars in a balanced way in the daily diet has important properties since it favors the rapid supply of glucose to the brain and muscle, carbohydrates being essential for the development of cognitive functions and physical activity. Cane sugar or sucrose has been the sweetener par excellence for centuries; however, its excessive intake has been linked to different conditions: obesity, diabetes and metabolic syndrome (Gil-Campos et al. 2015).

There are several reports on replacing cane sugar with non-caloric herbal high-intensity sweeteners, and these substances can provide the sweet taste but substitute the caloric effects of carbohydrates and provide antioxidant effects. *Stevia rebaudiana* Bertoni is a native South American shrub, and its leaves are a powerful alternative to caloric sweeteners. In addition to their sweetening properties, Stevia sp. Extracts have been linked to other therapeutic effects, as their phenolic compounds have potential antioxidant properties and their phytochemicals help reduce blood sugar, cholesterol, and blood pressure. However, when Stevia rebaudiana Bertoni is sold as a sugar substitute, many commercial brands include its steviosides in their formula and combine it with other artificial high-intensity sweeteners (EAI). Although the use of these sweeteners is widespread, their safety as food additives remains controversial, cytotoxic effects and metabolic effects have been reported, casting doubt on whether the consumption of these additives is the most appropriate tool for the control of metabolic diseases (Stephens -Camacho et al. 2018). The current results on the use of antioxidants to delay muscle fatigue in humans are contradictory and constitute the main focus of discussion about the role of reactive oxygen species (ROS) in muscle fatigue. However, some authors have reported on the direct antioxidant activity of Stevia rebaudiana Bertoni leaf extracts on oxidative stress markers in different organs. Therefore, studies of this nature are necessary on the role of the antioxidants in natural Stevia rebaudiana Bertoni as their role in synergy with artificial sweeteners in the inactivation of ROS and its effect on muscle fatigue (Fernández et al. 2009; Ruiz -Ruiz et al. 2017).

1.2 General objective

To evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on fatigue and oxidative stress of skeletal muscle.

1.3 Specific objectives

- Check the effect of natural and artificial *Stevia rebaudiana* Bertoni on rats' body weight and plasma glucose levels.

- To evaluate the contractile response and resistance to skeletal muscle fatigue by consuming natural and artificial *Stevia rebaudiana* Bertoni in rats.

- To evaluate the effect of natural and artificial *Stevia rebaudiana* Bertoni on oxidative stress and the antioxidant capacity of skeletal muscle in rats.

1.4 Materials and methods

1.4.1 Experimental animals

Twenty-four male rats of the Wistar strain with an initial weight of 300-320 g were used, kept in a room at a temperature of ~ 24 ° C, with a typical 12 h light / dark cycle with free access to food and water.

They were randomly divided into 4 groups (n = 6): (C) control, rats receiving unsweetened water; (S) sucrose: rats receiving sucrose solution (41.2 g / L); (SRA) Artificial *Stevia rebaudiana*: rats receiving solution with commercial sweetener of the brand “Svetia” (6 g / L); (SRN) Natural *Stevia rebaudiana*: rats receiving solution with the dried and powdered leaf of the *Stevia rebaudiana* Bertoni plant (4.4 g / L).

For 8 weeks, the rats received the solutions daily and were exchanged for new ones every 24 h; also, constant monitoring and recording was carried out simultaneously (1:00 pm) of the amount of solution and food consumed.

Once a week, the following were measured from the beginning of the treatments to the end: postprandial glycemia (GP) with a commercial glucometer (Accu-Check Performa ©), weight on a conventional scale and waist diameter with a measuring tape.

1.4.2 Animal ethics approval

The animals used were cared for by their nature and the experiment's objectives, following the recommendations of the Official Mexican Standard NOM-062-ZOO-1999 on the technical specifications for the production, care and use of laboratory animals, and approved by the Bioethics and Biosafety Committee of the UMSNH Chemical-Biological Research Institute.

1.4.3 Solutions and sweeteners

Three types of sweeteners of different natures were used. The concentrations of the solutions were chosen according to the substances sweetening power, tested in a preliminary test; based on the previous background, they were perceived with similar intensities of sweetness and without a detectable aftertaste.

- Control (C): Conventional drinking water without any added sweetener.

- Sucrose (S): Sucrose or cane sugar was used from a commercial brand of typical use, which meets the standard characteristics of sugar. 24.5 g were dissolved in 1 L of water.

- *S. rebaudiana* artificial (SRA): artificial sweetener of the “Svetia ©" brand that is used daily as a substitute for sugar, the content of this sweetener per 1 g of product (1 sachet) consisting: sucrose, extract of Stevia (steviol glucosides) 25 mg, isomalt 10 mg and sucralose 6 mg. 6 g (6 sachets) dissolved in 1 L of water were used.

- Natural *S. rebaudiana* (SRN): dried and powdered *Stevia rebaudiana* Bertoni plant leaves in their natural state, 4.4 g were used, this quantity was supplied as an infusion, mixed with hot water for 5 min and was filtered with the help of a funnel and filter paper, to finally made 1 L in its corresponding drinker.

The solutions were administered to the rats and were made available to them with free access in plastic drinkers with metal nozzles with a capacity of 1 L.

1.4.4 Euthanasia and muscle dissection

Once the treatment was finished, the rats were sacrificed by decapitation to dissect the Extensor Digitium Longus (EDL) and the Soleus muscle of the two hind limbs.

The first hind limb's dissected EDL muscle and soleus were preserved in Eppendorf tubes with Krebs-Ringer physiological solution (118 mM NaCl, 4.75 mM KCl, 1.18 mM MgSO4, 24.8 mM NaHCO3, 1.18 mM KH2PO4; pH 7.4) and brought to ultra-freezing at -80 ° C.

The dissected EDL muscle and soleus muscle of the second hind limb was placed and fixed with entomological pins in a Petri dish lined with a transparent resin bottom; this was previously filled with the Krebs-Ringer physiological solution (118 mM NaCl, 4.75 mM KCl, 1.18 mM $MgSO_4$, 24.8 mM $NaHCO_3$, 1.18 mM KH_2PO_4; pH 7.4) plus an addition of 10 mM C6H12O6 and 24.8 mM $NaHCO_3$. The solution was also supplied with carbon gas (95% CO_2 and 5% O_2). Mounted muscles were cleaned using a stereoscopic microscope to remove excess connective tissue and fatty tissue adhered to the muscle to record tension finally.

4.5 Tension recording

In an isometric tension recording chamber, the muscle was mounted through its tendons, one of the tendons was hooked to an optical transducer hook (World Precision Instruments, USA), the second tendon was attached to the bottom of the chamber. Once the muscle was assembled, the fibers were adjusted and tensioned 1.3 times their length at rest. At all times, the muscle was immersed in Krebs-Ringer physiological solution added with glucose and bicarbonate; the tissue received constant carbogen gas. Inside the recording chamber, two platinum-iridium electrodes were placed directly into the solution where the muscle is submerged, but without making direct contact with the muscle, connected to a stimulus isolating unit (Grass. USA), for fatigue induction stimulation, started 10 min after muscle placement.

In turn, the transducer was connected to an amplifier and a digital-analog interface (World Precision Instruments, USA), allowing the tension generated by each muscle to be acquired on a computer, using the MDAC software (Word precision Instruments, USA).

1.4.6 Fatigue protocol

The fatigue protocol was induced in skeletal muscle by repetitive electrical stimulation. Applied in EDL muscle pulses 100 V, 300 ms of duration and a frequency of 50 Hz; and in soleus muscle with 100 V pulses, 300 ms in duration and a frequency of 45 Hz by a stimulus isolating unit and a stimulator (Grass, USA). Tissue was stimulated until the tension decreased approximately 60-70% to the initial tension.

1.4.7 Homogenization

The skeletal muscle samples were thawed to remove the tendons and immediately homogenize in Eppendorf tubes with Krebs Ringer solution with a Dragon Lab D-500 homogenizer, and aliquots were made for the corresponding biochemical tests. The homogenates were deep-frozen at -80 ° C.

1.4.8 Biochemical tests

1.4.8.1 Measurement of reactive oxygen species levels

The production of reactive oxygen species was determined using the fluorescence probe 2 ', 7'-dichlorodihydrofluorescein diacetate (H2DCFDA). Muscle tissue homogenate protein (1 mg/ml) was resuspended in 5 µL of dichlorofluorescein and completed with ROS Buffer (10 mM HEPES, 100 mM KCl, 3 mM $MgCl_2$ and 3 mM KH_2PO_4; pH 7.4) for a 2000 µL total volume, were incubated cold with shaking for 15 min. Baseline fluorescence was recorded after samples were tempered; finally, the samples were shaken cold for 60 min to record the final fluorescence. Changes in fluorescence were measured at excitation/emission wavelengths of 485nm / 520nm on a Shimadzu RF-5301PC spectrofluorometer (Shimadzu, Kyoto, Japan). Data were expressed as fluorescence delta (ΔF) in arbitrary units.

1.4.8.2 Catalase activity

Catalase activity was analyzed by measuring the conversion of hydrogen peroxide to oxygen with a Clark type oxygen electrode connected to a biological oxygen monitor (5300A Biological Oxygen Monitor, YSI, Ohio, USA). 1 mg/ml of protein from the muscle tissue homogenates were resuspended in a 0.1 M phosphate buffer (pH 7.4) at 25 ° C and monitored for 1 min. Then, 6 mM H2O2 was added to the chamber, and the conversion of hydrogen peroxide to oxygen was measured with the oxygen electrode for 3 min.

1.4.9 Statistical analysis

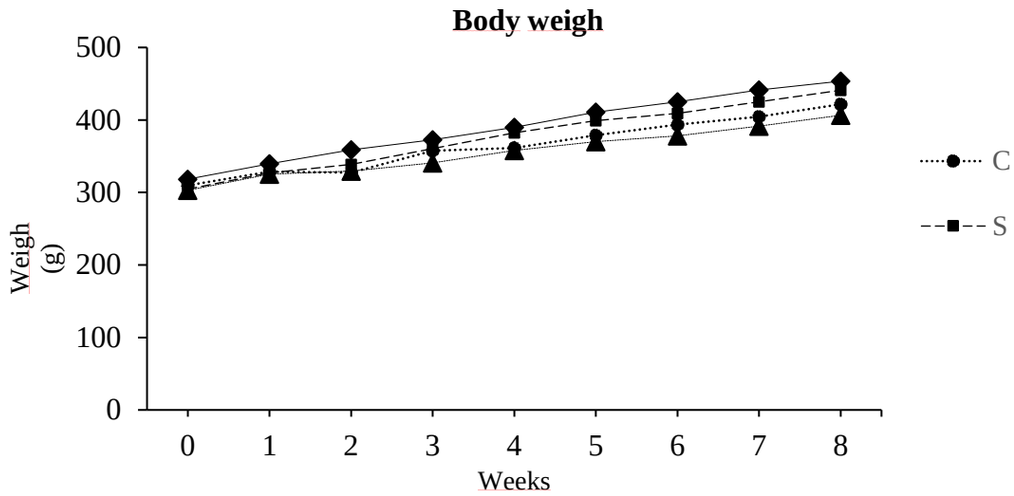
A 1-way ANOVA analyzed the results obtained with Tukey's posthoc test. Statistically significant differences were defined as $P < 0.05$.

1.5 Results

1.5.1 Effect of sucrose sweeteners, artificial and natural *Stevia rebaudiana* on body weight and postprandial blood glucose

At the end of the experiment, body weight was higher in the SRA group (453.5 ± 57.91 g) than in the C (421.7 ± 51.17), S (441 ± 17.24) and SRN (406.5 ± 39.90) groups; however, this increase it was not significant. On the other hand, the group of rats administered with sucrose also showed a more significant increase in body weight than groups C and SRN; however, the results are not significant. While the SRN group showed less weight gain than the control group, this was not significant either.

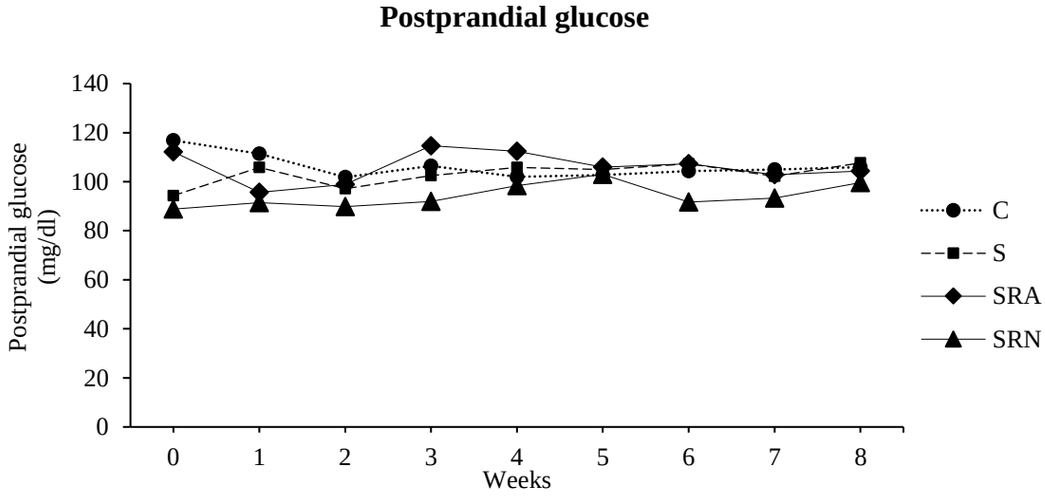
Graphic 1.1 Bodyweight. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = *Stevia rebaudiana* natura. C (421.7 ± 51.17), SRA (453.5 ± 57.91 g), S (441 ± 17.24) and SRN (406.5 ± 39.90), l. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Regarding the postprandial blood glucose level, no significant differences were observed throughout the treatment in any group. A decreasing trend was observed after the fifth week in the SRN group (91.67 ± 11.23) than in the other groups studied C (104.3 ± 8.52), S (107.3 ± 18.35) and SRA (107.3 ± 5.08); however, the differences were not significant and these values and all those reported during the 8 weeks are within the normal ranges of glucose in the blood in a healthy individual.

Graphic 1.2 Postprandial glucose. Throughout the 8 weeks of treatment with the oral administration of sweetening solutions

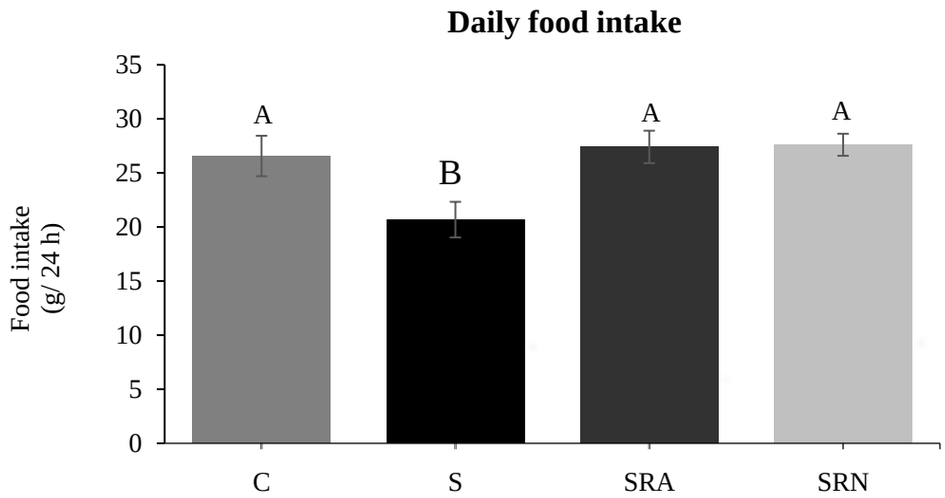


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*. n = 6; Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on food intake

Food consumption was significantly lower in group S (20.68 ± 1.65) than in the other groups studied C (26.57 ± 1.87), SRA (27.41 ± 1.50) and SRN (27.61 ± 1.02). The groups administered with the two types of *Stevia* sp. (SRA and SRA) presented a daily food intake similar to group C.

Graphic 1.3 Food intake. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

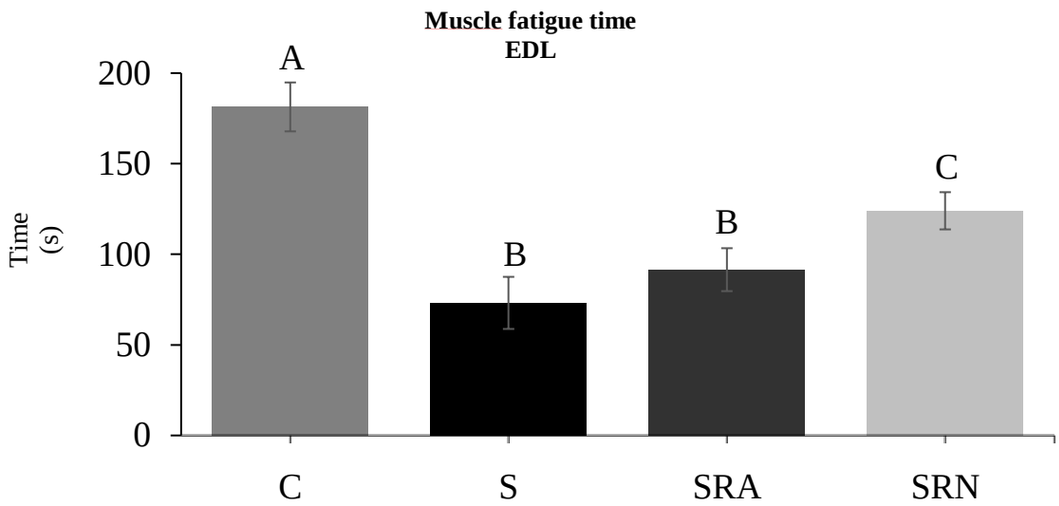


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on skeletal muscle fatigue resistance time

Regarding the time of resistance to fatigue in the long digitorum extensor muscle (EDL), all the groups administered with the sweeteners S (73.17 ± 14.38), SRA (91.50 ± 11.84) and SRN (124.5 ± 10.27) presented a significant reduction in the time to resist muscle fatigue compared to group C (181.3 ± 13.5). Furthermore, the S and SRA groups showed a more significant reduction in muscle fatigue than the SRN group.

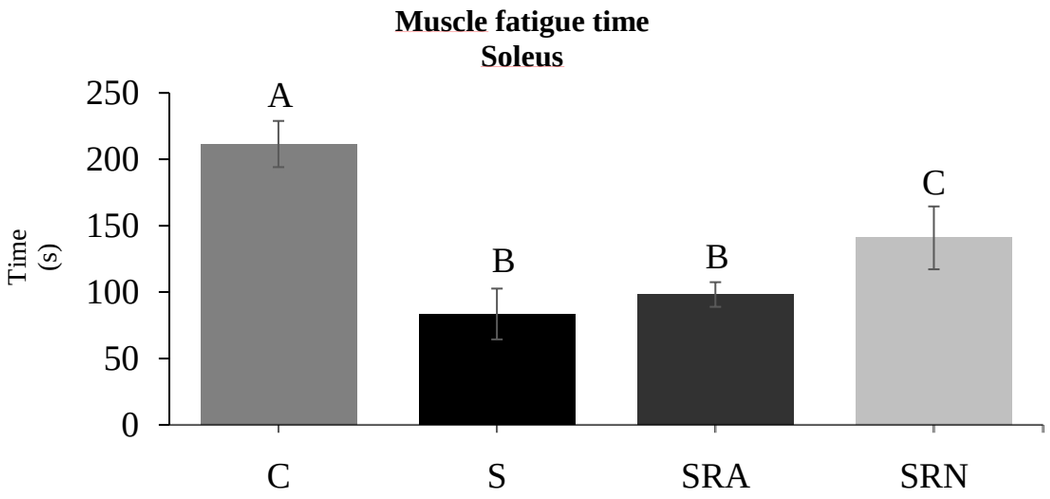
Graphic 1.4 Fatigue resistance time in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

On the other hand, the soleus muscle presented a greater resistance to fatigue in all the groups than the treated groups of the EDL muscle; this is probably due to its higher proportion of slow-twitch type I fibers. The administered sweeteners caused a significant reduction in the time of resistance to fatigue in all groups S (83.50 ± 19.13), SRA (98.17 ± 9.28) and SRN (140.8 ± 23.68) to control C (211.5 ± 17.40), being significantly lower in the S and SRA groups than in the SRN group.

Graphic 1.5 Fatigue resistance time in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



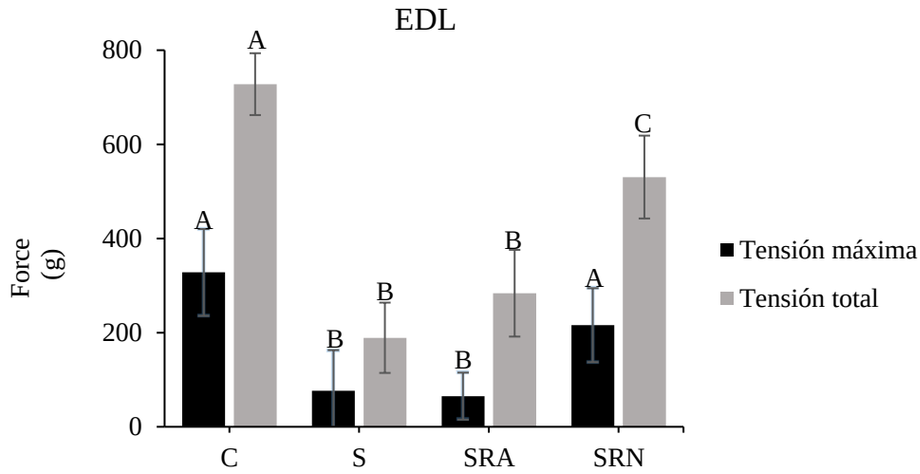
C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on maximum tension and total skeletal muscle tension

The EDL muscle presented a significant decrease in muscle contraction force over maximum tension in the groups treated with S (76.64 ± 86.23), SRA (65.13 ± 49.94) and SRN (215.9 ± 78.58) compared to group C (327.9 ± 92.21), with the largest significant decrease observed in the S group; also the maximum tension force was significantly greater in the SRN group than in the S and SRA groups.

Regarding the total tension, the groups S (189.1 ± 74.69) and SRA (283.8 ± 92.13), SRN (530.5 ± 88.01) presented a significant decrease in the total contraction force to the C (727.91 ± 65.71), being the action of SRN more favorable for the maintenance of muscle contraction compared to the sweeteners of S and SRA.

Graphic 1.6 Maximum tension and total tension of the EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

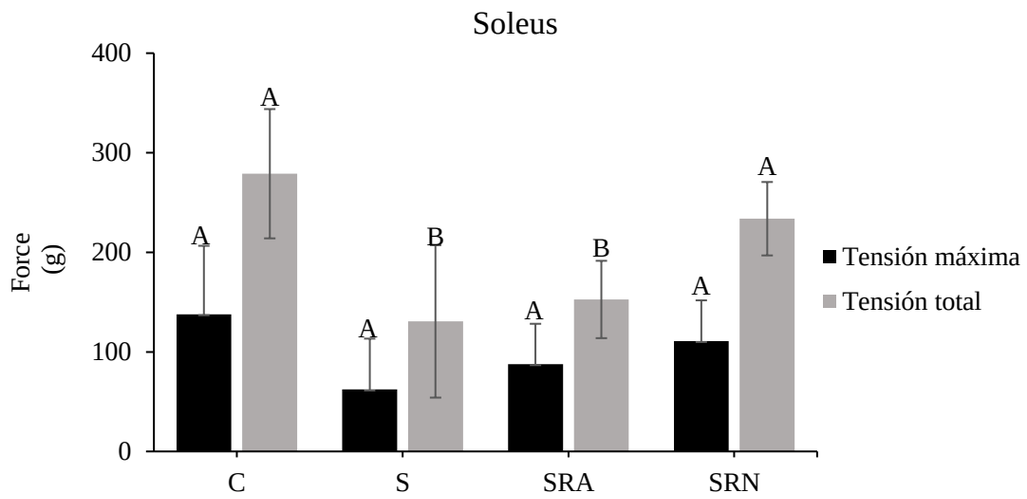


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

While in the soleus muscle, no significant differences were found in the maximum tension force between groups C (137.8 ± 68.73), S (62.23 ± 51.07), SRA (87.66 ± 40.54) and SRN (110.8 ± 40.90).

Regarding the total tension, the sweeteners administered S (130.6 ± 76.54), SRA (152.6 ± 38.85) and SRN (233.7 ± 36.89) affected the total contraction force due to a significant decrease compared to C (279.0 ± 64.92). The S and SRA groups presented the highest decrease, while the SRN the lowest decrease.

Graphic 1.7 Maximum tension and total tension of the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

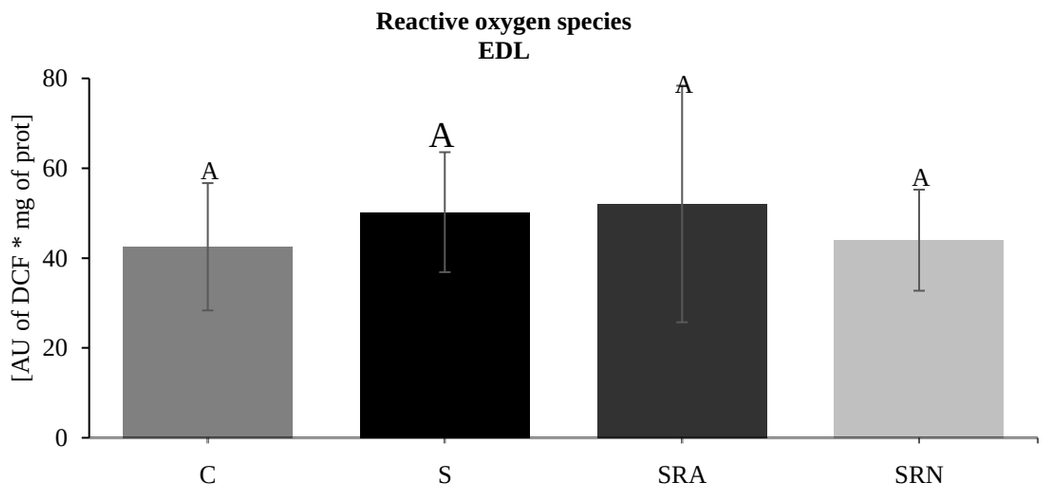


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on the level of reactive oxygen species of skeletal muscle

No significant differences were observed in ROS levels between groups C (42.53 ± 14.17), S (50.22 ± 13.35), SRA (52.05 ± 26.33) and SRN (43.99 ± 11.25) in the EDL muscle. An increasing trend was observed in groups S and SRA; however, these results are not conclusive.

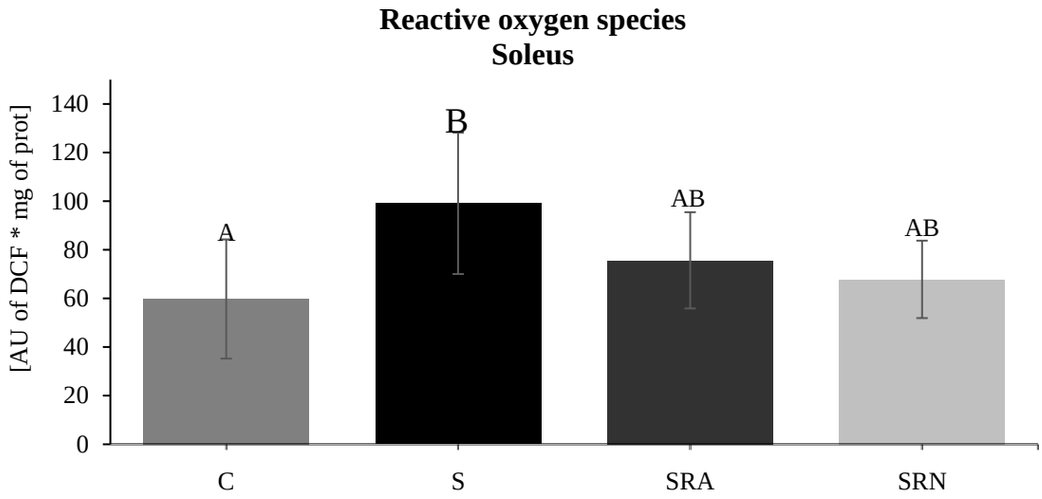
Graphic 1.8 Levels of reactive oxygen species in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test)

In the soleus muscle, ROS levels were significantly higher in group S (99.13 ± 29.08) than in group C (59.78 ± 24.52). Furthermore, the groups treated with Stevia sp. (SRA (75.68 ± 19.8) and SRN (67.84 ± 15.92) showed reductions in ROS levels compared to group S but not group C, these were not significant.

Graphic 1.9 Levels of reactive oxygen species in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions

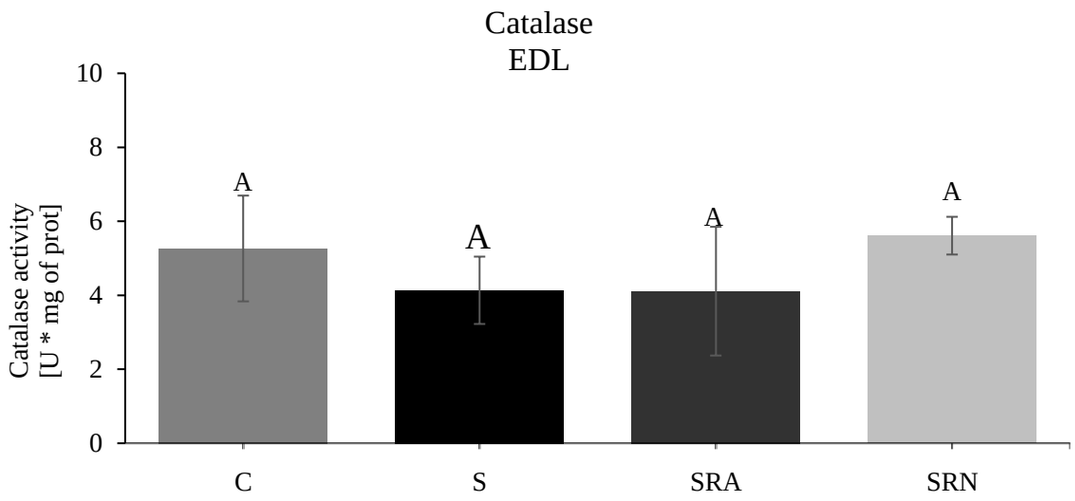


C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana;* n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

Effect of sucrose sweeteners, artificial and natural Stevia rebaudiana on the activity of the antioxidant enzyme catalase (CA) of skeletal muscle

In this study, we demonstrated that catalase activity in EDL muscle showed a decreasing trend in groups S (4.13 ± 0.91) and SRA (4.11 ± 1.74) compared to group C (5.26 ± 1.43) and SRN (5.61 ± 0.51); however, the results are not significant.

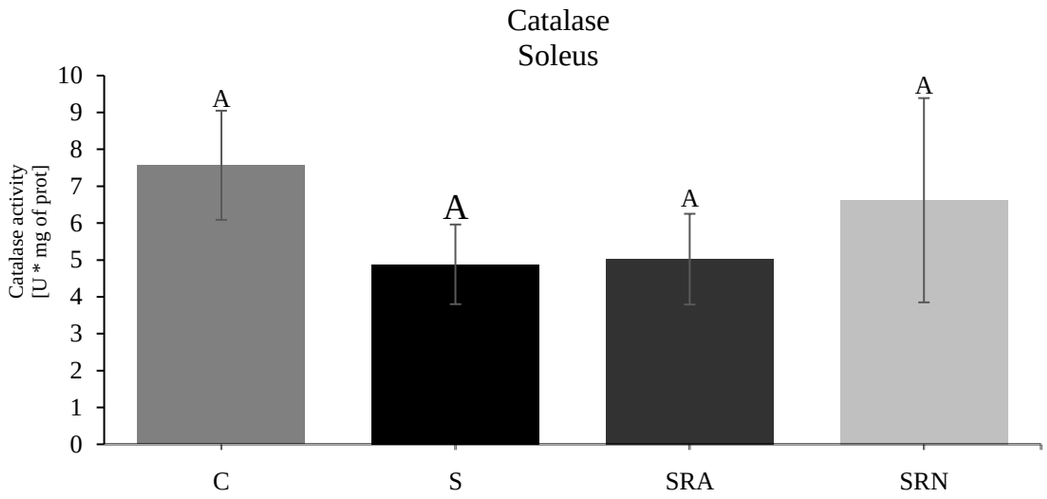
Graphic 1.10 Catalase levels in EDL muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

The catalase activity in the soleus muscle was lower in the groups administered with the sweeteners S (4.88 ± 1.08), SRA (5.02 ± 1.23) and SRN (6.62 ± 2.77), than in group C (7.56 ± 1.48), the activity in the SRN group being more improved, however, these results are not significant.

Graphic 1.11 Catalase levels in the soleus muscle. Throughout 8 weeks of treatment with the oral administration of sweetener solutions



C = control, S = Sucrose, SRA = Artificial *Stevia rebaudiana*, SRN = Natural *Stevia rebaudiana*; n = 6. Data are presented as the mean ± standard error, P <0.05. 1-way ANOVA, Tukey posthoc test).

1.6 Discussion

The growing concern about the increase in chronic diseases has led to a reduction in the consumption of simple sugars and an increase in the intake of artificial and natural non-caloric sweeteners, such as *Stevia rebaudiana* Bertoni, which is an excellent alternative, it is characterized by providing an intensely sweet flavor and have a zero caloric intake. However, the safety of these sweeteners as food additives remains unclear.

As previous studies have shown, the present study showed that the intake of Stevia sp. (SRN) at a dose of 4.4 g / L does not cause alterations in body weight and can be a compromising alternative to cane sugar or sucrose, however, steviol glycosides in combination with high intensity artificial sweeteners such as sucralose and Isomaltose (SRA) at a dose of 6 g / L, seems not to have the same effects on body weight, showing a trend of increase in body weight more significant than groups C and S, this may be due to the synergy not positive that steviol glycosides could be made in combination with the two artificial sweeteners, in addition, this is consistent with some antecedents that have reported that the intake of non-caloric sweeteners causes a positive body weight compensation due to physiological effects other than those produced due to the caloric consequences, a higher consumption of food after its ingestion, metabolic disorders and the stimulation of taste due to greater consumption of products sweets (Stephens-Camacho et al. 2018).

Furthermore, the supply of S, SRA and SRN sweeteners did not affect blood glucose levels, so Stevia sp did not show hypoglycemic effects as previously reported by Barriocanal et al. 2008, at least not within the tested concentration range and applied time. It is known that the antihyperglycemic, insulinotropic and glucagonostatic effects reported for the glycosides of Stevia sp. are highly dependent on high plasma glucose levels. Previous studies have reported that Stevia sp. have hypoglycemic effects in experimental animal models of DM, increasing the expression of GLUT4 in skeletal muscle, which increases glucose uptake and explains its hypoglycemic effects in DM; perhaps that is why in this study, no significant difference was observed due to that organism without any pathology were used (Gonzáles, 2013; Mouillot et al. 2020).

Also, in this study, it was shown that treatment with sucrose (S) at a dose of 41.2 g / L showed a significant reduction in the food consumption of rats; this may be due to the effects of sucrose on the systems of brain reward, it can bind to brain receptors that promote the release of dopamine, a neurotransmitter closely related to the generation of pleasurable sensations, which may have suppressed the rats' appetite in the weeks they received the sucrose solution.

While the consumption of *Stevia* sp (SRA and SRN) does not seem to modify appetite or food intake in rats, this contradicts a study by Farhat et al. 2019, who observed that Stevia sp might decrease the sensation of appetite, but this study was conducted with subjective tests. Furthermore, little is known about the differences in the activation of peripheral and central taste pathways, the central food and reward systems between sugar and non-caloric sweeteners. These results support the hypothesis that the consumption of sweet flavors in the absence of calories produces significantly different effects than the consumption of sweet flavors associated with calories (Durán et al. 2013; Mouillot et al. 2020).

The importance of studying skeletal muscle as a potential link between consuming the non-caloric artificial sweetener and Stevia sp is due to the relationship of food as a source to meet immediate energy needs, the reserve of nutrients and energy that the cells of the different organs and tissues use. Play an important regulatory role in metabolism; an altered energy pathway and substrate cause a higher incidence of the prevalence of muscle fatigue in chronic diseases. In this study, the sweeteners administered (S, SRA and SRN) caused a significant reduction in the fatigue resistance time in both fast and slow muscle, which could indicate that excessive intake of sugars regardless of their nature, whether natural or artificial, contribution or non-contribution of calories, causes a decrease in the time of fatigue resistance; however, a significant improvement was also shown within the deleterious effects of excess sweeteners. Administration of the SRN to the other two could be reflected in the antioxidant effects reported for the leaves of Stevia sp.

In addition, regarding the muscle contraction force, it has been described that the extracts of Stevia sp. They can aid skeletal muscle functionality by increasing muscle mass, strength and also improving mitochondrial function. In this research, it is shown that the concentrations of the sweeteners (S, SRA and SRN) applied caused in the EDL muscle a significant decrease in the force of muscle contraction, both in the maximum tension and in the total tension, which indicates that The different sweeteners in the study do not differ at least in causing this increase in muscle strength. However, it is also highlighted that the least harmful effect occurs in the SRN sweetener, which could once again indicate the critical role of leaf consumption whole of Stevia rebaudiana Bertoni due to its antioxidant phenolic properties found in it and not in industrially extracted glycosides.

Regarding the soleus muscle that presents type 1 slow-twitch fibers, no significant effect was found on the maximum tension in the treatment with sweeteners; however, on the total tension, the concentrations of S and SRA caused a significant decrease in the force of contraction, while the concentration of SRN significantly increased contractile dysfunctions almost the same as the control. The improved synthesis of proteins could explain these effects in the muscle fibers by the natural phytochemicals with the biological activity of the leaves of Stevia sp. (El-Mesallamy et al. 2017; El-Mesallamy et al. 2018).

The antioxidant activity of *S. rebaudiana* Bertoni is well established; however, the effect of these antioxidant compounds in association with high-intensity artificial sweeteners are not known. The sweetening compounds did not exhibit significant antioxidant activity in the EDL muscle since they could not decrease ROS levels to counteract the increase in oxidative stress induced by fatigue; there is an increase in the S and SRA groups concerning the control it is not significant.

In soleus muscle, the caloric intake of sucrose seems to affect ROS levels, which is consistent with reported data on the damage caused by excessive consumption of free sugars in the diet to different organs and systems (Cabezas-Zabala et al. 2016). As for Stevia sp, it does not produce a significant antioxidant defense, as it does not decrease ROS levels both in the treatment of RAS and in SRN. The SRA shows an increasing trend; however, it is not significant. This lack of antioxidant activity contrasts with the data reported by other authors, probably because the compounds used in this group are commercial sweeteners that contain steviol glycosides without appreciable amounts of polyphenols, naturally present in the leaves of Stevia sp., Probably responsible for antioxidant activity. However, the SRN group does not show significant protection against fatigue in both muscles; this could be because its effectiveness in other studies is due to its central effects in improving insulin secretion only in hyperglycemic individuals (Salvador-Reyes et al. 2014). These findings suggest that high-intensity sweeteners, especially SRA in a high dose, could have a deleterious effect on skeletal muscle through increases in the levels of reactive oxygen species of the soleus muscle (Rizzo et al. 2013; Ruíz-Ojeda et al. 2019).

In addition, it is known that muscle fatigue depletes the antioxidant defense system, thus promoting the generation of free radicals, which is why the antioxidant enzyme catalase (CAT) acts to protect cells against oxidative stress by degrading hydrogen peroxide in water and oxygen. , its activity varies depending on the fabric (Chance and Maehly, 1955). The catalase values presented in this study for skeletal muscle are in line with others previously reported (Paltian et al. 2019). In this research, we show that the glycosides of Stevia sp. in synergy with sucralose and isomaltose (SRA) caused a reduction in CAT activity together with group S in the soleus muscle and the EDL muscle. SRN seems to have an antioxidant effect due to its phenolic and flavonoid compounds; it should be noted that it does not present a significant increase in this activity, probably because the reported effects were in systems deteriorated by metabolic diseases (Chen et al. 2005). These results agree with other studies that have not demonstrated the antioxidant activity of Stevia sp. in healthy individuals.

The findings of this study, combined with evidence from other research on the sweetening effects of Stevia sp, suggest a possible beneficial role in the treatment of chronic diseases on muscle fatigue and the maintenance of health, but not of steviol glycosides in combination with artificial high-intensity sweeteners.

1.7 Conclusion

In conclusion, the leaves of Stevia sp. natural are an essential alternative for weight control and the development of antioxidant defense against muscle fatigue and its deleterious effects on skeletal muscle functionality compared to their extracts industrially extracted and marketed in synergy with other artificial sweeteners. More studies are needed to obtain more conclusive results because alternatives to sugar in the diet to avoid chronic diseases could increase the risk of these diseases.

1.8 Acknowledgment

To the Coordinación de la Investigación Científica of the UMSNH. RMP-CIC-2020.

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Chapter 2 Cashew bagasse (*Anacardium occidentale* L.) as a source of fiber-antioxidant and its possible use in lipoinflammation models

Capítulo 2 Bagazo de anacardo (*Anacardium occidentale* L.) como fuente de fibra-antioxidante y su posible uso en modelos de lipoinflamación

HERNÁNDEZ-PÉREZ, Susana´†, RAMÍREZ-HIGUERA, Abril´, CHÁVEZ-REYES, Yanet´´ and OLIART-ROS, Rosa María´*

´Tecnológico Nacional de México/ I.T. Veracruz. Food Research and Development Unit. Av. Miguel Ángel de Quevedo No. 2779, Col. Formando hogar. C.P. 91987. Veracruz, Ver., Mexico.

´´Instituto Tecnológico Superior de Las Choapas. Department of Food Industry Engineering. Carretera las Choapas-Cerro de Nanchital Km.6 Col. J. Mario Rosado, Las Choapas, Veracruz, C.P. 96980, Mexico.

ID 1st Author: *Susana, Hernández-Pérez* / **ORC ID:** 0000-0002-7942-5321, **Researcher ID Thomson:** AAR-9442-2021, **CVU CONACYT ID:** 839819

ID 1st Co-author: *Abril, Ramírez-Higuera* / **ORC ID:** 0000-0002-1430-2689, **CVU CONACYT ID:** 242658

ID 2nd Co-author: *Yanet, Chávez-Reyes* / **ORC ID:** 0000-0002-6186-9004, **Researcher ID Thomson:** A-2278-2013, **CVU CONACYT ID:** 286143

ID 3rd Co-author: *Rosa María, Oliart-Ros* / **ORC ID:** 0000-0003-1204-8792, **CVU CONACYT ID:** 26058

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S. Hernández, A. Ramírez, Y. Chávez and R. Oliart

* rosa.or@veracruz.tecnm.mx

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Abstract

Diet has a strong influence on health so that people with good eating habits and moderate exercise decrease the chance of developing diseases. As a result, the consumption of foods containing compounds with a value-added to the per se nutrient value is recommended; these compounds are known as bioactive compounds, such as fiber and antioxidants, which have been related to the decrease of oxidative stress and inflammation present in obesity and that, when not treated, they trigger multiple conditions such as diabetes, hypertension, metabolic syndrome, insulin resistance, and cardiovascular diseases. Cashew (*Anacardium occidentale* L.) is a fruit from Brazil that serves as a possible source of fiber-antioxidant by containing phenolic compounds and dietary fiber.

Fiber-Antioxidant, Cashew, Inflammation, Obesity

Resumen

La alimentación tiene una fuerte influencia en el estado de salud, de manera que personas con buenos hábitos alimenticios y que realicen ejercicio moderado, disminuyen la probabilidad de desarrollar enfermedades. Por lo que se recomienda el consumo de alimentos que contengan compuestos con un valor agregado al valor nutrimental *per se* del alimento, a estos compuestos se les conoce como compuestos bioactivos, como la fibra y los antioxidantes, los cuales han sido relacionados con la disminución del estrés oxidativo y la inflamación presentes en obesidad y que al no ser tratados desencadenaran en multiples padecimientos como diabetes, hipertensión, síndrome metabólico, resistencia a la insulina y enfermedades cardiovasculares. El Anacardo (*Anacardium occidentale L.*) es un fruto procedente de Brasil el cual funge como una posible fuente de fibra-antioxidante al contener compuestos fenólicos y fibra dietética.

Fibra-Antioxidante, Anacardo, Inflamación, Obesidad

2.1 Introduction

Obesity is a disease that afflicts 75.2% of Mexicans, who develop this condition through excessive intake of foods rich in refined carbohydrates and saturated fats, lack of physical exercise, and, to lesser extent, genetics (ENSANUT, 2018). This disease is visualized by the increase in adipose tissue in different areas of the body, with visceral adipose tissue being the most studied due to its direct participation in the development of diseases such as Diabetes Mellitus II, insulin resistance, and cardiovascular diseases (CVD). Adipose tissue is made up of adipocytes, endothelial cells, and immune cells such as macrophages and T lymphocytes. In obese patients, adipose tissue is modified and is unable to perform its functions correctly, since macrophages present a phenotypic change of a state anti-inflammatory to pro-inflammatory; various processes are also generated such as the secretion of adipokines involved in the inhibition of insulin, a decrease in leptin (the hormone that regulates appetite) and the inhibition of adiponectin known for its anti-inflammatory capacity. All this leads to the generation of an acute inflammatory process that, when not resolved, may evolve chronically (Olatz *et al.*, 2015; Clària *et al.*, 2012; Guilherme *et al.* 2008) resulting in the development of other diseases.

Some ways of treating obesity have focused on food restriction, however, studies have shown that in diets <800 Kcal/day adipokine levels persist and adipose tissue remains infiltrated by pro-inflammatory macrophages, concluding that the inflammatory process is not totally solved with caloric restriction, so it is recommended the intake of foods that provide extra compounds to the basic nutrients. Such is the case of fiber and antioxidants found in various vegetables. Both fiber and antioxidants are considered bioactive compounds that provide added value to the nutritional benefits of the food (Cencic and Chingwaru, 2010). Among its benefits are to improve the state of health and quality of life, as well as to reduce the risk of contracting diseases (Herrera *et al.,* 2014). The benefits of fiber lie in its ability to restore intestinal motility and promote the growth of beneficial bacteria that exert anti-inflammatory actions in the colon, blocking the growth and adherence of pathogens, as well as the production of short-chain fatty acids that serve as intermediaries in pro-inflammatory cascades (Escudero and Gonzáles, 2006). Currently, the fiber requirement is estimated between 25 and 35 g / day for an adult with an average intake of 2500 Kcals in order to obtain the aforementioned benefits (Sanchez and Romero, 2015). Antioxidants are beneficial due to their ability to give up electrons to stabilize free radicals, thus reducing the development of inflammation (Paredes and Roca, 2002).

Among the most common antioxidants are those of an exogenous nature that is acquired through the diet, such as polyphenols and phytoestrogens, found in red fruits, peaches, black tea, guava, and cashew, among others (Caballero and Gonzales, 2016).

Fruits have been studied for their high content of fiber and antioxidants, such is the case of cashew, a fruit from Brazil and to which a series of benefits and applications in the health area have been attributed. This document focuses on evaluating *Anacardium occidentale* L. as a possible source of fiber-antioxidant and its possible impact on an inflammation model, in order to provide information to support the treatment of obesity.

2.2 Obesity

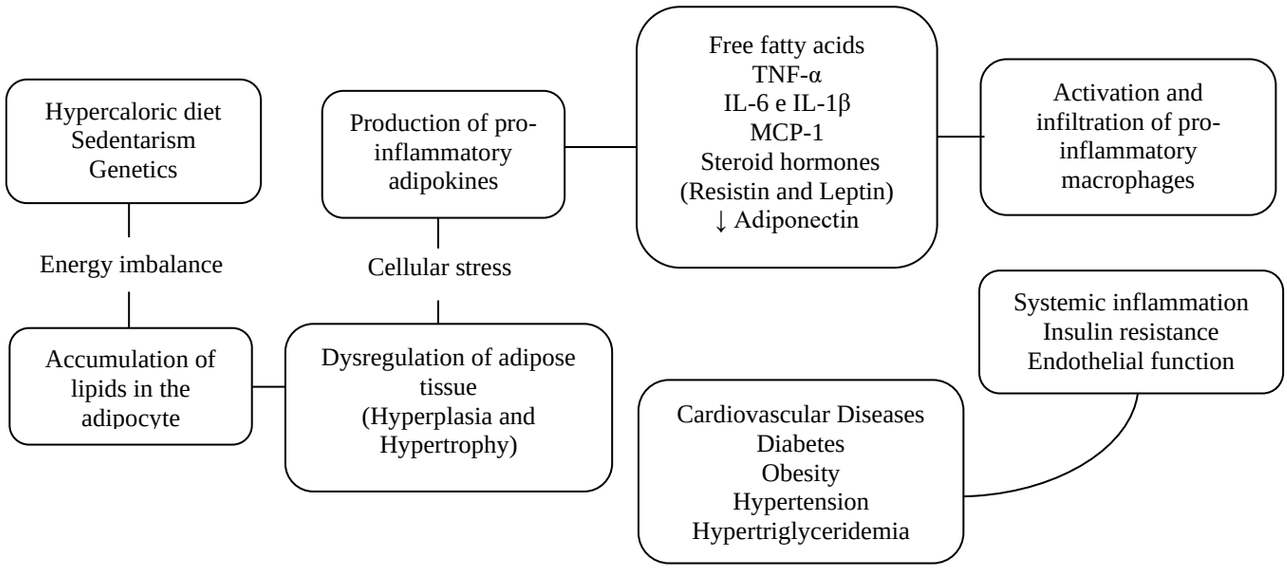
Obesity is a multifactorial chronic disease that is distinguished by the increase in adipose tissue in certain areas of the body, due to various factors such as a high consumption of foods rich in refined sugars and saturated fats, sedentary lifestyle, and in certain cases, environmental and genetic factors. It is diagnosed through the body mass index (BMI) where the weight (kg) and the height square (Cm^2) of the patient are taken into account. Therefore, a BMI $\geq 30 kg/m^2$ categorizes the patient as obese, while a BMI of 18.5 to $24.9 kg/m^2$ is considered adequate. However, it should be noted that diagnostic methods other than BMI are currently used to provide a better evaluation criteria, such as the relative composition of fat and musculoskeletal mass (Kim and Valdez, 2015), as well as a more detailed diagnosis that considers the metabolic and physiological abnormalities of the human body such as biomarkers related to inflammation and oxidative stress (adipocytokines and steroid hormones) (Zulet *et al.*, 2007; Ripka *et al.*, 2014). The location of the body fat or adipose tissue must also be taken into account since depending on where it is located, it can be associated with a greater or lesser risk of metabolic complications (Mathieu *et al.* 2009).

Currently, 75.2% of people in Mexico are overweight or obese, being Veracruz, Quintana Roo, Colima, Sonora, and Tabasco the states with the highest percentage of obese or overweight people. The importance of treating this condition lies in its consequences since various diseases such as insulin resistance, type 2 diabetes mellitus, CVD, metabolic syndrome, and polycystic ovary syndrome are triggered by the remodeling and increase of adipose tissue (Flores-Lazaro *et al.*, 2011).

2.1. Adipose tissue

The adipose tissue of the breasts and buttocks is susceptible to estrogens, while the visceral adipose tissue is related to the secretion of adipokines involved in inflammatory processes (lipoinflammation) and type 2 diabetes (Flores-lazaro *et al.,* 2011). In periods of overfeeding, the adipose tissue fat cells increase in size (hypertrophy), and new mature adipocytes are also generated from pre-adipocytes (hyperplasia) in order to store excess energy in the form of triglycerides. However, if these periods become constant and combined with sedentary lifestyle and genetics, a metabolic overload is generated that makes the adipocyte incapable of fulfilling its functions, causing dysregulation in the synthesis of adipokines, increasing inflammatory cytokines and decreasing adiponectin (adipokine with anti-inflammatory and anti-atherogenic capacity); the recruitment of inflammatory cells (macrophages M1) into adipose tissue begins, generating the activation of an intracellular inflammatory response and the overproduction of reactive species, causing a state of oxidative stress and inflammation (Bays *et al*., 2008; De Ferranti and Mozaffarian, 2008; Blüer, 2009) (Figure 2.1).

Figure 2.1 Mechanism of adipose tissue dysfunction and its metabolic impact



Source: Adapted from Flores-Lazaro et al., 2011

2.2. Adipose tissue and its participation in inflammatory processes

These dysfunctions described in Figure 2.1 are carried out through various substances secreted by adipose tissue such as fatty acids, prostanoids, cholesterol, retinol, steroid hormones, and protein factors, the latter known as adipokines, which serve as mediators between adipose tissue and adjacent and distant organs such as the endothelium, liver, muscle, pancreas, adrenal glands, and nervous system. A large part of adipokines are considered inflammatory factors such as tumor necrosis factor (TNF-α), interleukins 1-β, 6, 8, 10, 4, and 13 (IL 1-β; IL-6, IL-8, IL-10, IL-4, and IL-13), and monocyte chemoattractant protein 1 (MCP-1) which is directly related to the inflammatory response by inhibiting the expression of glucose transporter-4 (GLUT-4) and to the peroxisome-proliferator-activated receptor-γ (PPAR-γ) responsible for the differentiation and proliferation of adipocytes, and the increase of adiponectin, thus determining a link between inflammation and obesity (Bastarrachea *et al.*, 2007).

2.3 Dietary fiber

The main sources of dietary fiber in the human diet are fruits and vegetables since their cell wall is made of polysaccharides such as pectins, celluloses, and hemicelluloses (Caffall *et al.*, 2009), providing them with appreciable characteristics in the treatment and prevention of pathologies such as CVD, diabetes, colon cancer, and obesity (Viuda-Marctos *et al.*; Kendall *et al.*, 2010). Cellulose is a cell wall homopolymer constituted by D-glucose monomers linked by β-(1 → 4) glycosidic bonds that in turn form microfibrils and macro fibers linked by hydrogen bonds, which results in the fiber insolubility in water (Mudgil *et al*., 2013; Bajpai *et al*., 2017). Hemicellulose is made of more than one type of monosaccharide, giving rise to different heteropolymers such as xyloglucans, glucuronoxylans, glucomannan, and arabinoxylans (Scheller *et al*., 2010). Finally, pectins are the most complex group of cell wall polysaccharides since they are characterized by the presence of galacturonic acid, rhamnose, arabinose, and galactose, giving rise to homogalacturonans and rhamnogalacturonans (Arnous *et al*., 2009). It should be noted that pectins are the main component of the soluble fraction of dietary fiber. As mentioned above, the consumption of soluble fiber presents a series of health benefits such as a reduction in the glycemic response and cholesterol, while the insoluble fraction formed by cellulose and hemicellulose promotes a decrease in intestinal transit and an increase in fecal mass (Mudgil *et al*., 2013). Cellulose, hemicellulose and pectin have functional properties such as swelling capacity (Sw), water retention (WRC), and lipid adsorption (FAC), which play an important role in the regulation of digestive flow, the availability of nutrients, viscosity, and bolus formation (Elleuch *et al*., 2011).

The American Association of Cereal Chemists (2001) defines dietary fiber as “The edible part of plants or analogous carbohydrates that are resistant to digestion and absorption in the small intestine, with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, lignin, and associated substances from the plant. Dietary fibers promote physiologically beneficial effects on health by improving intestinal transit, cholesterol, and blood glucose levels”.

Dietary fiber can fall into the following categories:

- Polymers of edible carbohydrates that are consumed directly from food.

- Carbohydrate polymers obtained from food raw materials by physical, enzymatic, or chemical treatments that have a physiologically beneficial effect on health by generally accepted scientific evidence and provided to the competent authorities.

There are three classifications of dietary fiber (DF) regarding its composition, water retention capacity, and fermentation capacity. If we talk about its water retention capacity, fiber can be divided into soluble and insoluble, where soluble fiber is characterized by its ability to form gels, a property that slows gastric emptying and the absorption of nutrients, such as glucose, lipids, minerals, and bile acids in the intestine (Cherbut, 1998). Insoluble fiber passes through the colon unchanged, increasing the weight of the stool by absorbing water, making stools voluminous and soft, thus increasing intestinal regularity (Kin, 2001). Regarding its fermentative capacity, all fibers with the exception of lignin can be fermented by colonic bacteria, the main ones being soluble fibers. The ingestion of soluble fibers such as guar gum increases the weight of stool by 20%, improving gastrointestinal transit. The intake of fructooligosaccharides (prebiotic soluble fiber "FOS") can increase the proliferation of probiotic bacteria such as bifidobacteria (Bouhnik *et al*., 1996). Researchers such as Gibson *et al*. (1995) supplemented 15 g of inulin (FOS) / day to healthy volunteers, significantly increasing the number of bifidobacteria and reducing the number of Bacteroidetes, Clostridium and Fusobacterium. The National Cholesterol Education Program Adult Treatment Panel (NCEP ATP III) recommends increasing the intake of soluble fiber (10-25g / day) to lower serum cholesterol and therefore the risk of heart disease, and consumption of 20-30g / day total fiber to reduce 12-20% the probability of developing cardiovascular diseases.

One of the main functions of dietary fiber is to reach the large intestine and serve as a substrate for the resident bacteria in the colon, which produce short-chain fatty acids (SCFA), gases (hydrogen, carbon dioxide, and methane) and energy by using fiber as the main substrate. 90% of the SCFA produced by the microbiota are rapidly absorbed by the colonocyte, butyrate being the most used, followed by acetate and propionate (Roediger, 1982). Butyrate is metabolized to CO_2, ketone bodies, and water. It is the main source of energy, it stimulates the production of mucus, the absorption of ions, and the formation of bicarbonate. Likewise, butyrate exerts specific anti-inflammatory actions in the colon, decreasing the production of some pro-inflammatory cytokines (TNF), modulating the activity of the transcription nuclear factor enhancing the kappa light chains of activated B cells (NF-kB) in colonic cells *in vitro* (Inan *et al*., 2000).

As mentioned above, the intake of dietary fiber improves the intestine function, however, an excess can cause negative effects, so the American Diabetes Association recommends a daily consumption of 14g per 1000 Kcal in the diet to be able to obtain the benefits of fiber, such as control and reduction of hemorrhoids and constipation (Bosaeus, 2004), protection against colon cancer through the production of fatty acids (Eswaran *et al*., 2013), weight reduction due to increased satiety (Pereira *et al*., 2004), prevention of diabetes if consumed together with a diet low in fat and carbohydrates and decreased absorption of simple carbohydrates favoring blood glucose levels (Cho *et al*., 2013).

According to the European Parliament in regulation (CE) # 1924/2006 “A food can only be declared to have high fiber content, as well as any other declaration that may have the same meaning for the consumer, if the product contains at least 6g of fiber per 100g or 3g of fiber per 100 Kcal”.

At present, foods that have high fiber content and that in turn present bioactive compounds such as vitamins, antioxidants, minerals, have been investigated to improve the health of the consumer; examples are fruits like guava, apple, grape, peach, and cashew among others (Sudha *et al.*, 2007; Tseng and Zhao, 2013; Matias *et al*., 2015).

2.4 Antioxidants in food

A dietary antioxidant is a substance that is part of foods that can prevent the adverse effects of reactive species on normal physiological functions in humans (Patthamakanokporn *et al*., 2008). There are two main types of antioxidants: endogenous (enzymatic) and exogenous (non-enzymatic). Endogenous antioxidants are defense mechanisms developed by the body itself, among the best known are the enzymes catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), while exogenous antioxidants are known as dietary antioxidants, for example alfa-tocopherol that helps to prevent membrane peroxidation by stabilizing peroxyl radicals; vitamin C or ascorbic acid that acts in combination with vitamin E and carotenoids, as well as in conjunction with enzymatic antioxidants (Griffiths and Lunec, 2001); carotenoids especially β-carotene, which reduces genetic damage and mutations and inhibits tumor induction caused by UV rays and chemical agents (Krinsky, 1989). For their part, flavonoids and polyphenols are phenolic compounds known to be present in a wide variety of plants in higher concentrations than other antioxidants such as vitamin C and E, which makes them the main antioxidants acquired in the diet (Lotito and Frei, 2006). Although in *in vivo* studies it has been observed that antioxidants show a low availability and a high clearance rate (Manach *et al*., 2005), in some epidemiological studies a protective effect of the consumption of flavonoids has been reported against the risk of cardiovascular diseases (Knekt *et al*., 2002; Arts and Hollman, 2005) relating it to its antioxidant capacity in the face of the oxidative imbalance of these pathologies (Aviram and Fuhrman, 2002; Rietveld and Wiseman, 2003).

Polyphenols can be divided into several subgroups depending on their basic structure. Examples of this are flavonoids that include anthocyanins, flavonols, flavones, flavanones and isoflavones. Another subgroup is composed by the phenylpropanoids, which include hydroxycinnamic acid derivatives (caffeic, ferulic, synaptic, p-coumaric), as well as stilbenoids such as resveratrol and benzoic acid derivatives (gallic and ellagic acid, among others) and the anthocyanins responsible for the pigmentation of red fruits, some vegetables and proanthocyanidins (condensed tannins), and hydrolyzable tannins that confer the astringent flavor in some fruits (Tomás-Barberán, 2003). Within this classification, there are also extractable polyphenols (PE), so-called because they can be extracted with aqueous-organic solvents (Pérez-Jiménez *et al*., 2013), examples of which are flavonoids (catechins, proanthocyanidins, anthocyanidins, flavonoids, flavones, and isoflavones), phenolic acids, stilbenes, and lignans. Non-extractable polyphenols (PNE) remain retained in the residues after extraction (Saura-Calixto, 2013), examples of these are hydrolyzable tannins and proanthocyanidins associated with dietary fiber and protein. Of the total polyphenols consumed in the diet, 50% are PE that are absorbed in the small intestine while PNE (proanthocyanidins) reach the colon intact and can be fermented by the intestinal microbiota or broken down by some intestinal enzymes such as esterases (Pérez -Jiménez *et al*., 2013).

The most studied are hydrolyzable tannins, specifically those resulting from the esterification of gallic acid or ellagic acid, such as pentagalloyl glucose (PGG), which has a certain anti-cancer activity (prostate and lung cancer), and anti-diabetic activity since tannin has an effect similar to that of insulin, by binding to specific insulin receptors on the cell membrane, favoring the transport of glucose into the cell, even in the absence of insulin, in addition to a proven antioxidant activity *in vitro*. On the other hand, in *in vivo* models, it has been observed that the proanthocyanidins present in cranberry juice exhibit antibacterial activity, preventing the adhesion of *E. coli* to cell surfaces of the urinary tract (Álvarez *et al*., 2012).

2.5 Fiber-antioxidant and their influence on obesity

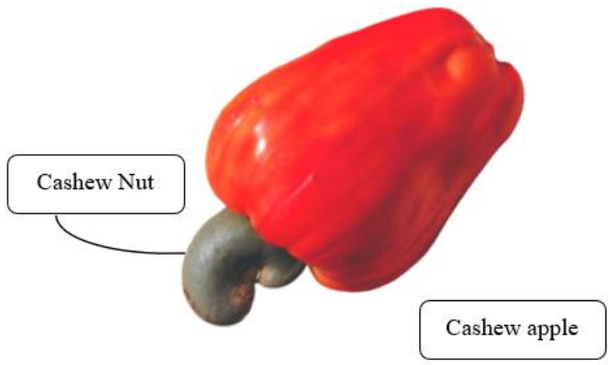
The term fiber-antioxidant (FA) was coined after observing that most of the dietary antioxidants are totally or partially absorbed in the small intestine, but an appreciable amount of these, mainly polyphenols and carotenoids, are transported together with the dietary fiber towards the large intestine, where they are released from the fibrous matrix by the action of the microbiota and can produce metabolites and an antioxidant environment (Mainai *et al*., 2009; Arranz *et al*., 2010). An example of this are *in vivo* studies that have shown that by consuming the fruit, peel, and grape pomace (rich in antioxidant fiber), it is possible to modulate the redox status and defense system of rats, due to the increase in the activity of the enzyme glutathione peroxidase in blood plasma and liver inducing a hepatoprotective effect (Bobek, 1999; Dani *et al*., 2008; Young *et al*., 2000).

A lipid-lowering effect has also been attributed to AF. Martín-Carrón *et al*. (2000) evaluated the effect of the addition to the diet of grape antioxidant dietary fiber (GADF) in adult Wistar rats on a cholesterol-free diet and another diet added with 10 g kg-1 of cholesterol. GADF intake increased weight and the amount of fat and protein excreted in the feces and did not affect animal growth and/or protein efficiency. In addition, it decreased total serum cholesterol and LDL-cholesterol concentrations in hypercholesterolemic rats. Hogan *et al*. (2010) studied the effect of FA on the modification of the bacterial profile in the cecum of rats fed for four weeks. The results showed that the intake of FA stimulates the *in vitro* proliferation of *Lactobacillus*, such as *Lactobacillus reuteri* and *Lactobacillus acidophilus*, but slightly affects the species composition of *Bifidobacterium*, therefore, these findings suggest that the consumption of a diet with food vegetables rich in FA can improve the gastrointestinal health of the host through modulation of the microbiota (Pozuelo *et al*., 2012).

2.6 Cashew (*Anacardium occidentale* L.) as a source of fiber-antioxidant

The cashew tree (*Anacardium occidentale* L.) belongs to the *Anacardiaceae* family that includes about 60 genera and 400 species. It is native to the northwest of Brazil, its leaves are evergreen and can reach between 8 and 12 m in height, and until the third year of life, it begins to bear fruit. According to the International Nut & Dried Fruit (INC, 2016), the cashew fruit consists of two parts: the pseudo fruit or apple, which represents the majority, and the nut or true fruit, the smallest (Figure 2.2) (CONABIO, 2020). The largest cashew producers worldwide are Vietnam, India, and Brazil with a maximum production of almost 3, 000,000 Ton/year (FAO, 2020). In México, the states with the highest production are Campeche, Guerrero, and Chiapas, producing a maximum of 3,000 Ton/year, however, in the states of Colima, Oaxaca, Sinaloa, Tabasco, and Veracruz, cashew nuts are found growing wild (SIAP, 2020).

Figure 2.2 Cashew Fruit (*Anacardium occidentale L.)*



Source: Own elaboration

The cashew pseudo fruit is firm and juicy, contains fiber, tannins, and carotenoids (Fonteles *et al*., 2017), a high concentration of sugars, strong flavor, low acidity, high astringency (Das and Arora, 2016) and high content of vitamin C, in juice (203.5mg / 100mL) and fresh pulp (229mg / 100g), almost four times more than orange (54.7mg / 100mL), lemon (33.7mg / 100mL), pineapple (14.70mg / 100mL) and mango (30.9mg / 100mL) (Akinwale, 2000; Figuereido *et al*., 2002). Other main components of the juice are esters (226.46 µg / kg) representing 45% of the total volatile mass, followed by terpenes (118.98 µg / kg), acids (45.23 µg / kg), aldehydes (39.10 µg / kg), alcohols (18.91 µg / kg), lactones (19.15 µg / kg), hydrocarbons (18.02 µg / kg) and ketones (11.05 µg / kg) (Telles *et al*., 2015). In the stage of physiological maturity of the pseudo fruit, polyphenols are found in concentrations of 1337.67 mg EAG / 100g of fresh pulp and flavonoids of 1018 mg EAG / 100g of fresh pulp, while in overripe stages their concentration decreases (614.33 mg EAG / 100g and 339.33 mg EAG / 100g of fresh pulp, respectively) (Flores, 2018). Bioaccessibility studies have been carried out on some minerals in cashew juice such as copper, iron, and zinc, finding that only 15%, 11%, and 3.7% are bioaccessible after the digestion process (De Lima *et al*., 2014).

2.6.1 Uses and applications of cashew

After extraction of the juice by pressing, 25% of a residue called bagasse is generated with a high concentration of fiber, which varies from 41.53% ps (Matias *et al*., 2005), to 33% in fresh bagasse (Rodrígues *et al*., 2007). Various authors have reported the effect of the administration of cashew bagasse in murine models, such as the case of Carvalho *et al*. (2017), who administered 10% of sonicated and lyophilized bagasse to a group of healthy rats, causing an increase in body weight and elevation of serum triglyceride and LDL cholesterol levels after 15 weeks. Later, Carvalho *et al*. (2019) administered natural cashew fiber (IcF) and cashew fiber after undergoing a low molecular weight metabolite (cFSM) extraction process to obese male Swiss mice for 15 weeks. At the end of the treatment, a reduction in glycemia and serum levels of insulin and ghrelin was found, while the animals fed IcF showed hyperlipidemia, hyperleptinemia, and increased abdominal fat, concluding that the elimination of low molecular weight metabolites in cashew bagasse is correlated with the improvement of the health of obese mice. For their part, Beejmohun *et al*. (2015) used 200 mg of the ethanolic extract of lyophilized bagasse in obese rats, reducing body weight, liver weight, and adipose tissue. With regard to industrial uses, Guedes-Oliveira *et al*. (2016) evaluated washed cashew fiber as a fat substitute in food, demonstrating its viability as a texture modifier. Its possible use as a prebiotic has also been evaluated since it satisfactorily modulates the intestinal microbiota, increasing the *Lactobacillus* and *Bifidobacterium* genera (Dantas *et al*., 2017). Cashew bagasse evaluations have been carried out after microwave drying at 390W and it has been found that it has a higher content of phenolic compounds (777 ± 0.01 mg EAG / 100g dehydrated bagasse) compared to the control (681mg EAG / 100g sample fresh), and that the color and other bioactive compounds such as carotenoids are preserved (Morales *et al*., 2019). Studies have also been carried out on the various parts of the cashew tree (Table 2.1) such as leaves, stem, roots, bark, gum and in both nut shell and liquid (CNSL), demonstrating antitumor, antibacterial activity, fungicide, anticorrosive, decontaminant, anti-inflammatory, and healing, among others (Sokeng *et al*., 2001; Lorenzi, 2004; Perone, 2012; Gómez and Pereira, 2016).

Table 2.1 Studies on uses and applications of cashew in various areas

Part of the tree	Health Benefit	References
Stem and leaves	Treatment of eczema, psoriasis, scrofula, dyspepsia, venereal diseases, sexual impotence, bronchitis, cough, menstrual and intestinal cramps and skin-related disorders.	Franca *et al*., 1993
Stem, leaves and fruit	Anti-inflammatory	Sokeng *et al.*, 2001
Bark	Treatment of leprosy, burns and as a healing	Perone, 2012
	Decreased levels of alanine and aspartate aminotransferase, protects against death from sepsis	Olajide *et al.*, 2004
Leaves	Antibacterial activity	Gómez y Pereira, 2016
Gum	Fungicide and insecticide	Lorenzi, 2004
	Anti-inflammatory activity	Yamassaki *et al.*, 2015
	Blood pressure reduction	Mothe *et al.*, 2006
	Antitumor activity	Mothe y Calazans, 2008
Juice	Antioxidant potential and mutagenic activity	Ferguson, 2001
Bagasse	Inhibits fat storage and the development of insulin resistance	Beejmohun *et al.*, 2015
	Prebiotic effect in *Lactobacillus johnsonii*	Vergara *et al.*, 2010; Dantas *et al.*, 2017
Fiber	Prevents the increase of body weight, liver and abdominal fat, reduces ghrelin, leptin, TNF- α and adiponectin levels	Carvalho *et al.*, 2019
	Adjuvant in the treatment of *Heamonchus contortus* (intestinal nematode)	Lopes *et al.*, 2018
CNSL (Cashew Nutshell liquid)	Elimination of plantar warts caused by human papilloma	Ñurinda y Valle, 2020
	Antibacterial activity on *Streptococcus mutans*	Ponce, 2011
	Antimicrobial activity on *Staphylococcus aureus*	Tello, 2011; Vicanco, 2011
Ánacardic acids	Increases glucose absorption in cells	Tedon *et al.*, 2010
	Increased expressión of PPAR-γ	*Chung *et al.*, 2019

CNSL: Corrosive oil obtained from pressing cashew nut shells. Source consulted: Own elaboration.

2.7 Conclusions

Both antioxidant compounds and fiber have been extensively studied throughout history. Fiber has been recognized for its direct health benefits, as well as its importance in modulating the intestinal microbiota, however, the relationship with antioxidant compounds such as non-extractable polyphenols have not been fully clarified. For their part, antioxidant compounds such as condensed and hydrolyzable tannins have proven their effectiveness in diseases such as cancer, diabetes, and chronic non-communicable diseases. Therefore, studies involving the bioavailability of antioxidants bound to fiber are of utmost importance to better understand the interaction of these compounds and their positive relationship to health. In the case of lipoinflammation or inflammation of adipose tissue, the antioxidant fiber shows promising results in improving health since by promoting an antioxidant environment and the release of SCFA by the microbiota, free radicals formed in inflammatory processes can be fought. Some foods have been proposed as sources of fiber antioxidant, as they have high fiber content linked to antioxidant compounds, such as guava, mango, and cactus. Therefore, the cashew is proposed as a possible source of fiber antioxidant since it has high concentrations of condensed tannins, vitamin C, and fiber, comparable with fruits such as guava, pineapple, orange, and apple that stand out for their use as functional foods in chronic degenerative diseases related to inflammation. It is also recommended to undertake the pertinent studies to cashew bagasse in order to evaluate its application in a model of obesity induced by a diet high in fat and carbohydrates in order to elucidate its anti-inflammatory potential.

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Chapter 3 The relevance of the source of animal or vegetable proteins on the metabolic syndrome and its comorbidities

Capítulo 3 La relevancia de la fuente de proteínas animales o vegetales sobre el síndrome metabólico y sus comorbilidades

MARCOS-MÉNDEZ, Dora Alicia´†, RAMIREZ-HIGUERA, Abril´*, OLIART-ROS, Rosa María´ and GUADARRAMA-LEZAMA, Andrea Yazmín´´

´Tecnológico Nacional de México / I. T. Veracruz, Food Research and Development Unit, Av. Miguel Angel de Quevedo No. 2779, Col. Formando hogar, 91987 Veracruz, Ver, Mexico.

´´Universidad Autónoma del Estado de México, Paseo Colón esq. Paseo Tollocan s/n, Col. Residencial Colón, C.P. 50120, Toluca, Estado de México, Mexico.

ID 1st Author: *Dora Alicia, Marcos-Méndez* / **ORC ID:** 0000-0002-5662-5478, **CVU CONACYT ID:** 1027248

ID 1st Co-author: *Abril, Ramírez-Higuera* / **ORC ID:** 0000-0002-1430-2689, **CVU CONACYT ID:** 242658

ID 2nd Co-author: *Rosa María, Oliart-Ros* / **ORC ID:** 0000-0003-1204-8792, **CVU CONACYT ID:** 26058

ID 3rd Co-author: *Andrea Yazmín, Guadarrama-Lezama* / **ORC ID:** 0000-0002-9264-4887, **CVU CONACYT ID:** 296381

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D. Marcos, A. Ramírez, R. Oliart and A. Guadarrama

* abril.rh@veracruz.tecnm.mx

A. Marroquín, J. Olivares, A. Dector L. Cruz. (Coord.) CIERMMI Women in Science TXIV Biology, Chemistry and Life Sciences. Handbooks-©ECORFAN-México, Querétaro, 2021.

Abstract

Metabolic Syndrome (MS) is one of the most serious health problems worldwide since 25% of the population suffers from it and 80% of these are at risk of cardiovascular diseases and diabetes mellitus. MS is defined as a series of metabolic abnormalities constituted by arterial hypertension (HTN), abdominal obesity, dyslipidemias, glucose intolerance and/or insulin resistance (IR). Proteins are long chains of amino acids and have a characteristic three-dimensional structure that is essential for their specific function. These are a source of bioactive peptides that can have beneficial effects on health. Bioactive peptides are small peptide chains composed of 2 to 15 amino acid residues, obtained by industrial food processing or during gastrointestinal digestion; after oral administration, they exert their beneficial effect on the cardiovascular, digestive, immune, and nervous systems. Therefore, the objective of this review is to describe investigations about the positive effects of different kinds and sources of protein, fractions, or peptides in MS.

Metabolic syndrome, Proteins, Bioactive peptides

Resumen

Actualmente el Síndrome Metabólico (SM) es uno de los problemas de salud más graves a nivel mundial, ya que el 25 % de la población lo padece y el 80% de éstos tiene riesgo de presentar enfermedades cardiovasculares y diabetes mellitus. El SM incluye una serie de anormalidades metabólicas como la hipertensión arterial (HTA), obesidad abdominal, dislipidemias, intolerancia a la glucosa y/o resistencia a la insulina (RI). Las proteínas son largas cadenas de aminoácidos y tienen una estructura tridimensional característica indispensable para que desempeñe su función específica. En los últimos años, estas macromoléculas han sido de gran interés debido a que son una fuente de péptidos bioactivos que pueden tener efectos benéficos en la salud. Los péptidos bioactivos son pequeñas cadenas peptídicas compuestas por 2 a 15 residuos de aminoácidos, su liberación es a través del procesado industrial de los alimentos o durante la digestión gastrointestinal; tras la administración oral, ejercen su efecto benéfico en los sistemas cardiovascular, digestivo, inmunológico y nervioso. Es por lo anterior que el objetivo de esta revisión es proporcionar información acerca del efecto de las proteínas y péptidos bioactivos provenientes de fuentes de origen animal y vegetal sobre algunos componentes del SM.

Síndrome metabólico, Proteínas, Péptidos bioactivos

3.1 Introduction

MS includes a series of metabolic abnormalities such as arterial hypertension (HTA), central obesity, dyslipidemias characterized by elevated triglyceride concentrations and decreased concentrations of high-density lipoprotein cholesterol (HDL- C), glucose intolerance, and/or insulin resistance (IR), as well as an increase in pro-inflammatory molecules (Pierlot *et al.*, 2017). Currently, it is one of the most serious health problems, since 25% of the world population suffers from it; among them, 80% have the risk of developing cardiovascular diseases and diabetes mellitus (Fernández, 2016).

Proteins are physically and functionally complex macromolecules, serving multiple crucial functions. They are long chains of amino acids composed of 50-55% carbon, 20-23% oxygen, 6-7% hydrogen, 0.2-0.3% sulfur, and are an important source of nitrogen (12-19%) that can be assimilated by the organism (Walstra and Van Vliet, 2010). Each protein has a characteristic three-dimensional structure that is essential for its specific function (Feduchi, 2011).

Currently, it is known that in addition to their nutritional and functional value, proteins are a source of bioactive peptides that can have beneficial effects on health. These peptides can be obtained through lactic fermentation methods, chemical hydrolysis and enzymatic processes (Daliri *et al.*, 2017).

Bioactive peptides are made up of 2 to 15 amino acid residues and their production can be through industrial food processing or during gastrointestinal digestion. After oral administration, the bioactive peptides exert their beneficial effect on the cardiovascular, digestive, immune, and nervous systems. They have different activities such as antimicrobials, opiates, antioxidants, antihypercholesterolemic, antithrombotic, antihypertensive, hypoglycemic, among others.

They have a positive impact on human health, beyond their nutritional function, in relation to the composition and sequence of the amino acids that compose them (Alvarado, 2010). Due to the different health benefits of oral administration of bioactive peptides, proteins of animal and vegetable origin have been used for their isolation by enzymatic hydrolysis (Mulero *et al.*, 2011).

According to recent population studies, the prevalence of MS components is increasing worldwide, so it is necessary to find non-pharmacological alternatives that allow their prevention and treatment. This chapter presents a review of the effect of bioactive proteins and peptides from animal and plant sources on some components of MS.

Table 3.1 shows the effects of plant and animal proteins, because they can interfere with different mechanisms for lowering blood pressure, improving diabetes or dyslipidemia.

Table 3.1 Health effects of animal vs vegetable protein consumption

Animal protein	Vegetal protein
- Reduction of glucose levels - Prevention of liver and kidney toxicity - Increase in catabolism of hepatic fatty acid - Increase hepatic gene expression of the fernesoid X receptor (Fxr) - Reduction of low-density lipoprotein (LDL) cholesterol levels - Inhibition of angiotensin-converting enzyme I (ACE I) - Improved ex vivo vasodilation	- Inhibition of the DPP-IV enzyme - Reduction of glycosylated hemoglobin levels - Significant reduction in triglyceride levels - Improvement of hepatic stagtosis - Lower expression of transcription factor SREBP-2 - Reduction of low-density lipoprotein (LDL) cholesterol levels - Inhibition of angiotensin-converting enzyme I (ACE I)

Source: Own elaboration

3.2 Animal protein

Foods of animal origin generally contain a high Digestible Indispensable Amino Acid Index (DIAAS) and a high Protein Digestibility Corrected Amino Acid Score (PDCAAS), which places animal protein as having high biological value. This means that they contain a favorable ratio and quantity of the essential amino acids. The DIAAS method is used to know the quality of dietary proteins for regulatory purposes, this is defined as% DIAAS = 100 x [(mg of the amino acid of the diet indispensable digestible in 1g of the protein of the diet) / (mg of the same amino acid in 1g of the reference protein)]. The PDCAAS method also assesses the quality of the protein; however, this method has been found to overestimate the protein quality of some products. Foods of animal origin, in addition to being a good source of proteins and essential amino acids, are important sources of cobalamin or vitamin B12, zinc, phosphorus, and heme iron (Quesada and Gómez, 2019).

3.2.1 Effect of consumption of animal protein on glucose metabolism

Various investigations have suggested that proteins or protein hydrolysates can regulate blood glucose levels under different conditions, however, the mechanisms have not been fully elucidated.

There are different mechanisms by which bioactive proteins or peptides can reduce blood glucose levels. In the study of Nasri *et al.* (2015) the therapeutic effect of undigested goby fish muscle proteins (UGP) and their hydrolysates (GPHs) was investigated in rats fed a high-fat, high-fructose (HFFD) diet. In the rats that were fed with HFFD, hyperglycemia associated with an increase in oxidative stress was observed, while in those that were co-administered with GPHs, a significant decrease in their blood glucose levels and α-amylase activity was found; however, in UGP fed rats, blood glucose values did not decrease, which may be due to differences in length, chain and amino acid sequence of the peptides used. In addition to the hypoglycemic activity that GPH provides, results suggested its use for the prevention of liver and kidney toxicity induced by the consumption of a high-fat and high-fructose diet. Akhavan *et al*. (2010) evaluated the effect of the consumption of whey protein (WP) and its hydrolysate (WPH) on the blood concentrations of glucose and insulin in healthy young adults, and showed that the WP in small amounts and when consumed before a meal, reduces postprandial blood glucose levels as well as food intake; however, the consumption of WPH did not reduce blood glucose levels, suggesting that non-insulin tropic mechanisms require the stimulation that arises from the digestion of intact proteins.

3.2.2 Effect of consumption of animal protein on lipid metabolism

According to what has been mentioned by different researchers, proteins, hydrolysates or peptides can have effects on lipid metabolism. Ramsvik *et al.*, in 2013, evaluated the effect of krill protein hydrolysate (KPH) on lipid and bile acid (BAs) metabolism in mice. The animals were fed a high-fat diet for six weeks. One group was supplemented with casein (control) and the other with KPH. In the KPH group, hepatic fatty acid catabolism increased as the activity of the target PPARα CPT-2 gene increased. An increased hepatic gene expression of the farnesoid X receptor (FXR) was also found in KPH-treated mice compared to the control group. FXR is responsible for maintaining bile acid homeostasis and controlling plasma TAG levels by activating the PPARα gene. It has also been reported that PPARα induces the expression of genes that encode enzymes involved in the oxidation of mitochondrial fatty acids, so that the regulation of KPH of BAs may have the ability to modulate the oxidation of hepatic fatty acids, reducing the concentration of triglycerides in plasma. Mice with FXR deficiency have an increased synthesis of Apolipoprotein B (ApoB), resulting in a higher concentration of TG in serum and liver.

In 2012, Xue *et al.* determined the effects of chickpea albumin hydrolysate (CAH) on the lipid profile of mice fed a high-fat diet. The mice were divided into the following groups: control normal diet, high-fat diet, and CAH with a low (LD), medium (MD), and high dose (HD). The triglyceride content of the three CAH groups decreased by 15.50% (LD), 19.01% (MD), and 36.55% (HD), compared to the high-fat diet group; triglyceride levels of the CAH-HD group were similar to those of the normal diet group. The high-fat diet also caused an increase in low-density lipoprotein (LDL) cholesterol levels, however, after four weeks of CAH administration these levels were decreased in a dose-dependent manner. For high-density lipoprotein (HDL) cholesterol, levels increased showing a dose-dependence. The researchers concluded that the mechanism of action of CAH for cholesterol reduction cannot be fully understood yet and suggested that it may involve the enhancement of LDL cholesterol catabolism through hepatic receptors and/or the increased lipoprotein lipase activity.

3.2.3 Effect of consumption of animal protein on blood pressure

Studies on the relationship between the consumption of protein, protein hydrolysates, or bioactive peptides and the control of blood pressure associate the effect with the inhibition of the angiotensin-converting enzyme (ACE).

A study by Girgih *et al.* in 2015 evaluated the ACE inhibitory activity of a cod hydrolysate (CPH) and its bioactive peptides (CF3) obtained through proteolysis. The experimentation was carried out in four groups of rats (CHP, CF3, captopril, and phosphate-buffered saline), to which the aforementioned were administered by oral gavage, and systolic blood pressure was measured at 2, 4, 6, 8, and 24 hours. It was found that the CF3 peptide reduced blood pressure within the first 2 hours after oral administration and was maintained until after 24 hours, which suggests that its absorption was faster in the gastrointestinal tract of rats compared to CPH.

Another study that evaluated the effect of animal protein and on blood pressure was carried out by Jahandideh *et al.* in 2016, who used an egg white hydrolysate (EWH) in spontaneously hypertensive rats. Three groups were assigned, the control group, low dose EWH (250 mg/kg body weight), and high dose EWH (1000 mg/kg body weight). Treatments were administered orally once a day for 12 days, and blood pressure (BP) was measured on days 0, 3, 6, 9, and 12. It was reported that both EWH doses decreased BP values compared to the control group, but the reduction was only significant in those rats that were administered with the high dose. This could be associated with improved ex vivo vasodilation that reduced oxidative stress because spontaneously hypertensive rats show impaired vasodilation. It also reduced ACE, as well as the expression of the angiotensin II receptor.

3.2.4 Vegetal protein

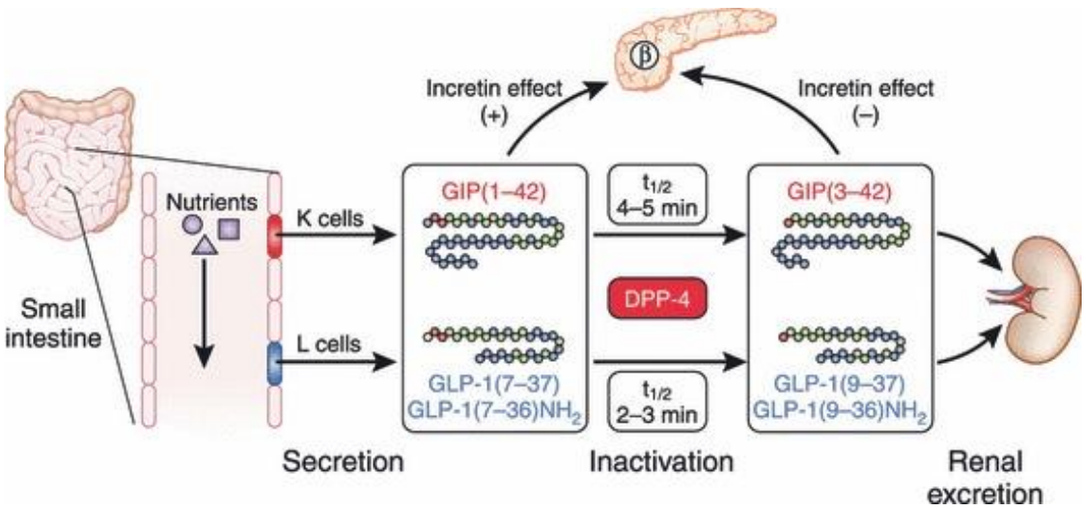
Vegetable proteins are presented in a wide variety, availability and low cost, and great versatility in terms of physicochemical characteristics, which is why it is sought to improve their functionality through chemical or enzymatic modifications. They are obtained from crushed seeds or defatted flour by different methods (Bonino, 2016). Grains are the most abundant and valuable source of vegetable protein since, in addition to being of high quality, they have an adequate content of essential amino acids.

Although proteins of vegetable origin can be considered as an incomplete protein source due to their null or limiting content of some amino acids, it is possible to obtain high-quality proteins by combining vegetable sources (Quesada and Gómez, 2019).

3.2.5 Effect of consumption of vegetal protein on glucose metabolism

After food intake (Figure 3.1), insulin secretion is stimulated by the combined action of glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). These are the main hormones of the incretin effect, which exert their metabolic effects by activating their receptors. It has been suggested that GLP-1 modulation can help normalize blood glucose levels in individuals suffering from type 2 diabetes (DM2) (González *et al.*, 2018). However, these hormones are degraded by the enzyme dipeptidyl peptidase IV (DPP-IV), turning them into inactive peptide fragments, so their circulating half-life is very short (Romero, 2007). Therefore, the inhibition of DPP-IV can be considered as an alternative for the management of DM2, as well as the blockade of salivary α-amylase and intestinal α-glucosidase.

Figure 3.1 Mechanism of inhibition of the DPP-IV enzyme



Source: Seino et al., 2010

González *et al.* in 2018, investigated *in vitro* the effect of soybean sprout peptides on the inhibition of DPP-IV, salivary α-amylase, and intestinal α-glucosidase. Germinated soy protein was digested for six days (6GSPD) and peptide fractions were obtained by ultrafiltration. Results showed that the inhibition of DPP-IV activity by 6GSPD was dose-dependent. In addition, peptides of 5-10 and >10 kDa were effective in inhibiting the DPP-IV enzyme but peptides of 5-10 and <5 kDa showed better inhibition of the enzymes α-amylase and α-glucosidase, suggesting that the amino acid composition of the peptides determines the interaction with the enzymes in the inhibitory mechanism.

Hernández *et al.*, in 2020 evaluated the effect of consuming a bean protein concentrate (BPC) and a cooked bean flour (WCB) in male Wistar rats given a control diet (casein) or a high-fat diet with 5% sucrose in drinking water. Rats fed the high-fat diet and supplemented with BPCs had 33.6% less body weight, a 11% loss of body fat, and a 5.5% increase in lean mass compared to those fed the same diet but supplemented with casein. Animals fed a high-fat diet and supplemented with WCB had a lower percentage of body fat and a higher percentage of lean mass compared to BPCs group. These results were associated with a significant increase in energy expenditure in BPCs and WCB groups. In addition, a significant reduction in triglyceride, glucose and insulin levels were found in both groups compared to the control group, despite being fed with the high-fat diet, suggesting a lower expression of lipogenic genes in the liver.

In 2019, Marthandam *et al.* studied the efficacy of a decapeptide obtained from potato protein hydrolysated with alcalase (APPH) administered to male mice induced to diabetes by streptozotocin, and observed that animals administered with APPH showed regulated blood glucose levels, and a reduction in the levels of glycated hemoglobin (HbA1c), triglycerides (TG), and total cholesterol (TC).

Another important aspect was the significant improvement in the morphology of the liver and kidney when compared with the diabetic group, who showed loss of normal architecture of the hepatocytes, necrosis, hydropic degeneration, and congestion of the central vein, as well as diabetic nephropathy.

3.2.6 Effect on lipid metabolism

In vivo studies have determined the different effects of vegetal protein on lipid metabolism, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein (LDL), and triglycerides (TG). Moriyama *et al.* in 2004, evaluated the effects of soybean β-conglycinin and glycinin on the lipid profile in normal and obese mice. They observed that triglycerides and glucose levels in the group of mice with a diet supplemented with β-conglycinin were lower than in control (casein) and conglycinin groups. The researchers reported that the activities and expression of the enzymes related to β-oxidation were higher in the β-conglycinin group than in the other groups. Furthermore, the activity of the fatty acid synthase in the β-conglycinin group was significantly lower when compared to other groups. The aforementioned suggests that the activities of the enzymes involved in fatty acids metabolism could alter the levels of TG in the liver and probably the levels of TG as VLDL (very low-density lipoprotein).

Amaral *et al.,* in 2014, evaluated the effect of the consumption of 11S globulin isolated from chickpea on the lipid profile of adult male Wistar rats with hypercholesterolemia and found that serum triglycerides levels were 28.77% lower than those of the hypercholesterolemic group, and similar to control group. The researchers mentioned that 11S globulin could be involved in triglycerides metabolism, which could be attributed to the possible regulatory effects of this fraction on the transcription factor SREBP-1 (sterol regulatory element-binding protein) found associated with genes involved in fatty acid biosynthesis. Another important observation was the improvement in liver steatosis in the group treated with 11S globulin.

In 2016, Zhang *et al.* evaluated the effects of defatted rice bran protein (DRBP), fresh rice bran protein (FRBP), DRBP hydrolysate (DRBPH), and FRBP hydrolysate (FRBPH) on the lipid profile of mice fed a high-fat diet. In all protein-supplemented groups, decreased levels of very-low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) cholesterol were observed. In the group supplemented with FRBPH, a lower expression of the transcription factor SREBP-2 was observed; SREBP-2 is responsible of cholesterol biosynthesis regulation. Authors concluded that rice bran proteins, as well as their hydrolysates, intervene in mice lipid metabolism, since they can reduce cholesterol biosynthesis.

3.2.7 Effect of vegetal protein consumption on blood pressure

Different *in vitro* investigations have been carried out to analyze the effect of vegetal proteins on the angiotensin-converting enzyme (ACE). Boye *et al.,* in 2010, characterized the proteins present in red lentils, obtaining different fractions such as albumins, legumes and vicilins, which underwent tryptic hydrolysis to release bioactive peptides. Each fraction showed an ACE inhibition property, however, legumin showed higher activity compared to the other fractions, which could be due to its particular peptide composition or higher protein content.

A study conducted in 2014 by García *et al.* evaluated different proteases (alcalase, savinase, protamex and corollase 7089) at different hydrolysis times, to obtain multifunctional hydrolysates from lentil protein concentrates. Once the hydrolysates were obtained, their antioxidant and ACE inhibitor activities were measured. It was found that the hydrolysate produced by the protease Savinase after 2 h of hydrolysis (S2) showed the highest ACE inhibitory activity and antioxidant activity, concluding that the peptides of this hydrolysate could preserve or improve its multifunctionality in the gastrointestinal tract. Authors identified peptides with hydrophobic amino acids, which makes them potential contributors to the double bioactivity detected.

García *et al.* in 2017, identified peptides with antioxidant and ACE inhibitory activity, released by hydrolysis of lentil protein by protease Savinase, and studied the functional stability of bioactive peptides after simulation of gastrointestinal digestion. The most abundant peptides identified were in the vicilin, convicilin, and legumin fractions.

The researchers found that 3 peptides (LLSGTQNQPSFLSGF, NSLTLPILRYL, TLEPNSVFLPVLLH) showed the highest antioxidant activity (0.013-1.432µmol Trolox eq./µmol peptide) and ACE inhibition (IC 50 = 44-120µM). Inhibition of ACE is based on the formation of hydrogen bonds between the C-terminal residues of bioactive peptides and the residues of the catalytic site of ACE. To confirm gastrointestinal stability, bioactive peptides from lentils were treated *in vitro* with the enzyme pepsin and subsequently with pancreatin at 37ºC in simulated gastrointestinal digestion, and it was found that the action of digestive enzymes increased the double activity of the peptides.

3.3 Conclusions

Metabolic Syndrome (MS) is a condition that afflicts an alarming percentage of society. The drugs used for the treatment of MS and its comorbidities have, in the long term, a series of adverse health effects. Therefore, it is necessary to identify alternative food sources that are less aggressive and that can alleviate and/or prevent the appearance of the syndrome.

There is evidence that suggests that bioactive peptides, whether of animal or plant origin, could be an alternative that helps in the treatment of MS and its associated comorbidities. However, the mechanisms of action of these have not yet been fully elucidated, which opens up a field of opportunity for new research using *in vitro* and *in vivo* models.

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Capítulo 4 Andanzas de un elemento mágico: el ciclo biogeoquímico del manganeso

Chapter 4 Wanderings of a magic element: the biogeochemical cycle of manganese

DAMIÁN-SALINAS, Daniela†, RIVERA-RODRÍGUEZ, Dulce Arely, LIZÁRRAGA-MENDIOLA, Liliana and VÁZQUEZ-RODRÍGUEZ, Gabriela A.*

Universidad Autónoma del Estado de Hidalgo, Área Académica de Química, México.

Universidad para el Bienestar Benito Juárez García, Área de Conocimiento de Procesos Agroalimentarios, México.

Universidad Autónoma del Estado de Hidalgo, Área Académica de Ingeniería y Arquitectura, México.

ID 1st Author: *Daniela, Damián-Salinas* / **ORC ID**: 0000-0002-2955-2752

ID 1st Co-author: *Dulce Arely, Rivera-Rodríguez*, **ORC ID:** 0000-0001-7428-3291, **CVU CONACYT:** 708668

ID 2nd Co-author: *Liliana, Lizárraga-Mendiola*, **ORC ID**: 0000-0001-6320-8923, **CVU CONACYT:** 99262

ID 3rd Co-author: *Gabriela A., Vázquez-Rodríguez* / **ORC ID**: 0000-0001-8351-8451, **CVU CONACYT:** 25767

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* gvazquez@uaeh.edu.mx

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Resumen

Esta revisión se refiere al ciclo biogeoquímico del manganeso y a las características químicas de este elemento que hacen posible tal ciclo, en particular sus transformaciones redox. A través de un recorrido por las cinco esferas ambientales, a saber, la corteza terrestre (y específicamente el suelo), las distintas partes de la hidrosfera, la biosfera, la antroposfera y la atmósfera, se analizan las principales formas que adquiere este elemento en cada uno de estos compartimientos, entre las que destacan los óxidos de manganeso (MnOx). Se presenta también la formación de depósitos submarinos de MnOx en la interfase corteza/hidrosfera, puesto que representan el mayor reservorio de este elemento en la corteza terrestre. Al revisar las particularidades de las reacciones redox del manganeso en la hidrosfera se discute su especiación en los distintos tipos de agua naturales y en qué circunstancias su presencia se vuelve objeto de preocupación. En el apartado dedicado a la biosfera se revisa cómo la historia terrestre del manganeso está íntimamente entrelazada con la aparición de la fotosíntesis y por consiguiente con la oxigenación de la atmósfera. Asimismo, se examina cómo la química del manganeso fue crucial en proveer de manera fortuita una defensa contra los radicales libres que han acompañado, desde su aparición, al oxígeno molecular y al metabolismo aerobio. Se examinan también algunas transformaciones redox microbianas, el papel del manganeso como nutriente y aspectos relevantes de su toxicología. En las secciones correspondientes a la antroposfera y a la atmósfera se recapitulan algunos usos socioindustriales del manganeso, que se extienden varias decenas de miles de años, y a qué se debe que se encuentre este metal en la atmósfera. La revisión concluye con un repaso de los mecanismos no redox que movilizan este elemento entre el suelo y el agua.

Biogeoquímica, Química ambiental, Biosfera, Oxígeno, Reacciones redox

Abstract

This review is devoted to the biogeochemical cycle of manganese and the chemical characteristics of this element that make such a cycle possible, particularly its redox transformations. Through a journey of the five environmental spheres, namely the Earth's crust (and specifically the soil), the different parts of the hydrosphere, the biosphere, the anthroposphere, and the atmosphere, the main manganese species in each of these compartments are analyzed, among which manganese oxides (MnOx) stand out. The formation of submarine deposits of MnOx at the crust/hydrosphere interface is also presented since they represent the largest reservoir of this element in the Earth's crust. In reviewing the manganese redox reactions in the hydrosphere, its speciation in different types of natural water is presented, as well as the circumstances that turn this element into a matter of concern. The section dedicated to the biosphere shows how the terrestrial history of manganese is intimately intertwined with the emergence of photosynthesis and the oxygenation of the atmosphere. It also examines how manganese chemistry was crucial in fortuitously providing a defense against the free radicals that have, since its emergence, accompanied molecular oxygen and aerobic metabolism. Besides, some microbial redox transformations, the role of manganese as a nutrient, and relevant aspects of its toxicology are examined. Socio-industrial uses of manganese, which span several thousands of years, are summarized in the anthroposphere section. The article concludes with an overview of the non-redox mechanisms that mobilize this "magic" element between soil and water.

Biogeochemistry, Environmental chemistry, Biosphere, Oxygen, Redox reactions

4.1 Introducción

El manganeso (Mn), con número atómico 25 y peso atómico de 54.94, es un elemento que, al igual que el magnesio (Mg), podría deber su nombre a la región griega de Magnesia (Μαγνησία), donde eran abundantes los minerales de ambos elementos (Emsley, 2011). Otra etimología probable es que provenga del griego mangania (μαγγανεία), que significa magia (Lingappa et al., 2019). Es un metal de tono grisáceo con una estructura cúbica sólida (Figura 4.1), que naturalmente se encuentra combinado con otros compuestos como el oxígeno, el azufre o el cloro (Gómez-Miguel y Sotés, 2014). Solo se le encuentra en estado puro en meteoritos (Lugo-López, 2017).

Figura 4.1 Manganeso puro. Imagen con licencia CC-BY-SA-3.0

Fuente: http://bumialamindo.blogspot.com/2014/11/manganese.html

El Mn representa el 0.1% de la corteza terrestre, por lo que es el duodécimo elemento más abundante en ella y el segundo metal de transición más común en el planeta después del Fe (Rudnick y Gao, 2003; (Das et al., 2011). Se encuentra en el grupo VII B, período 4, de la tabla periódica y presenta una configuración electrónica $3d^5 4s^2$ en su capa externa. Gracias a esta configuración electrónica es posible encontrarlo en once estados de oxidación, que van desde -3 a +7. Esta versatilidad redox es la marca característica del ciclo biogeoquímico del manganeso. Sin embargo, sus formas más comunes en el ambiente son Mn(II), Mn(IV) y Mn(VII), ya sea en minerales, materia orgánica e incluso como micronutriente de todos los seres vivos (Mazuelos-Vela, 1959; Tabla 4.1).

Tabla 4.1 Principales especies de manganeso encontradas en el medio ambiente.

	Mn (II)	Mn (III)	Mn (IV)
Especies acuáticas	$[Mn^{II}(H_2O)_6]^{2+}$	Mn (III)-L[a]	Nanopartículas de óxidos de Mn (IV)
	$[Mn^{II}(OH^-)]^+$	Nanopartículas de óxidos de Mn (III)	
	Mn (II)-L[a]		
Especies minerales	Mn^{2+} como constituyente traza en minerales ígneos	Bixbita $(Mn^{3+}, Fe^{3+})_2(O^{2-})_3$	Óxidos de Mn (IV)
	Kutnohorita/calcita manganona	Braunita	$Mn^{4+}(O^{2-})_2$
	$(Mn^{2+}, Ca^{2+})(CO_3^{2-})$	$Mn^{2+}Mn_6^{3+}(SiO_4^{4-})(O^{2-})_4$	Los poliformos comunes incluyen pirolusita, todorokita, hollandita, criptomelano y birnesita
	Rodocrosita		
	$Mn^{2+}(CO_3^{2+})$		
	Rodonita		
	$Mn^{2+}(SiO_3^{2+})$		

[a] Ligandos conocidos que forman complejos acuáticos del manganeso y que incluyen pirofosfato, bicarbonato, citrato, tartrato y ácidos húmicos.
[b] Puede incluir algo de Mn (III).

Fuente: Lingappa et al. (2019)

Transitoriamente pueden existir otros estados de oxidación del Mn, que como intermediarios reactivos no se acumulan en los sistemas naturales. Es el caso de la forma Mn(V), que surge justo antes de la formación del enlace O-O tras la ruptura del agua de la fotosíntesis (Lingappa et al., 2019). De tal suerte que el comportamiento químico de este elemento se ha resumido como dominado por *"…las transiciones redox entre la forma reducida de Mn, relativamente soluble y la forma oxidada altamente insoluble…"* (Laxen et al., 1984).

De manera menos simplista, varias de las transformaciones redox del Mn pueden implicar cambios de fase tales como precipitación y disolución, así como la adsorción o desorción de partículas o superficies. Al especiarse de modo tan diverso, puede ser difícil seguir su rastro a nivel global y hacer un recuento pormenorizado de los papeles que desempeña en cada uno de los procesos en los que participa.

Sin embargo, algunos investigadores han logrado descifrar algunos de los procesos de los que es partícipe este elemento fundamental, gracias a que comparte características similares con metales como el hierro, entre las que destaca su sensibilidad redox. Al ser ambos metales tan parecidos, con frecuencia se les encuentra incluso en los mismos lugares, como en aguas subterráneas (Homoncik et al., 2010).

Actualmente es un hecho que el Mn es un metal que forma parte de numerosos procesos, tanto bióticos como abióticos, en ambientes tanto acuáticos como terrestres e incluso en el aire. Además, es un elemento fundamental de los seres vivos al fungir como un micronutriente esencial para plantas, microorganismos y animales (Gómez-Miguel y Sotés, 2014). De hecho, en la clasificación geoquímica original de Goldschmidt se le considera un elemento biófilo, puesto que se encuentra concentrado en y por seres vivos (Hollabaugh, 2007). Como tiene distintas aplicaciones industriales, su movilización debido a actividades antropogénicas también es relevante.

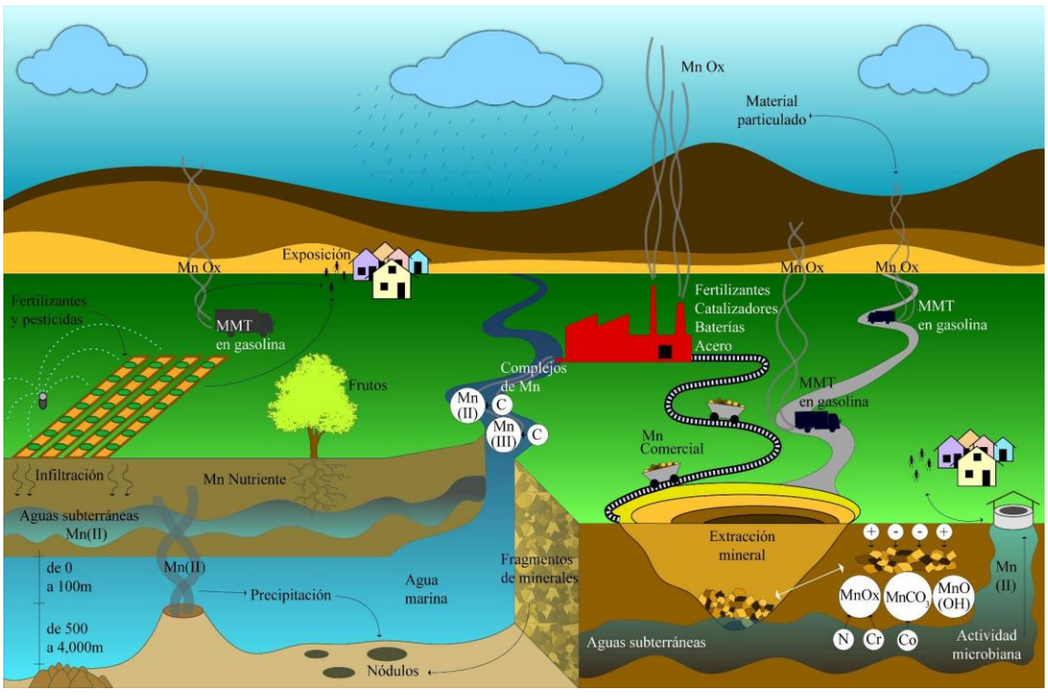
El objetivo de este capítulo es mostrar una perspectiva general del ciclo biogeoquímico del manganeso. Se presentan sus principales reservorios en las distintas esferas ambientales, a saber, la corteza terrestre y los suelos, la hidrosfera, la biosfera, la antroposfera y la atmósfera. Se discute la especiación química del elemento en cada uno de estos reservorios, así como los mecanismos que lo dirigieron hacia ellos, con énfasis en los procesos redox. También se le presta singular atención a la formación de depósitos de manganeso en la interfase corteza/océanos, a su papel en la reacción más importante que ocurre sobre la Tierra, i.e. la fotosíntesis, y a las numerosas aplicaciones que tiene este elemento en las actividades humanas actuales. Por último, se presentan algunos principios básicos que gobiernan su transporte entre los suelos y la fase acuosa, y se aportan conclusiones y perspectivas.

4.2 Reservorios ambientales de manganeso

Tradicionalmente se ha considerado que las principales fuentes de Mn son los minerales de la corteza continental. Se trata de un elemento litófilo que se encuentra en la fase silicatada de los meteoritos, mientras que en la litosfera superior es oxífilo y, como se señaló arriba, biófilo (Mazuelos-Vela, 1959). Sin embargo, desde tiempos recientes se resalta su abundancia en la corteza oceánica, que se estima 16 veces superior a la de la corteza continental (Glasby, 2006).

A continuación, se describirán algunas de las formas más comunes de este elemento en las distintas esferas ambientales, que se resumen en la Figura 4.2, al igual que los diversos mecanismos fisicoquímicos, biológicos y antropogénicos que dan cuenta del movimiento del Mn de una esfera a otra.

Figura 4.2 Principales mecanismos implicados en el ciclo biogeoquímico del manganeso y presentados en esta revisión



Fuente: Imagen de elaboración propia

4.2.1 Corteza terrestre y suelos

En la corteza terrestre se puede encontrar al manganeso en rocas máficas y ultramáficas, así como en minerales detríticos tales como silicatos máficos, magnetita, ilmenita y en su mayoría en los óxidos secundarios formados por concreciones o recubrimientos superficiales de los minerales primarios (Gómez-Miguel y Sotés, 2014). También se le puede encontrar como constituyente minoritario de minerales ígneos en sustitución del Fe(II) (Lingappa et al., 2019). Los minerales más relevantes son los óxidos (MnOx, tales como la pirolusita, MnO_2), los carbonatos (como la rodocrosita, $MnCO_3$) y los oxohidróxidos de manganeso (como la manganita, MnO(OH), Tabla 4.2). Estos se clasifican según su grado en altos (más del 44-48%), medios (35-44%) y bajos (25-35%) (Das et al., 2011). Las reservas mundiales de estos minerales se han estimado superiores a $3 \cdot 10^9$ toneladas (Emsley, 2011), aunque el Servicio Geológico de los Estados Unidos (USGS por sus siglas en inglés) proporciona una estimación mucho menor ($1.3 \cdot 10^6$; USGS, 2021). Alrededor del 80% de las reservas mundiales se concentran en Sudáfrica (40%), Brasil (20%) y Australia (17.7%) (USGS, 2021). No obstante, en los últimos años se ha llegado a la conclusión que la mayor cantidad de Mn está en la corteza oceánica en forma de nódulos polimetálicos (Figura 4.3), en donde podrían existir alrededor de $1 \cdot 10^{11}$ toneladas de MnOx, específicamente al noreste del Océano Pacífico (Glasby, 2006). En la sección 4.1.1 se presentan algunos aspectos básicos de la formación de estos nódulos.

Tabla 4.2 Minerales de manganeso más comunes

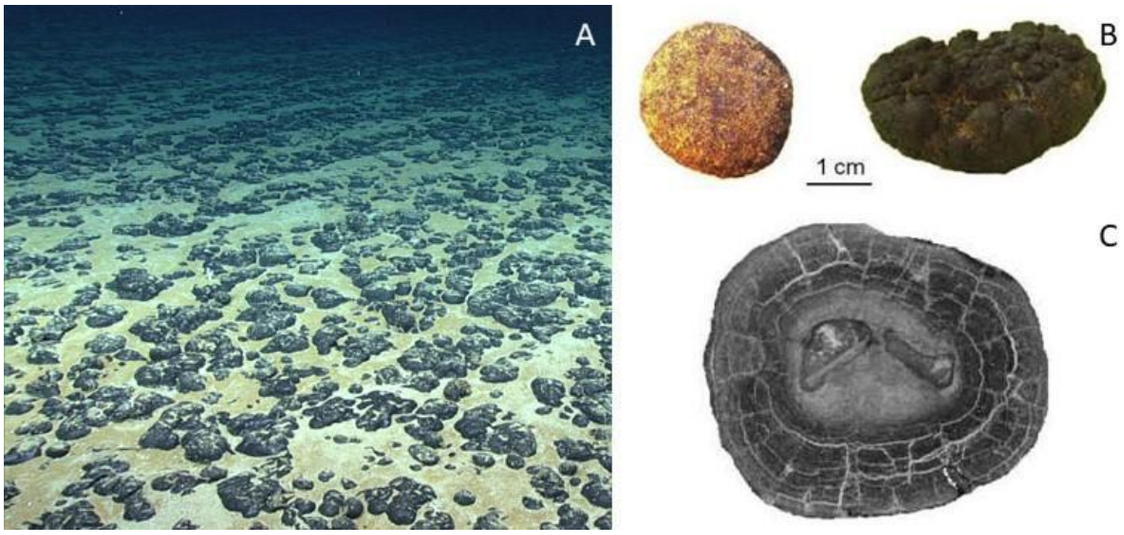
Dominantes en rocas		**Neoformados en suelos y Mn accesorio**		
Mineral	**Fórmula**	**Estructura**	**Mineral**	**Fórmula**
Bixbita	Mn_2O_3	*Capas y pseudocapas*	Birnesita	(Na, Ca) $Mn_7O_{14} \cdot 2.8H_2O$
Blenda de Mn	MnS		Vernadita	$\delta MnO_2 \cdot nH_2O$
Braunita	$3Mn_2O_3.MnSiO_3$	*Híbridos*	Litioforita	$LiAl_2(Mn_2^{4+}Mn^{3+})O_6(OH)_6$
Hauerita	MnS_2	*En túnel*	Criptomelano	$K(Mn^{4+}Mn^{3+})_8(O \cdot OH)_{16}$
Hausmanita	Mn_3O_4		Hollandita	$Ba(Mn^{4+}Mn^{3+})_8O_{16}$
Knebelita	$(MnFe)_2SiO_4$		Todorokita	$(Mn^{4+}Mn^{3+})_6O_{12} \cdot 3H_2O$ (a_0=9.75A) $(Mn^{4+}Mn^{3+})_{14}O_{28} \cdot 9H_2O$ (a_0=24.4A)
Manganita	$Mn_2O_2 \cdot H_2O$	Mn accesorio en: *granates, olivinos, piroxenos, anfíboles, calcita*		
Pirolusita	MnO_2			
Rhodamita	~$MnSiO_3$			
Rodocrosita	$MnCO_3$			
Tefroita	~$MnSiO_3$			

Fuente: Gómez-Miguel y Sotés (2014)

Además de existir en diferentes estados de oxidación, los minerales de Mn poseen diferentes estados cristalinos o pseudocristalinos, e incluso algunos de sus óxidos suelen coprecipitar con óxidos de hierro. Dicha interacción entre compuestos permite que se desarrollen propiedades importantes tales como su comportamiento anfótero, lo que da paso a su interacción tanto con aniones como con cationes (Bradl, 2004). En el suelo el manganeso se encuentra principalmente en rocas ígneas y metamórficas, ya que al estar expuesto a la superficie tiende a oxidarse y a formar diferentes especies minerales (Post, 1999).

Las concentraciones totales de Mn en suelos se han estimado entre 40-900 mg de Mn/kg (Pinsino et al., 2012). Esto es importante, ya que la composición del suelo influye en procesos tan cruciales como la nutrición de las plantas y la movilidad de nutrientes clave como los fosfatos, entre otros. A su vez, la composición edáfica modifica la especiación y la movilidad del Mn, que puede encontrarse como manganeso mineral, como complejo orgánico, manganeso intercambiable o disuelto. Estas formas del manganeso edáfico se explicarán a continuación (Gómez-Miguel y Sotés, 2014).

Figura 4.3 A) Lecho submarino de nódulos polimetálicos; B) Acercamiento a nódulos; C) Corte transversal de un nódulo



Fuente: A) Imagen de la NOAA Office of Ocean Exploration and Research, tomada en 2019 por la Southeastern U.S. Deep-sea Exploration, https://oceanexplorer.noaa.gov/okeanos/explorations/ex1907/logs/nov7/nov7.html; B) y C) Olivares-Cruz et al. (2014)

- **Manganeso mineral.** El Mn es un metal que tiene afinidad por los minerales primarios, arcillas, óxidos e hidróxidos; sin embargo, se le suele encontrar asociado a otros metales, como es el caso de las concreciones de ferromanganeso (ver sección 2.1.1), de donde se libera por alteración, lo que produce minerales secundarios.

- **Manganeso complejado orgánicamente**. Es preciso mencionar que los metales divalentes como el manganeso tienen la capacidad de formar complejos debido a que en el suelo se encuentran sustancias orgánicas cargadas negativamente (que pueden ser naturales como los ácidos húmicos o antropogénicas como el EDTA), tanto solubles como insolubles. Estas especies pueden modificar la estabilidad, su actividad catalítica, su toxicidad y su movilidad en su trayecto por el medio ambiente (Bradl, 2004).

- **Manganeso intercambiable.** Esta forma del manganeso permanece en el complejo de intercambio del suelo (específicamente en las arcillas) como Mn^{2+}, ya que es la forma que comúnmente adquiere en la disolución del suelo. Se trata de una forma de manganeso que no se modifica de modo apreciable solo con añadir más metal, debido a que se oxida fácilmente a Mn(IV) y su concentración en el suelo disminuye en cuanto el valor de pH aumenta.

- **Manganeso en solución.** En el suelo, el ion Mn^{2+} y los MnOx se encuentran en equilibrio, el cual dependerá del estado de oxidación del suelo, el pH y la adsorción en áreas orgánicas. Esta forma del manganeso aumenta en cuanto se presenta acidez y condiciones anaerobias, ya que, en caso contrario, la alcalinidad y la presencia de O_2 favorecerá la conversión a formas menos solubles como Mn(IV).

Estas especies tienen distinta movilidad, y a través de su capacidad de adsorción y captación tanto de oligoelementos como de metales tóxicos, participan en la nutrición vegetal e incluso en la composición del agua subterránea (Post, 1999). En la mayoría de los suelos, alcalinos o ácidos, la especie predominante en solución es el ion Mn^{2+} (Bradl, 2004). Asimismo, es sabido que el manganeso es capaz de movilizarse mayormente en suelos ácidos y ricos en materia orgánica, y en zonas templadas y subárticas (Post, 1999).

4.2.1.1 Interfase corteza/océanos: formación de depósitos submarinos de manganeso

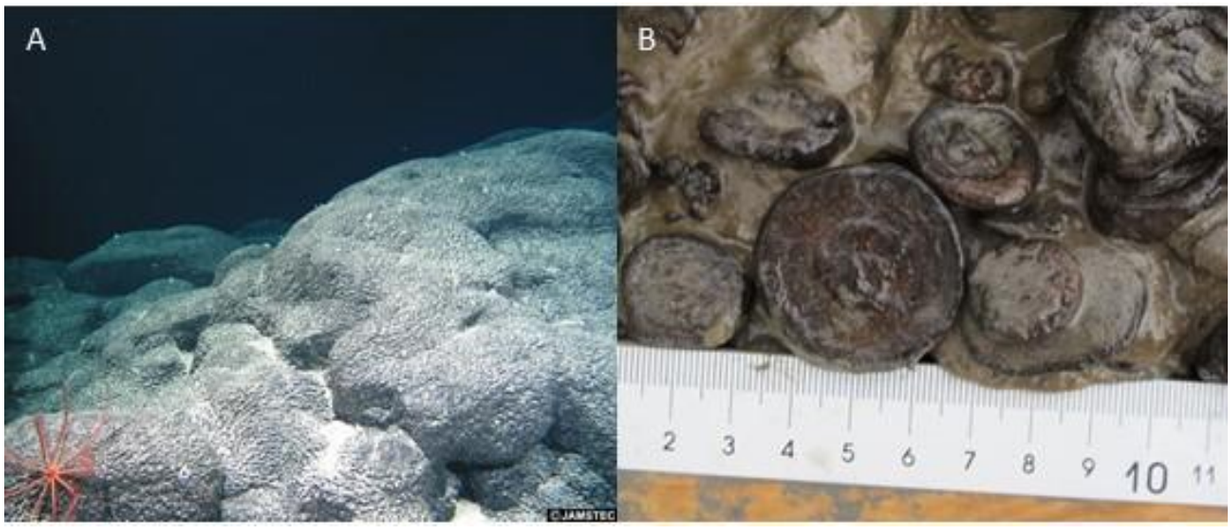
En los océanos existen tres tipos principales de depósitos de manganeso, que se describirán a continuación:

- **Nódulos polimetálicos:** Antes se les conocía como nódulos de manganeso por la abundancia relativa de este elemento en su composición (Olivares-Cruz et al., 2014). Se trata de concreciones rocosas que se forman a profundidades de más de 4,000 m, generalmente en cuencas oceánicas profundas y alejadas de los continentes en donde prevalezcan bajas velocidades de sedimentación. Los nódulos normalmente crecen a partir de un núcleo, tal como un fragmento de roca volcánica, un diente de tiburón o incluso un nódulo preexistente, alrededor del cual se acumulan capas concéntricas de óxidos de hierro y manganeso (Figura 4.3). Se ha estimado que crecen a razón de 0.8 mm cada millón de años (Glasby, 2006).

- **Costras de manganeso:** Se acumulan en los montes submarinos y mesetas ubicados a profundidades mayores a 1,000 m, donde las corrientes evitan la acumulación de sedimentos (Glasby, 2006) (Figura 4.4A).

- **Concreciones de ferromanganeso:** Estos depósitos se forman en ambientes oceánicos de poca profundidad (como los mares Báltico y Negro), en lagos de zonas templadas, en ríos y en suelos (Gasparatos, 2012). Crecen mucho más rápido que los nódulos polimetálicos, de los que se distinguen en forma, mineralogía y composición (Glasby, 2006) (Figura 1.4B).

Figura 4.4 A) Costras de manganeso en el Monte Takuyo Daigo, en el Océano Pacífico;
B) Concreciones de ferromanganeso tomadas del Mar Báltico



Fuente: A) Imagen de la Japan Agency for Marine-Earth Science and Technology, tomada en 2009, http://www.jamstec.go.jp/gallery/e/geology/resource/004.html;
B) Imagen de Joonas Virtasalo, proyecto Fermaid, https://twitter.com/jouko_nieminen/status/1275330366715375616.

Según el modo de formación, los depósitos de manganeso también pueden clasificarse como sigue (Glasby, 2006):

- **Depósitos hidrogénicos.** Esta vía ocurre de modo muy lento (alrededor de 2 mm cada millón de años) en ambientes oxidantes. Si se trata de nódulos de manganeso, estos se forman en arcillas rojas, mientras que las costras se depositan sobre sustratos rocosos. Estos depósitos tienen una composición Mn/Fe cercana a 1 debido a que el agua de las profundidades oceánicas tiene una relación alta entre la composición Mn/Fe.

- **Depósitos diagenéticos.** Este tipo de depósitos se forman por procesos diagenéticos producidos por la circulación de fluidos, algunos procesos físico químicos, o fuentes de energía requeridas que ocurren dentro de los sedimentos subyacentes, los cuales provocan el suministro ascendente de elementos a lo largo de la estructura de tales sedimentos. Crecen a velocidad alta (entre 10-100 mm cada millón de años).

- **Depósitos hidrotermales.** Estos precipitan directamente desde las fuentes hidrotermales, donde prevalecen altas temperaturas debido a un alto flujo de calor; lo anterior es característico de volcanes submarinos o dorsales oceánicas. Por esta vía hay una formación a alta velocidad (superior a los 1,000 mm cada millón de años) de depósitos con un bajo contenido de oligoelementos.

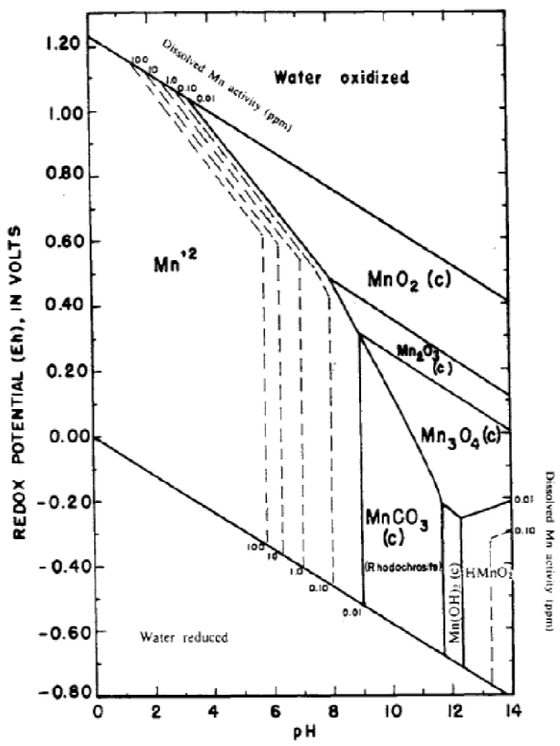
4.2.2 Hidrosfera

Se ha reportado una concentración promedio en agua dulce de 8 µg Mn/kg, mientras que en zonas anóxicas de lagos (i.e., el hipolimnion de lagos estratificados) pueden encontrarse concentraciones superiores a 1 mg/kg. En agua de mar, la concentración promedio que se ha reportado es de 0.2 µg/kg (Bowen, 1979).

Las principales fuentes naturales de Mn a la hidrosfera son geogénicas, i.e. la disolución de minerales ricos en este metal, como los citados en el apartado anterior, bajo el efecto de concentraciones adecuadas de CO_2 y de gradientes redox (Rodríguez-Díaz et al., 2005). Más específicamente, la migración del manganeso desde la corteza terrestre hasta sistemas acuáticos depende de factores como el pH y el oxígeno disuelto, que en ciertos niveles (i.e., 4.7-5.5 y 0 mg/L, respectivamente) facilitan la liberación de Mn^{2+} (Brezonik, 2003). En el medio marino, los iones Mn^{2+} liberados por la disolución de minerales se añaden a los aportados por los sistemas acuosos hidrotermales; de hecho, cerca del 90% del Mn que ingresa a los océanos tiene un origen hidrotermal (Glasby, 2006). El Mn^{2+} es la especie disuelta más común en sistemas acuáticos (en agua marina también es relevante la especie $MnCl^{+}$), y que permite su migración desde ambientes menos a más oxidantes. De hecho, solo el Mn^{2+} puede estar como ion libre en solución acuosa. En este estado de oxidación también puede estar soluble como complejo orgánico o inorgánico (Glasby, 2006).

La Figura 4.5 presenta un diagrama de Pourbaix para un sistema con características semejantes a las de numerosos tipos de aguas naturales, puesto que considera 100 mg/L de alcalinidad, que para un rango de pH de 6.4 – 10.3 se encuentra principalmente en forma de HCO_3^- (Hem, 1963). Las fronteras representadas en esta figura representan que la baja solubilidad del $MnCO_3$ condiciona la movilidad del manganeso en agua natural y a pH usuales en ésta (6 – 9). La Figura 1.5 también indica que el manganeso debe existir predominantemente como Mn^{2+} a un pH de 5.5 y como Mn(IV) a pH superiores a 6, si el potencial de óxido reducción (Eh) es de 0.80 V y con cualquier actividad comprendida entre 0.1 y 100 ppm de Mn. A un Eh inferior a 0.50 V y una actividad de 10 mg Mn/L, el Mn(IV) puede predominar a valores de pH superiores a 8 (Hem, 1963). La especie que predomina en el intervalo de pH de 0 hasta 8, a condición de contar con valores de Eh reducidos, es el Mn^{2+} como ion libre en el agua. Esto se cumple en aguas subterráneas o en las profundidades marinas, donde el potencial redox es bajo debido a la escasa concentración de O_2 disuelto.

Figura 4.5 Diagrama de Pourbaix para el Mn en solución. La actividad del Mn disuelto total varía entre 0.01 y 100 ppm, y la del ion HCO_3^- es 100 ppm. El ion SO_4^{2-} está ausente



Fuente: Hem (1963)

Una explicación para esta resistencia de los iones de Mn^{2+} a la oxidación es la alta energía de activación requerida para la reacción. Otra explicación es que es el Mn^{2+} puede estar extensivamente complejado y de este modo estabilizado por iones inorgánicos como Cl^-, SO_4^{2-} y HCO_3^- (Hem, 1963) o por compuestos orgánicos tales como aminoácidos, ácidos húmicos y otros (Graham, 1959). En cuanto a los óxidos de Mn (IV), estos son insolubles en agua. Tienen propiedades anfotéricas que afectan su afinidad por varios cationes, especialmente por metales pesados como Co^{+2}, Ni^{+2} y Cu^{+2}. Asimismo, son conocidos como secuestrantes de cationes metálicos. Por ejemplo, la vernadita (δMnO_2) tiene un área superficial de alrededor 260 $m^2 \cdot g^{-1}$, que le confiere una elevada capacidad para adsorber Ni^{2+}, Cu^{2+} y Zn^{2+} de aguas naturales (Glasby, 2006).

En general, se asume que el Mn (III) puede estar en solución acuosa solo si está complejado, puesto que el ion libre Mn^{3+} tiende a dismutar entre los estados de oxidación +2 y +4 (Ecuación 1; Ehrlich, 2001).

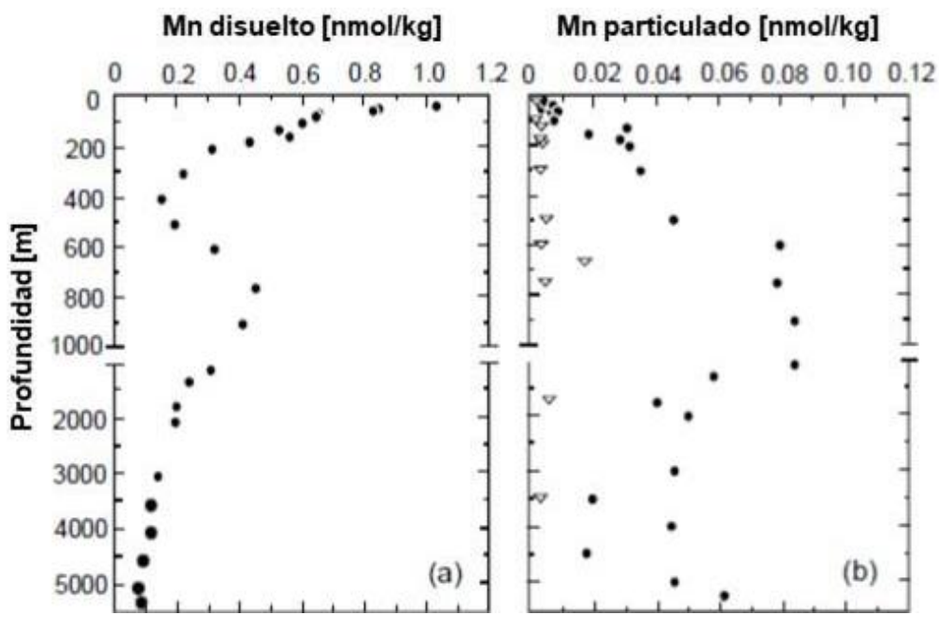
$$2Mn^{3+} + 2H_2O \rightarrow Mn^{2+} + MnO_2 + 4H^+ \quad (1)$$

Algunas posibles fuentes de Mn(III) son: la descomposición del fitoplancton que, como se explicará en el apartado 2.3.1, contiene este metal en el centro generador de oxígeno; su formación como intermediario en la oxidación Mn(II) ➔ Mn(IV) y, finalmente, la reducción de MnOx con iones S^{2-} (Trouwborst et al., 2006). Una vez formado, el Mn(III) suele estabilizarse por complejación con ligandos aniónicos, en particular con átomos donadores de oxígeno como los pirofosfatos (provenientes de la descomposición de biomoléculas energéticas tales como el ATP o el ADP). El Mn(III) estabilizado puede actuar como oxidante y como reductor, y se le ha encontrado abundantemente en medios subóxicos. Por ejemplo, en el Mar Negro, se encontró que la totalidad del Mn presente (5 mM) estaba como Mn(III) soluble, y que este se formaba principalmente en dos alturas de la zona subóxica por sendos mecanismos distintos: en la parte superior por oxidación de Mn(II), y en la parte inferior por reducción de $Mn^{IV}O_2$ y subsecuente estabilización por ligandos naturales (Trouwborst et al., 2006).

4.2.2.1 El manganeso en agua marina

La concentración de manganeso en el mar abierto se encuentra en un rango que va desde 0.1 μg/L en los niveles superiores hasta 0.02 μg/L en aguas profundas, lo que se ha atribuido a aportes de este metal en forma de polvo desde la atmósfera (Schlesinger y Bernhardt, 2020). Su reparto entre especies disueltas y particuladas también depende de la profundidad de la columna de agua (Figura 1.6). De este modo, mientras aproximadamente el 99% del manganeso presente en capas oceánicas superficiales se encuentra disuelto, en aguas profundas esta proporción disminuye a 80% (Glasby, 2006). El resto del manganeso presente en mares se encuentra en su forma precipitada en los fondos marinos.

Figura 4.6 Distribución vertical de a) Mn disuelto y b) Mn particulado en agua marina



Fuente: Glasby (2006)

4.2.2.2 El manganeso en aguas subterráneas

La presencia de Mn en las aguas subterráneas está controlada por factores tales como la geoquímica de las rocas, la composición del agua y la actividad microbiana. La meteorización de las rocas portadoras de Mn puede dar como resultado concentraciones elevadas de este metal en suelos y sedimentos. En el acuífero, el agua subterránea disuelve estos materiales, que liberan Mn al agua. Las características del agua subterránea, como el pH, el potencial redox, el oxígeno disuelto y la materia orgánica, controlan la concentración y la especiación de Mn. La especie más probable es Mn^{2+} a pH inferior a 7 y con un potencial redox de hasta 800 mV (Homoncik et al., 2010). Finalmente, la actividad microbiana tiene un papel importante, ya que intensifica la oxidación o la reducción de Mn. Como todos los mecanismos antes mencionados actúan de manera similar en Mn y Fe, estos metales suelen encontrarse simultáneamente en aguas subterráneas, y con frecuencia el Fe en niveles más altos que el Mn (Homoncik et al., 2010). Las altas concentraciones de estos metales afectan los posibles usos del agua subterránea, ya que en contacto con la atmósfera se oxida y ocasiona cambios organolépticos (color, sabor y olor); además, en el agua de uso municipal tiñe prendas de vestir y causa incrustaciones en tuberías (WHO, 2011).

En un estudio acerca de la presencia de Fe, Mn y otros metales traza en agua subterránea del sistema de acuíferos del norte de los E.U.A., el Mn fue el cuarto elemento más frecuente, detrás del Ba, Sr y Li, en un total de 1590 muestras. En ellas se encontró un rango de concentraciones de Mn que iban de menos de 0.001 a 28 mg/L (Tobiason, 2016). Para las aguas subterráneas de la cuenca de México, las concentraciones de Mn variaron entre 0.0003 y 0.960 mg/L (Domínguez-Mariani et al., 2015), lo que indica que el límite para agua potable establecido por la normatividad mexicana (0.15 mg/L) se supera con frecuencia. Igual sucede en otros acuíferos mexicanos: en los pozos individuales del sitio Peñón-Texcoco se midió una concentración máxima de 4.61 mg/L, aunque la mezcla total del agua de 15 pozos arrojó una concentración media de 1.52 mg/L (Piña-Soberanis et al., 2003), mientras que en el sitio de Santa Cruz, Hgo., se determinaron concentraciones comprendidas entre 3.72 y 5.76 mg/L (Rivera-Rodríguez et al., 2019). También se supera la normatividad para agua potable en los pozos que abastecen a las ciudades de Guaymas (0.1 – 1.50 mg/L) y Navojoa (1.30 mg/L), ambas en Sonora, y a Veracruz, Ver. (0.39 – 0.54 mg/L; Piña-Soberanis et al., 2003).

4.2.2.3 El Mn en aguas superficiales

Como se expuso antes, las aguas subterráneas pueden presentar altas concentraciones de Mn (II); en cambio, en aguas superficiales esto no es común (de Joode et al., 2016). Puede, no obstante, ser abundante en el hipolimnion de lagos estratificados[1] (Granger et al., 2014). Durante la estratificación, se generan diferencias en la concentración de Mn(II) entre el epilimnion y el hipolimnion: en el primer estrato, con mayor temperatura y un pH más alcalino por el crecimiento de algas, se favorece la oxidación bacteriana del Mn(II) a Mn(IV) (ver sección 2.3.2), que precipita luego en el hipolimnion; en éste, por el contrario, prevalecen temperaturas más frías y pH ácidos con bajo potencial redox que conducen a la presencia de Mn(II) (Bertone et al., 2016). De ahí que Balistrieri et al. (1992) hayan encontrado concentraciones de Mn disuelto hasta de 2.6 mg/L a más de 25 m de profundidad del Lago Sammamish, en Washington, al inicio de la temporada otoñal. Igualmente, durante el verano, en el Lago Bennery (Canadá) la estratificación termal conduce a la formación de Mn(II) disuelto en concentraciones superiores a 1 mg/L, de tal suerte que una planta potabilizadora que recibe el agua desde una tubería localizada en el fondo del lago debe implementar procesos de oxidación adicionales para remover el metal (Granger et al., 2014).

[1] La estratificación termal es un fenómeno estacional o permanente por el cual los embalses acuáticos se separan en tres capas distintas y estables frente a la mezcla vertical del agua. El fenómeno se desencadena por la luz solar que recibe la superficie del embalse, y que causa variaciones en la densidad del agua. Esto forma una capa de agua cálida en la superficie, llamada epilimnion, mientras que se forma una capa de agua más densa y fría en el fondo del embalse, conocida como hipolimnion. Entre las dos se sitúa una interfase denominada termoclina. En un lago estratificado, además, se presentan gradientes en la concentración de oxígeno disuelto debidos, por una parte, a que en el epilimnion existe una mayor difusión de aire atmosférico, y, por la otra, a que en este estrato ocurre un mayor crecimiento de algas oxigénicas. Así, el hipolimnion se caracteriza por menores concentraciones de oxígeno disuelto.

4.2.3 Biosfera

4.2.3.1 El manganeso, la fotosíntesis y el oxígeno

El mayor efecto que el conjunto de los seres vivos, *i.e*. la biosfera, ha tenido en el planeta es la aparición y posterior acumulación de O_2 en la atmósfera, evento conocido como la Gran Oxigenación y que inició hace alrededor de 2.35 millones de años (Knoll, 2003). Los ancestros de las cianobacterias modernas adquirieron la capacidad de retirar electrones del agua, que fue luego transferida por endosimbiosis a algas y plantas. Gracias a esta nueva capacidad biológica, que produce O_2 como residuo, la atmósfera se fue enriqueciendo en este elemento, lo que a su vez posibilitó la aparición del metabolismo que más genera energía, es decir, el aerobio. En esta cadena de acontecimientos, quizás los más significativos de la historia de nuestro planeta solo después de la aparición de la vida en sí, el Mn tuvo y sigue teniendo un rol central (Lingappa et al., 2019).

La fotosíntesis oxigénica moderna se basa en el complejo generador de oxígeno, que es el sitio activo del fotosistema II y está constituido por un clúster de Mn_4CaO_5 (Figura 4.7). Este complejo, cuya estructura fue apenas dilucidada con precisión por Umena et al. en 2011, hace posible que los cuatro electrones que se extraen de una molécula de agua y producen O_2 sean aceptados por los átomos de Mn, que cambian cíclicamente de estado de oxidación.

Figura 4.7. Modelo del clúster de Mn en el complejo generador de oxígeno.
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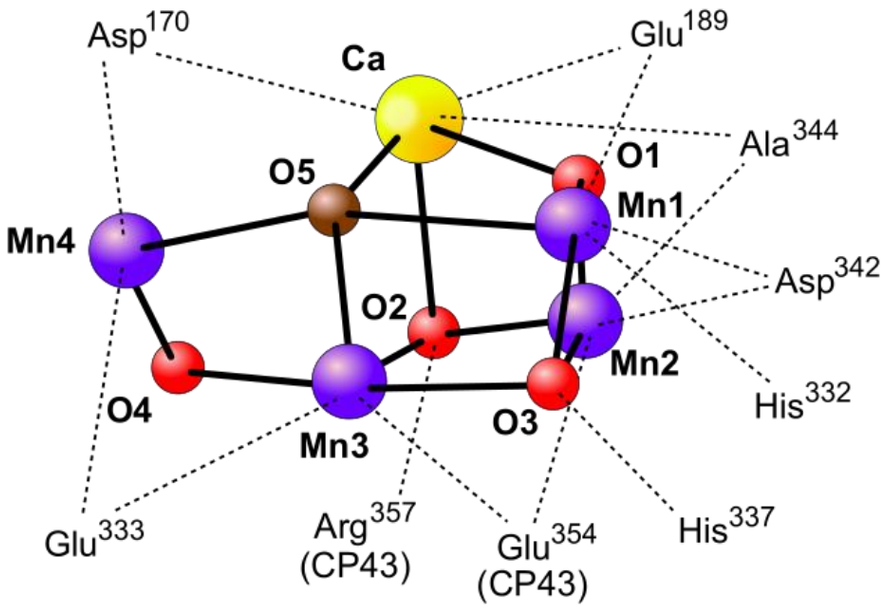


Fuente de consulta: https://steemit.com/science/@ritch/artificial-photosynthesis-part-1-understanding-the-structure-of-the-oxygen-evolving-complex

Según evidencia cada vez mayor, el manganeso fue crucial en el surgimiento de una versión primitiva del fotosistema II, que no utilizaba agua como donador de electrones sino Mn^{2+} y tampoco producía O_2. Este innovador sistema, semejante a la fotosíntesis anoxigénica que existe aún hoy, habría podido surgir gracias a las grandes cantidades de Mn^{2+} que, previamente a la Gran Oxigenación, se encontraban disueltas en los océanos (estimadas en 120 μM), y luego habría evolucionado hacia el complejo generador de oxígeno actual (Lingappa et al., 2019).

La oxigenación de la Tierra trajo consigo los devastadores efectos del oxígeno y de sus especies reactivas (i.e., $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$, $^1O_2$) en los seres vivos, conocidos como estrés oxidativo. Los organismos que primero debieron lidiar con este problema aprovecharon las propiedades antioxidantes de moléculas intracelulares con las que ya contaban, lo que también involucra al manganeso. Numerosos complejos orgánicos e inorgánicos de Mn^{2+} reaccionan con especies reactivas del oxígeno, inactivándolas; por ejemplo, los complejos formados intracelularmente a partir de ligandos tales como los iones lactato o fosfato eliminan especies reactivas del oxígeno en *Lactobacillus plantarum*; esta bacteria anaerobia facultativa llega a acumular concentraciones de Mn(II) de 30-35 mM (Horsburgh et al., 2002).

Sin embargo, los organismos aerobios y las plantas basan su defensa contra las especies reactivas del oxígeno en metaloenzimas como las catalasas, peroxidasas y superóxido dismutasas. Una enzima de este último grupo, la manganeso superóxido dismutasa, está presente en las mitocondrias de todos los organismos aerobios, desde las bacterias hasta los humanos. Esta enzima intercepta el anión superóxido (i.e., $O_2^{\bullet-}$), y gracias a la capacidad redox del cofactor Mn^{3+}, lo dismuta en su sitio activo por el mecanismo representado en las Ecuaciones 2-3 (Serrato-Ruge, 2013):

$$O_2 \cdot^- + Mn^{3+} \rightarrow O_2 + Mn^{2+} \quad (2)$$

$$2H^+ + O_2 \cdot^- + Mn^{2+} \rightarrow H_2O_2 + Mn^{3+} \quad (3)$$

Así, el manganeso sería también un actor central en el desarrollo de estrategias contra el estrés oxidativo. Por ejemplo, *Deinococcus radiodurans* es una bacteria extremadamente resistente a la radiación ionizante, la cual daña biomoléculas y produce una gran cantidad de especies reactivas del oxígeno que intensifican este daño. *D. radiodurans* puede recuperarse de dosis de radiación gamma que son letales para la mayoría de los seres vivos, y lo hace mediante la acumulación intracelular de iones de manganeso en detrimento de la concentración de iones de hierro. De manera opuesta, los microorganismos más sensibles a la radiactividad tienen mayores concentraciones de hierro que de manganeso (Sun et al., 2010).

Otro ejemplo sorprendente del papel del manganeso en la fisiología bacteriana lo ofrece *Borrelia burgdorferi*, que causa la enfermedad de Lyme. El sistema inmune humano combate la infección restringiendo el hierro que esta bacteria necesita para fabricar sus propias metaloenzimas; sin embargo, se ha descubierto que parte de la infecciosidad de *D. burgdorferi* se debe a que no requiere hierro y usa manganeso en su lugar (Emsley, 2011). Otros agentes patógenos tales como *Treponema pallidum*, que causa la sífilis, podrían haber adoptado estrategias similares para contrarrestar la restricción de hierro (Horsburgh et al., 2002).

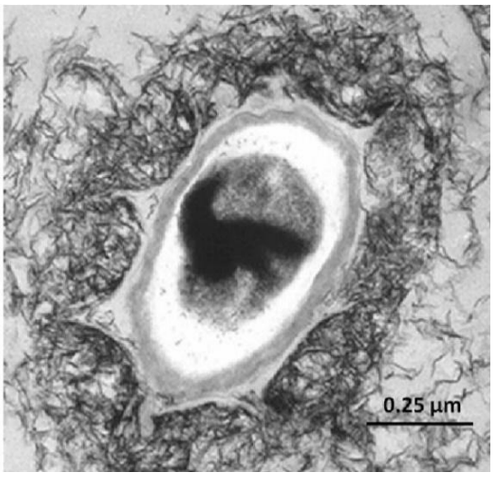
Por último, otra metaloenzima de origen fúngico, la manganeso peroxidasa, es responsable de oxidar el Mn(II) proveniente de la descomposición de la materia vegetal a Mn(III). Luego de ser estabilizado por ligandos orgánicos (i.e., iones oxalato), el Mn(III) se convierte en un oxidante que puede transformar a la lignina en compuestos aromáticos de bajo peso molecular (Lingappa et al., 2019). Dado que se ha encontrado una fuerte correlación entre el contenido de Mn y la descomposición de detritus vegetales en ecosistemas forestales boreales, templados y semiáridos, las metaloenzimas de manganeso tendrían también un papel importante en el balance terrestre del carbono (Keiluweit et al., 2015).

4.2.3.2 Transformaciones redox microbianas

Como se indicó en el apartado 4.2.2, la oxidación abiótica del Mn(II) a Mn(IV) por parte del O_2 está limitada por una alta energía de activación, por lo que, a pH 8 y 25°C, esta oxidación en un medio homogéneo es 10 millones de veces más lenta que la del Fe(II) (Morgan, 2000). De lo anterior se deduce que, aunque aún está por dilucidarse el papel catalítico de las nanopartículas de MnOx (Lingappa et al., 2019), esta reacción ocurre principalmente por vía microbiana. Así, la mayoría de los MnOx de origen natural serían resultado de la oxidación biológica de Mn(II) o de su alteración subsecuente (Tebo et al., 2007). De hecho, se ha mostrado que las tasas de oxidación de Mn(II) se incrementan de 3 a 5 órdenes de magnitud en presencia de bacterias u hongos oxidantes de Mn(II) (Soldatova et al., 2012).

Existen numerosos géneros bacterianos y fúngicos que oxidan Mn(II), aunque no se ha identificado a ningún miembro del linaje *Archaea* que tenga esta capacidad. Las bacterias que oxidan al manganeso son ubicuas en el ambiente y pertenecen a *phyla* variados: Firmicutes, Actinobacteria, Bacteroidetes y Proteobacteria (Piazza et al., 2019). En las bacterias se han identificado dos familias de enzimas, ambas exocelulares, implicadas: las oxidasas multicobre y las peroxidasa ciclooxigenasas. Como se muestra en la Figura 1.8, esto conlleva una acumulación del MnOx en el exterior de las células. En la oxidación directa del Mn(II) catalizada por estos microorganismos no hay conservación de energía. Dado que la formación de MnOx no está asociada al crecimiento, su papel fisiológico queda aún por establecer, aunque se ha supuesto que aporta protección a las bacterias contra metales tóxicos, radiación UV, virus o depredación (Soldatova et al., 2012).

Figura 4.8. Fotografía obtenida por TEM de esporas de *Bacillus* sp. SG-1 cubiertas por MnO_2



Fuente: Soldatova et al. (2012)

Desde el punto de vista termodinámico, se había establecido la posibilidad de que existiera un metabolismo quimiolitotrófico basado únicamente en la oxidación del manganeso (Ecuación 4), lo cual no había sido demostrado.

$$Mn^{2+} + \frac{1}{2}O_2 + H_2O \rightarrow Mn^{IV}O_{2(s)} + 2H^+;\ \Delta G^{\circ\prime} = -68 \text{ kJ/mol Mn} \quad (4)$$

Recientemente, se encontró un consorcio microbiano autótrofo y aerobio que acopla la oxidación exocelular de Mn(II) a su crecimiento, tras lo cual produce un MnOx cercano a la birnesita (Yu y Leadbetter, 2020). Los autores del estudio asignaron el nombre de 'Candidatus Manganitrophus noduliformans' a la especie mayoritaria del consorcio, y designaron a la especie minoritaria como *Ramlibacter lithotrophicus*. La primera especie está afiliada al *phylum* Nitrospirae, cuyos miembros son capaces de aprovechar exiguas diferencias de potenciales redox entre donadores y aceptores de electrones inorgánicos; en cuanto a la segunda, es una betaproteobacteria que por sí sola no oxida al Mn(II).

Por otra parte, la reducción del manganeso es un metabolismo bien conocido que conserva energía; se trata de una forma de respiración anóxica en la que el Mn(IV) funge como aceptor externo de electrones, que por lo general ocurre en ambientes con abundantes $Mn^{IV}Ox$. Esta forma de respiración, acoplada a la oxidación de materia orgánica, es llevada a cabo por microorganismos que no la realizan de modo exclusivo, es decir, que pueden emplear otros aceptores de electrones, como el oxígeno molecular, los iones nitrato o compuestos azufrados. Entre los microorganismos más estudiados que respiran $Mn^{IV}Ox$ se encuentran *Shewanella oneidensis* y *Geobacter sulfatorreducens* (Lingappa et al., 2019).

4.2.3.3 Importancia del manganeso como micronutriente

El Mn es un elemento esencial: las células de todos los seres vivos lo contienen en pequeñas cantidades (del orden de los µg/g; Emsley, 2011), razón por la cual se le considera un micronutriente. Sin embargo, una deficiencia en este metal, en organismos que van desde bacterias hasta plantas y animales, inhibe el crecimiento y disminuye la expectativa de vida (Kemmitt, 1975). Las concentraciones intracelulares de Mn típicamente son del orden de 10^{-7}M, aunque pueden alcanzar 10^{-3}M (como en el ejemplo de *L. plantarum* citado arriba). El manganeso intracelular puede encontrarse unido, por una parte, a moléculas orgánicas pequeñas, polifosfatos o carbonatos o, por otra parte, a proteínas tales como las metaloenzimas ya mencionadas. En total se han identificado 125 manganoenzimas; algunas de ellas requieren absolutamente el Mn, mientras que otras permiten su sustitución por otros iones divalentes (Lingappa et al., 2019).

4.2.3.4 Toxicología del manganeso

En adultos, el manganeso se considera no tóxico excepto hacia el cerebro, donde se concentra fácilmente, especialmente en los ganglios basales, y causa síntomas parecidos a los de la enfermedad de Parkinson cuando se le inhala durante períodos prolongados e incluso en cantidades moderadas (Nádaská et al., 2010). Esto puede ocurrir sobre todo a través de la exposición ocupacional, en trabajadores de la minería y de la industria del acero, y en agricultores (ver sección 2.4). Las dosis relativamente altas de manganeso afectan la replicación del ADN y causan mutaciones en células microbianas y de mamíferos; en estos, las cantidades excesivas de manganeso afectan la fertilidad y son tóxicos tanto para embriones como para fetos (Gerber et al., 2002). En niños, la acumulación de manganeso en cabello y sangre se ha asociado con déficits cognitivos y de comportamiento, inteligencia verbal disminuida, comportamiento hiperactivo y dificultades de aprendizaje (de Joode et al., 2016; Gunier et al., 2014).

4.2.4 Antroposfera

Los MnOx se han usado desde tiempos prehistóricos, y una de sus más antiguas aplicaciones podría ser la intensificación de la combustión. Los gases derivados de la pirólisis de la madera pueden ser oxidados por el MnO_2, que al reducirse libera O_2 y disminuye la temperatura de ignición. Las evidencias arqueológicas de la presencia de MnO_2 en hogueras de asentamientos neandertales sugiere que estos homínidos lo usaron para facilitar la combustión, lo cual seguramente fue de vital importancia dadas las bajas temperaturas que imperaban durante la última glaciación (Heyes et al., 2016). Asimismo, los creadores del arte paleolítico, como el que se encuentra en las cuevas de Lascaux (Francia) o Ekain (España), emplearon los MnOx como pigmentos hace alrededor de 30,000 años (Chalmin et al., 2006).

Mediante la adición de pirolusita (MnO_2), los egipcios y romanos removían del vidrio el tono verde causado por el Fe^{2+} aportado por la arena de origen (Post, 1999). Durante la Edad media europea, varios MnOx se comercializaban como el “jabón de los vidrieros”, en particular entre los fabricantes de Venecia. Fue a partir de la pirolusita que Johan Gottlieb Hahn aisló por primera vez al metal puro en 1774 (Emsley, 2011; McCray, 1998).

A mediados del siglo XIX, el Mn ya se usaba como endurecedor en la fabricación del acero y de otras aleaciones (Post, 1999). En promedio, el acero contiene 0.6% en peso de Mn, aunque en el acero de alta dureza, como el que se emplea en vías férreas, cajas fuertes o las rejas de prisiones, puede representar hasta 13% (Emsley, 2011). A la fabricación de acero, para la cual el Mn no tiene sustituto, se destina más del 80% de la extracción mundial de los minerales de este elemento, que en 2020 alcanzó 18,500 toneladas y se localizó principalmente en Sudáfrica, Australia y Gabón (USGS, 2021). También se añade 1.5% de Mn al aluminio con el que se fabrican latas de bebidas para prevenir su corrosión (Emsley, 2011).

Varios MnOx naturales y sintéticos tienen aplicaciones en la fabricación de baterías. La nsutita es un óxido natural que se usa en las pilas secas de zinc carbono, mientras que para la fabricación de pilas alcalinas se usa un MnOx sintético (Post, 1999). Otra aplicación industrial del Mn es como catalizador, y es un fertilizante común, sobre todo en forma de $MnSO_4$, para paliar las deficiencias de Mn en algunos suelos que luego afectan a los animales que pastan en ellos (Emsley, 2011). Asimismo, un compuesto orgánico de este elemento, el MMT (por las siglas en inglés de metilciclopentadienil tricarbonil de manganeso) se añade como antidetonante de gasolina en algunos países, aunque su uso es controversial y está en descenso (Hoekman y Leland, 2018; OMS, 2011).

Los fungicidas Maneb y Mancozeb son fungicidas conocidos como etilenbisditiocarbamatos que contienen hasta 21% de Mn y que son muy usados en viñedos, silvicultura, huertos y cultivos variados, así como en el tratamiento de semillas. Se aplican en grandes cantidades a ciertos cultivos: por ejemplo, en Salinas Valley, California, se aplican 150,000 kg anuales de estos fungicidas, en su mayoría a cultivos de lechugas (Gunier et al., 2014), mientras que solo en 10,500 has productoras de plátano de la sierra de Tabasco, México, se rocían más de 1,000 toneladas de mancozeb al año (Domínguez-Rodríguez et al., 2015). Por lo tanto, se considera a estos pesticidas como fuentes importantes de este metal en el medio agrícola (de Joode et al., 2016).

Un área que reviste gran interés en la investigación actual se enfoca en el desarrollo de la fotosíntesis artificial, en el que es crucial contar con un catalizador eficiente que oxide el agua y produzca H_2 como fuente de energía limpia. El clúster de Mn en el complejo generador de oxígeno (Figura 1.7) ha servido como modelo para estos nuevos catalizadores, muchos de los cuales son nanodepósitos de MnOx. Entre estos se encuentran MnOx coloidales, clústeres de MnOx nanoestructurados soportados en sílice mesoporosa y láminas de óxidos de calcio y manganeso nanoestructuradas (Najafpour et al., 2012).

Otras novedosas aplicaciones de este metal se relacionan con el campo médico. El mangafodipir (dipiridoxildifosfato de manganeso) se ha propuesto como un agente de contraste en resonancia magnética nuclear que podría sustituir a los complejos de gadolinio en la visualización de ciertos tejidos. Para este compuesto y un análogo cálcico, el calmangafodipir, también se han encontrado resultados prometedores en el tratamiento del cáncer (Karlsson et al., 2015). Por último, recientemente se encontró que un nanodepósito de Mn capaz de proveer de manera regulada iones Mn^{2+} aumenta la respuesta de interferón de tipo I en el tratamiento del SARS-CoV-2. Al aumentar la inmunidad antiviral y disminuir el daño celular producido por el coronavirus, este nanodepósito podría constituir un coadyuvante de la vacunación (Sun et al., 2021).

4.2.5 Atmósfera

El Mn puede encontrarse en la atmósfera proveniente de fuentes naturales, tales como el intemperismo de las rocas de la corteza terrestre, los aerosoles marinos, los incendios forestales y la actividad volcánica (Howe et al., 2004). Entre las fuentes antropogénicas destacan las emisiones de la industria siderúrgica, que como se señaló arriba es la principal consumidora de Mn. Las emisiones de esta industria son la principal fuente de Mn en material particulado urbano. Otras fuentes antropogénicas importantes son las plantas generadoras de energía a base de carbón y la quema de combustibles fósiles, en particular donde se haya adicionado MMT a la gasolina. En el medio rural, la aplicación de los fungicidas maneb o mancozeb puede originar la acumulación de Mn en el polvo que se encuentra dentro de los hogares cercanos a los campos de cultivo (Gunier et al., 2014).

Las fuentes ya mencionadas, así como el procesamiento de los minerales de Mn, liberan este metal a la atmósfera en forma de partículas, cuyo alcance depende tanto de su tamaño como de la velocidad y la dirección del viento. Las concentraciones de Mn en el aire de regiones rurales (en promedio, 40 ng/m^3) son inferiores a las urbanas (65 – 166 ng/m^3), que a su vez son inferiores a las concentraciones en áreas con una fuerte influencia de una fuente específica (tal como una fundición), las cuales pueden alcanzar los 8000 ng/m^3 (Howe et al., 2004).

4.3 Movilidad del manganeso en el ambiente

Como se indicó en la sección anterior, el mecanismo principal por el cual el manganeso se mueve de un reservorio ambiental a otro es mediante cambios en su estado de oxidación (en particular +2 y +4), que para este elemento implican también cambios de fase. En esta sección se abordará otra vía de movilidad destacada, la sorción, que ocurre de modo importante en suelos, aunque cabe señalar que puede ocurrir en otros medios, como en los sedimentos y en columnas acuosas.

De modo general, se denomina sorción al paso de metales como el manganeso de una solución acuosa a una fase sólida contigua. Este término agrupa tres procesos distintos, que se detallarán a continuación (Bradl, 2004).

1. **Adsorción.** Se refiere a la acumulación bidimensional del metal en la interfaz sólido-agua; este mecanismo implica diferentes interacciones:

- Reacciones de complejación superficial. Estas reacciones conducen a la complejación del metal en su esfera interna de coordinación con los grupos funcionales de la superficie de adsorción a modo de ligandos.

- Interacciones electrostáticas. El metal se compleja en su esfera externa de coordinación con grupos funcionales de la superficie de adsorción.

- Expulsión hidrófoba de complejos metálicos que contengan grupos funcionales orgánicos altamente no polares.

- Adsorción de metales complejados con un polielectrolito a un tensioactivo.

Es posible también describir este proceso de acumulación de manganeso a los suelos desde una fase acuosa por medio de dos mecanismos básicos (Bradl, 2004):

- ***Adsorción específica***. Se refiere al mecanismo mediante el cual ocurren reacciones selectivas y poco reversibles, que involucran la quimisorción de complejos en la esfera interna de coordinación. Esta interacción permite una unión fuerte e irreversible entre iones de metales pesados y materia orgánica e incluso minerales de carga variable. Es posible describir este fenómeno como una reacción entre los grupos funcionales de la superficie (i.e., silanol, hidroxilo u otros grupos orgánicos) y un ion en solución, que forma una unidad estable. Este tipo de adsorción suele basarse en reacciones entre el metal y los grupos –OH presentes en las superficies edáficas (*S*), las cuales están cargadas negativamente a pH elevado. La Ecuación 5 representa lo anterior para el caso del manganeso:

$$S - OH + Mn^{2+} + H_2O \leftrightarrow S - O - MnOH_2^+ \quad (5)$$

- ***Adsorción no específica***. Se trata de un fenómeno electrostático por el cual se intercambia el metal presente en el agua edáfica por cationes cercanos a la superficie de adsorción. Esta interacción es reversible porque se basa en el establecimiento de enlaces covalentes débiles entre el metal y las superficies del suelo cargadas.

2. **Precipitación superficial.** Se caracteriza por el crecimiento de una nueva fase sólida, la cual se repite en tres dimensiones y forma una red. En esta fase el metal se adsorbe inicialmente sobre la superficie del suelo y precipita sobre ella en forma de óxidos, carbonatos, hidróxidos, sulfuros o fosfatos. Este proceso se ha descrito mediante un modelo en dos fases: la primera se refiere a la formación de un complejo superficial entre el metal y la superficie (*S*) (Ecuación 5) y, la segunda, a la precipitación del metal sobre la superficie. Esta segunda fase se representa en la Ecuación 6 específicamente para el manganeso:

$$S - O - MnOH_2^+ + Mn^{2+} + H_2O \leftrightarrow S - O - MnOH_2^+ + Mn(OH)_{2(s)} + 2H^+ \quad (6)$$

3. **Fijación**. Este tipo de adsorción implica la difusión de un metal desde la fase acuosa a la fase sólida. Al igual que la precipitación superficial, este mecanismo tiene una naturaleza tridimensional. Los metales pesados que se adsorben específicamente en minerales arcillosos y óxidos metálicos pueden difundirse en las estructuras reticulares de esos mismos minerales. Los metales quedan entonces fijados dentro de los poros de la estructura mineral, por lo que puede asimilarse al proceso denominado *absorción*.

De modo general, los mecanismos que incrementan la sorción del manganeso al suelo son (Bradl, 2004):

- La oxidación de Mn(II) a MnOx o la precipitación de compuestos insolubles en suelos sometidos a humedad y secado;

- La fijación (absorción) del manganeso en la red cristalina de minerales arcillosos, así como la adsorción en sitios de intercambio.

La adsorción de manganeso es consistente con los modelos de Langmuir y de Freundlich, y es favorecida a valores altos de pH; esto se explica por una mayor hidrólisis del Mn(II) que aumenta tanto la precipitación de este elemento como la carga negativa en el complejo de intercambio. Los minerales arcillosos adsorben fuertemente al manganeso, lo que también se incrementa a pH altos. De igual forma, la adsorción se facilita en medios edáficos con alta capacidad de intercambio catiónico, mayor contenido de materia orgánica y mayor presencia de óxidos de hierro amorfos; estas condiciones suelen reunirse en los horizontes superficiales del suelo (Bradl, 2004).

En suelos calcáreos, la quimisorción del manganeso sobre el $CaCO_3$ puede ser significativa; en este tipo de suelos, especialmente en aquellos con pobre conductividad hidráulica y abundante materia orgánica, las plantas pueden padecer insuficiencia de manganeso (Howe et al., 2004).

4.4 Conclusiones

En este trabajo se revisaron los mecanismos que movilizan al manganeso en el medio ambiente y lo localizan en ciertos reservorios, como los depósitos de manganeso submarinos. Se enfatizó la importancia de la química redox de este elemento, que hizo posible la fotosíntesis, la oxigenación de la atmósfera terrestre y la aparición del metabolismo aerobio. Aunque el manganeso tiene efectos nocivos en las personas sobreexpuestas, sobre todo a raíz de su presencia en pesticidas y en el MMT (aditivo de gasolina), se resaltó el enorme potencial de los compuestos basados en este elemento en aplicaciones que van desde la fotosíntesis artificial hasta el combate contra el SARS-CoV-2.

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Chapter 5 Methods of physical control of pathogenic microorganisms in hospital areas

Capítulo 5 Métodos de control físico de microorganismos patógenos en áreas hospitalarias

SÁNCHEZ-OTERO, María Guadalupe*†, PANIAGUA-MARTÍNEZ, Ingrid, TEMICHE-CHI, Diana Michel and ALEXANDER-AGUILERA, Alfonso

Universidad Veracruzana, Faculty of Bioanalysis, Iturbide s/n Esq. Carmen Serdán, Col.Centro, 91700 Veracruz, Veracruz, Mexico.

ID 1st Author: *María Guadalupe, Sánchez-Otero* / **ORC ID:** 0000-0002-7916-7158, **CVU CONACYT ID:** 49392

ID 1st Co-author: *Ingrid, Paniagua-Martínez* / **ORC ID:** 0000-0003-0955-941X, **CVU CONACYT ID:** 269088

ID 2nd Co-author: *Diana Michel, Temiche-Chi* / **ORC ID:** 0000-0001-6717-5823

ID 3rd Co-author: *Alfonso, Alexander-Aguilera* / **ORC ID:** 0000-0001-8415-6923, **CVU CONACYT ID:** 123506

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* guadsanchez@uv.mx

A. Marroquín, J. Olivares, A. Dector L. Cruz. (Coord.) CIERMMI Women in Science TXIV Biology, Chemistry and Life Sciences. Handbooks-©ECORFAN-México, Querétaro, 2021.

Abstract

Hospitals are establishments that are open 24 hours a day, 365 days a year, and are responsible for providing the necessary care to patients, there are hospitals of different levels and each one of them fulfills its mandate with different equipment and materials. Cleaning and disinfection are important issues to address, so this paper explains under reliable information, the different physical methods that have been implemented to improve the cleaning process every day and to control the levels of viable pathogen microorganisms installed on surfaces or equipment, there are also chemicals that damage health, the above is distributed in different hospital areas where there is contact between health personnel, patient, administrative workers, family members, and others. The administration of physical disinfection methods such as sterilization by dry or wet methods, radiation, filtration, electricity, have shown to be effective over the years and have been reflected in the controls carried out by Mexico's Secretary of Health or various institutions responsible for implementing cleaning protocols and that these are applied. Within a hospital, everything must be planned for good work performance and that the impact is favorable, with the information provided by this research is expected to achieve a social impact especially in health centers or hospitals, so that the problems that occur every day are decreasing.

Hospital areas, Pathogens, Physical methods of control

Resumen

Los hospitales son establecimientos que están abiertos las 24 horas del día, los 365 días del año, y son los encargados de brindar la atención necesaria a los pacientes, existen hospitales de diferentes niveles y cada uno de ellos cumple su mandato con diferentes equipos y materiales. La limpieza y desinfección es un tema importante a tratar, por lo que en este trabajo se explica bajo información fidedigna, los diferentes métodos físicos que se han implementado para mejorar el proceso de limpieza cada día y controlar los niveles de microorganismos patógenos viables instalados en las superficies o equipos, también existen productos químicos que dañan la salud, lo anterior se distribuye en las diferentes áreas hospitalarias donde hay contacto entre el personal de salud, el paciente, los trabajadores administrativos, los familiares, entre otros. La administración de métodos de desinfección física como la esterilización por métodos secos o húmedos, radiación, filtración, electricidad, han demostrado ser efectivos a lo largo de los años y se han visto reflejados en los controles realizados por la Secretaría de Salud de México o diversas instituciones encargadas de implementar los protocolos de limpieza y que estos sean aplicados. Dentro de un hospital, todo debe ser planeado para un buen desempeño laboral y que el impacto sea favorable, con la información que aporta esta investigación se espera lograr un impacto social sobre todo en los centros de salud u hospitales, para que los problemas que se presentan día a día vayan disminuyendo

Áreas hospitalarias, Patógenos, Métodos físicos de control

5.1 Introduction

There are problems within a hospital that might be caused by different factors, such as lack of economic resources, lack of infrastructure, personal and union situations, and internal attention, among others. From the above, important challenges are derived in terms of public health, according to the Diagnostic Study of the Right to Health conducted by the National Council for the Evaluation of Social Development Policy (CONEVAL), in México, physical and economic access to health services must be improved, infrastructure in health institutions must be increased, health education must be promoted, and the quality and effectiveness of medical services must be improved (CONEVAL, 2014).

Cleanliness and asepsis have also been considered among the biggest challenges to overcome, since all areas need to be perfectly innocuous, considering that the hospital environment is a source of infection due to the transitory diseases that are handled in the different health centers. Hospital is a space where there are several people involved, patients, family members, and medical staff who, under any circumstance, perform their work, usually present innocuous scenarios from which derives a source of infection depending on the emergency attended, so the intrahospital areas should remain dirty as little time as possible (López, 2013).

The topic of cleaning is complex since there is a cycle in which the personnel, the materials used (wound dressing supplies, laboratory material, among others), and the disinfection methods such as chemical, physical, among others are involved, the latter have had to improve and evolve to fulfill their purpose: which is to reduce at minimum the presence and the viable number of pathogenic microorganisms in hospital areas, (Rodriguez, 2018). Therefore, to provide information to health personnel, regarding physical disinfection, this work presents a review regarding the types of physical methods that are used in different health facilities, hospitals, clinics, and offices involved in health care.

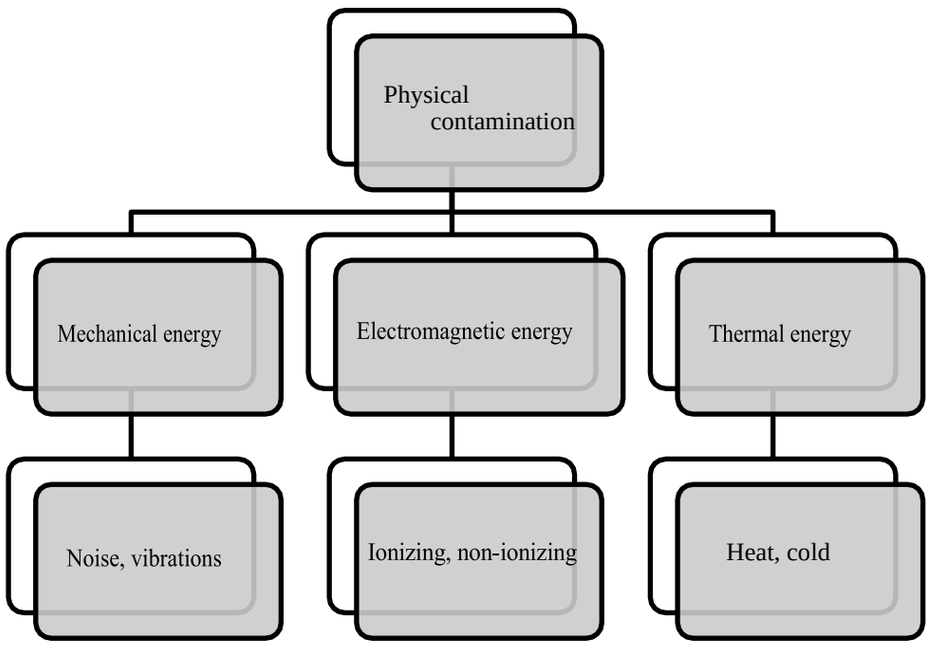
5.2 Contaminants in hospital areas

According to the World Health Organization (WHO), a hospital is a medical and social organization whose mission is to provide the population with complete medical-health care, both curative and preventive. It is also a center for medical-health personnel training and biosocial research (WHO Guide, 2002). Due to these activities, hospitals generate multiple types of waste, which derive from the care provided to patients in different areas. (Puchau, 2018).

5.2.1 Physical contaminants

Physical contaminants refer to the different forms of energy present in the work environment, by which workers can be affected, they are depicted in Figure 5.1 (Puchau, 2018).

Figure 5.1 *Most common physical contaminants*



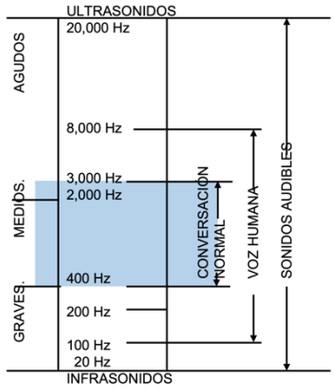
Source: Adapted from "Manual Básico de Seguridad y Salud en el trabajo "Puchau. 2018, Basic Handbook of Safety and Health at Work

5.2.1.1 Mechanical energy: Noise

It is defined as a sequence of uncoordinated sounds, unpleasant to the human ear, sound it as energy generated from an emitter, and move through space through a physical medium, to reach the receiver (passing through a transmission medium) (Puchau, 2018). The main characteristics of sound (noise) are acoustic pressure level, which refers to the intensity of the sound, it is measured in decibels (dB); and frequency, which is the number of times the sound is repeated with the same or different acoustic pressure and is measured in hertz (Hz).

Humans can only hear sounds in a certain range of frequencies, namely those between 20 and 20,000 Hz, a range known as “audible frequency spectrum”, this is depicted in Figure 5.2, normal conversations are typically between 400 and 3000 Hz (Puchau, 2018).

Figure 5.2 Audible frequency spectrum



Source: Taken from "Manual Básico de Seguridad y Salud en el trabajo" Puchau. 2018, Basic Handbook of Safety and Health at Work

A vibration can be defined as the oscillation of a particle around a point in any physical medium, if the medium is the air we get the sound, if the medium is solid, we have what is meant by the vibration ofthe material (Forteza, 2010). Vibrations are characterized by both frequency, those between 1 and 1500 Hz are of interest; and amplitude, characterized by the acceleration of the movement, measured in m/s^2. Vibrations are measured by an accelerometer, which is only sensitive to movement in one direction in space.

5.2.1.2 Radiations

There is electromagnetic energy, these are physical phenomena consisting of the emission, propagation, and absorption of energy by matter, both in the form of waves (electromagnetic radiations) and subatomic particles (corpuscular radiations) (Puchau, 2018). Radiations are divided into non-ionizing and ionizing, non-ionizing radiations, non-ionizing radiations are differentiated by the type of frequency at which itis expressed, which are given different names, such as ultraviolet, visible, infrared, microwave and radio wave radiations, as shown in Table 5.1. Therefore, it is said that non-ionizing radiation is incapable of causing an ionization effect in the body cells and c) ionizing radiation, unlike the previous radiation, we have ionizing radiation, this type of energy becomes ionizing when there is a "shock" so that it results in electrically charged particles (ions).

Ionizing radiation can be electromagnetic, such as X-rays and gamma rays, or corpuscular, such as α andβ particles, which are components of the atoms that are emitted. Exposure to ionizing radiation can causevery serious and irreversible damage to health, even provoking cancer) (Regulation on health protection againstionizing radiation, 2001).

The effect produced by ionizing radiation has only a "cause-effect" relationship, it will depend on the dose ofwhich there are levels, called "threshold dose". Below this level, there might not be direct effects depending onthe dose received, but the probability of long-term effects increases. For this reason, ionizing radiation does have an impact on the body's cells, as it is more energetic (Forteza, 2010).

Table 5.1 Non-ionizing radiation classification

	Source	Effects
Ultraviolet	Sun, Hg vapor lamps, germicidal lamps, photocopiers, others.	Skin: erythema, burns, and cancer. Eyes: conjunctivitis.
Infrared	Sun, ovens, incandescentlamps, others.	Heat shock and delayed effects on the crystallinelens.
Radiofrequency microwave	Ovens, microwaves, radio, TV broadcasts.	Possible affectation of biological membranes and alterationsin gene transmission.
Laser	Devices capable of emitting visible, infrared, or UV	Skin and eyes.

Source: Adapted from "Reglamento sobre protección sanitaria contra radiaciones ionizantes" by Boletín official del estado, number 178. 2001, Reglamento sobre protección sanitaria contra radiaciones ionizantes

5.2.1.3 Thermal energy

Human beings need a 37°C temperature to survive and keeping their homeostasis, human body has self-regulation of temperature, regardless of the surrounding conditions so physical and physiological mechanisms can be performed correctly (Puchau, 2018).

The effect of an imbalance in thermoregulation leads to consequences that could be serious and have a high impact on the human body, such as those depicted in Table 5.2.

Table 5.2 Imbalance of thermoregulation

	Description	Effects
Thermal stress(heat)	A thermal destabilization, manifested by blood vasodilatation, the opening of sweatglands, and loss of mineral salts.	Cramps, effects on the skin, exhaustion, heat stroke (death)
Cold stress	It can cause blood vasoconstriction, deactivation of sweat glands, and poorcirculation.	Hypothermia, resulting in frostbite of limbs and death (<28ºC).

Source: Adapted from: "Prevención de riesgos laborales" Francisco José Forteza Oliver; María de las NievesPiña Capó. 2010, Prevention of occupational hazards.

Finally, it is considered that, for the good development of physiological mechanisms of the human being,it is necessary to have a good relationship with the thermal environment, having a comfortable zone,which has the characteristics of the most suitable temperature being this same pleasant It is worth mentioning that not all humans will have the same thermoregulatory response, either due topersonal susceptibility or poor adaptation to new spaces, whether hot or cold. (Puchau, 2018)

5.2.1.4 Pathogenic microorganisms

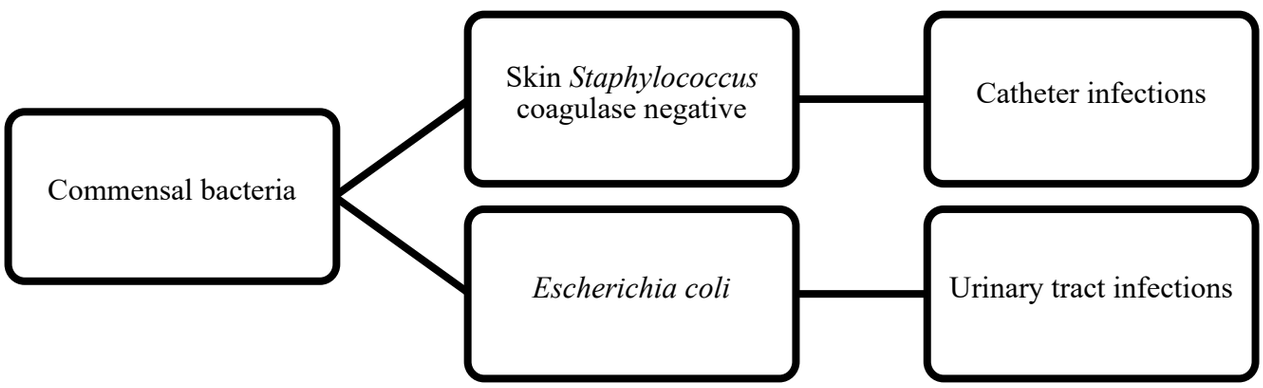
Biological agents are understood as microorganisms, including genetically modified microorganisms, cell cultures, and human endoparasites, susceptible to cause any type of infection, allergy, or toxicity. Microorganisms are any microbiological entity, cellular or not, capable of reproducingor transferring genetic material (Law. 664/1997, 1997).

Infectious microorganisms vary in different patient populations, different health care facilities, differentfacilities, and different countries (WHO Guideline, 2002).

In the case of working with this type of biological agent in a hospital, it is the protocol to use measures suchas personal protective equipment (PPE) or take sanitary measures on handwashing (Law. 664/1997, 1997).

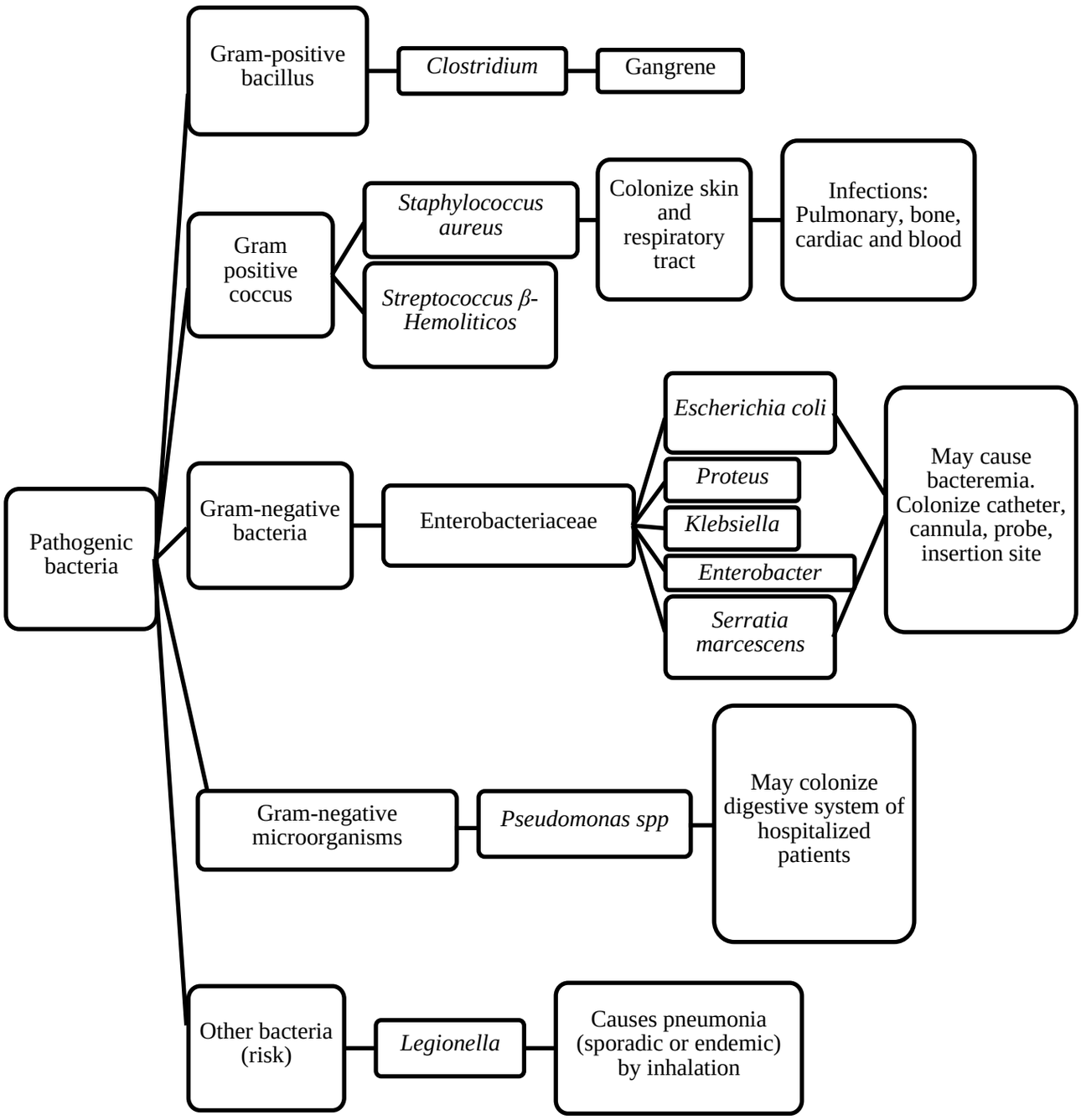
As a result of the above, under different and numerous studies it has been possible to identify the microorganisms that contribute to hospital contamination, as shown in Figures 5.3 and 5.4.

Figure 5.3 Most common commensal bacteria in hospitals causing infections



Source: Adapted from "On the protection of workers against risks related to exposure to biological agents at work" by State Official Gazette, number 124. 1997, On the protection of workers against risks relatedto exposure to biological agents during work

Figure 5.4 Most common pathogenic bacteria in hospital areas



Adapted from: Prevention of nosocomial infections by World Health Organization. 2002, Practicalguide Prevention of nosocomial infections

5.2.2.1 Nosocomial or in-hospital illnesses

Nosocomial infections (NI) are infections acquired by patients admitted to a hospital for a reason other than admission, therefore it is understood that a hospital can be a focus of these nosocomial infections, and this represents a major health and economic problem today (Leralta, 2017).

Infections occurring more than 48 hours after hospitalization are considered nosocomial. According to NOM-045-SSA2-2005 for epidemiological surveillance, prevention, and control of nosocomial infections, infections acquired by neonates who become infected by passage through the birth canal, those that develop within 30 days following a surgical intervention or that occur in the year following the performance of a surgery in which an implant was placed are also considered nosocomial, as shownin Table 5.7.

Table 5.3 Relationship of microorganisms with the focus of infection

	Respiratory infection	Urinary tract infection	Bacteremia	SurgicalWound
Escherichia coli	X	XXXX	XX	XXXX
Staphylococcus aureus	XXXX	----	XXXXX	XXXXXXXX
Pseudomona aerugiosa	XXX	XXX	X	XX
Enterococcus faecalis	----	X	XXXX	XXX
Klebsiella. pneumonie	XX	XX	XXX	X
Staphylococcus epidermidis	----	----	XXXXXXXX	XXXXX

Note: The "X" relates the nosocomial microorganism with the type of infection it produces to a greater or lesser extent, depending on the translocation of the microorganisms from their usual location to a new sterile location, they can produce some infections or others

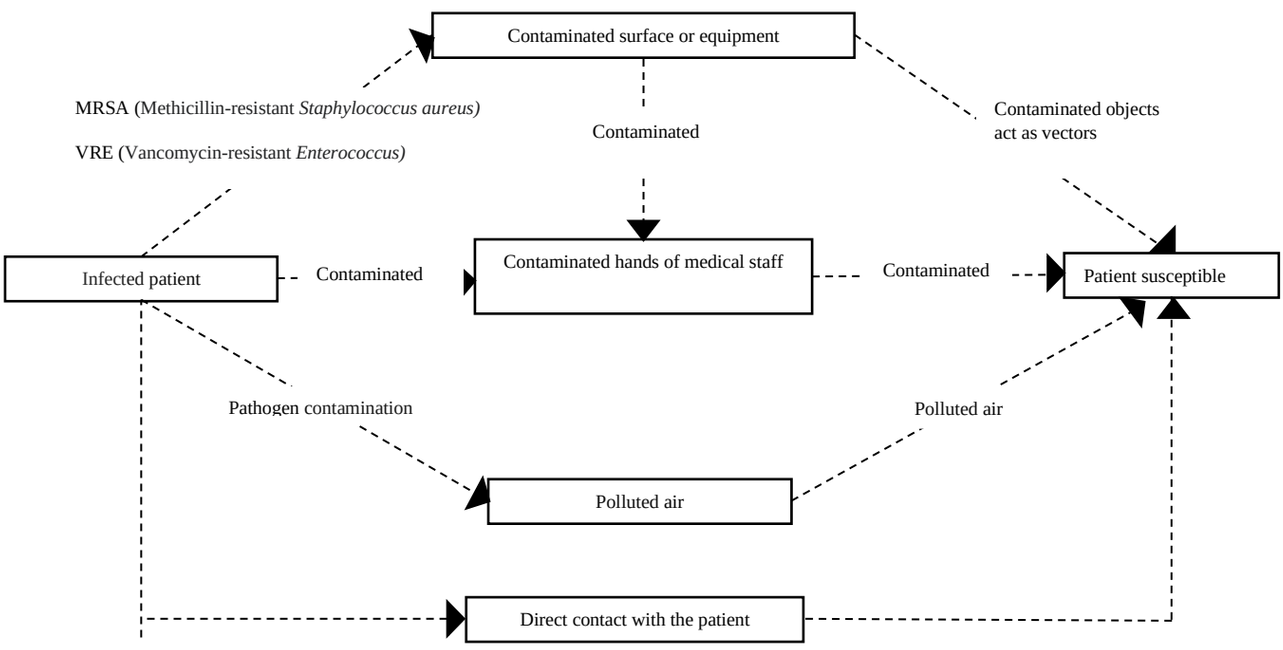
Source: Adapted from "Nosocomial infections, importance of Pseudomona aeruginosa" by Claudia Leralta Gonzales. 2017, Nosocomial infections, importance of Pseudomona aeruginosa

5.2.3 Hospital equipment

5.2.3.1 Personal computers as sources of infection

Patient-to-patient transmission of nosocomial pathogens has been associated with transient colonization of healthcare workers, and studies have suggested that contamination of healthcare workers' clothing is an important part (Treakle *et al*., 2009), as is the use of medical equipment and instruments, as well as technological equipment such as cell phones or watches. Most studies have identified various types of pathogenic germs, and some objects, being in close contact with patients and other individuals, could serve as reservoirs of bacteria easily transmitted and disseminated both within the hospital and in external homes (Magdaleno *et al*., 2011). Under the analysis of the presence of nosocomial infections, the consequences are the impact on the patient's health and life, the consequent increase in health care costs, and lawsuits against the medical practice. There is sufficient evidence to explain the sequence of events that occur in the possible role ofsurfaces of medical accessories and devices in the chain of NIs, as shown in Figure 5.5 Pathogens are shedfrom infected or colonized patients (sometimes from staff) in the hospital environment (General, S., 2016).

Figure 5.5 Nosocomial infection acquisition route



Source: Adapted from "Stethoscope, gown and tie, and the risk of nosocomial infections" Baptista and Zamorano 201.

5.2.3.2 Shared diagnostic hospital equipment

For the formation of a hospital, assets are needed to ensure the optimal functioning of the hospital, which include: a)medical equipment, which includes devices that are used for specific purposes of prevention, diagnosis, treatment, or rehabilitation of an illness or injury; it can be used alone or in combination with some accessories, consumables, or other medical equipment, b)furniture, which is designed to keep patients in a comfortable position during a process of exploration or medical care; of safeguarding, holding, organizing, and mobilizing medical supplies, the above items are shown in Appendix 1, c)communication and information systems and industrial equipment, for example, electric generators, heaters, water pumps, refrigeration, and air conditioning systems, elevators, laundry, kitchen, and other similar equipment (Secretaría de Salud, 2016).

5.2.4 Cleaning procedure in hospital areas

The cleanliness of the patient's environment is an important factor in their early recovery from illness. The hospital environment, as mentioned above, is predisposed to harboring potential pathogens, the main reasons being the volume of sick patients and the pace of patient care activities performed by healthcare workers. The complexity of the hospital is the reason for all medical surfaces and equipment to require dailycleaning. The ability of certain pathogens to remain viable for long periods on inert surfaces (some microorganisms can survive weeks or months in the hospital environment), make careful sanitization of areas difficult and even more important (Pimienta, 2017).

There is a classification with concerning the risk of transmission of infections based on the activities performed in each one. This classification helps in some strategies against the transmission of nosocomialinfections. The purpose of this classification is to guide on the complexity, timeliness, and detail of the sanitation services in the areas in question so that the cleaning and disinfection process is appropriate to the risk, in addition to facilitating the development of procedures for cleaning and disinfection of intrahospital surfaces (Pimienta, 2017).

A) High-risk areas (critical): space where delicate patients are located, who has undergone surgery or suffer from a chronic degenerative disease, where immunosuppressed patients can be found, for example operating room, toco-surgical unit, intensive therapy unit, dialysis, hemodynamics, burns, transplants, isolation units, analysis laboratory, blood bank, nursery, materials, and sterilization center, milk bank, among the most important ones. B) Intermediate risk areas (semi-critical): here patients who have undergone a disease with a low level of transmissibility or diseases that do not generate direct contagion share space, examples of this type of areas are nurses' stations, consulting rooms, bathrooms, elevators, and corridors. C) Low-risk areas (non-critical): these are areas that are generally used by health personnel or other hospital workers, for example dressing rooms, bedrooms, offices, administrative areas, warehouses, library, among others (Pimienta, 2017).

Cleaning is the mechanical removal of all foreign matter in the environment, on surfaces and objects, using manual or mechanical washing. The purpose of cleaning is to reduce the bioburden (number of microorganisms) through mechanical removal. Usually, water and detergent are used for this process. It is recommended that a detergent be used to ensure the effectiveness of the cleaning process (Molina, 2003). Cleaning generally comprises 3 types of action:

1) Mechanical action such as scrubbing, brushing, or pressure washing, 2) Chemical action refers to the use of detergents, enzymatic detergents, and water, necessary to inhibit and diminish bioburden and dustparticles and 3) Thermal action specifically refers to the use of heat (hot water) from mechanized washingmachines where they exist (Molina, 2003).

5.2.5 Floor cleaning

5.2.5.1 Two-bucket technique

It involves cleaning with the use of floor cleaning cloths and floor dryer, using two buckets, which comes to optimize the work of the cleaning staff and the disinfection of surfaces, avoiding back and forth movements for a water change and cleaning of the cloth, in the best case, or the use of dirty water to continue cleaning, by not resorting to water change (Pimienta, 2017).

Wet Sweeping (Mopping)

1) Mopping the edges, with horizontal movements, it is important not to go over the same place twice, to reinforce the mop should be rinsed until it is clean, and go over it again. 2) Take care not to leave puddles or wet places that favor bacterial growth. 3) Verify the state of the drains and remove all dirt onthe floor such as chewing gum, stains, etc. (PAHO, 2011).

Common areas are mopped only with clean water and a well-washed and wrung-out mop. Areas with spills of body fluids should have sodium hypochlorite at a concentration of 5000 ppm, as this is used forcleaning. It is important to verify that the implements are very clean when cleaning in another area or room, to avoid cross-contamination (PAHO, 2011).

Soaping, rinsing and drying

Lathering, is the action of rubbing the surface with soap or detergent to remove all the dirt, thisstage, one of the buckets contains water and the other soap or detergent, therefore, rinsing has the purposeof removing the soap or detergent, in this last stage, the two buckets contain only water, finally, it is leftto dry (Pimienta, 2017). After finishing mopping, it is necessary to verify that the buckets used for the water change are placed upside down to avoid bacterial growth (PAHO, 2011).

Minimum frequency of floor cleaning

All cleaning should be structured in an official guide, which should be followed to the letter, these being appropriate to each need that the hospital has, some common points are: inpatient rooms should be cleaned once a day when stained and upon discharge of the patient, laboratories require daily cleaning. Floors will be cleaned with a disinfectant detergent solution, operating rooms require a special cleaning regimen and the frequency of this should be standardized (Quiroga, 2018).

5.2.6 Surface cleaning

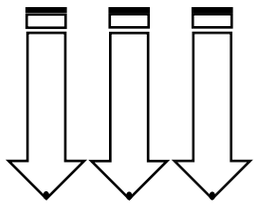
Contaminating particles are not always visible, they are distributed in various media such as air, furniture, walls, among others. Therefore, a fundamental part of cleaning is shaking; being this a common technique, a safe process is needed (Molina, 2003). There are considerations for dust cleaning, this process should be carried out in recurrent and terminal cleanings, put on personal protection elements (according to the biosafety manual), do not shake the cloth so as not to disperse the dust, start cleaning from the upper parts, continue towards the lower parts, continue with flat surfaces, sides, and supports and verify that all the spaces where dust cleaning was performed are in perfect conditions. Based on the above, cleaning techniques have been implemented:

1) Aseptic technique: It is so-called because it is a technique that can prevent contact with pathogenic microorganisms.

2) Dragging technique: perform a movement from top to bottom on vertical surfaces, from right to left or vice versa, trying not to pass the cloth through the same place, as shown in Figure 5.6.This technique is used for dust cleaning. It is important to emphasize the chipping (part in which a surface loses its coating) and cracks in which dirt can remain accumulated.

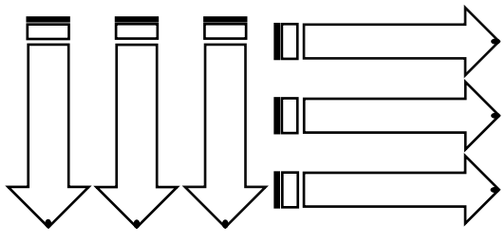
3) Grid technique, the flannel used in this procedure must be moistened in sodium hypochlorite solution (in a concentration according to the service or area), this will be used to exert friction directly in the surfaces with a downward movement, next with another clean flannel, previously moistened in water, movements are made to the right, exerting friction to remove the sodium hypochlorite, as shown in Figure 5.7. (Forteza, 2010, JaveSalud, 2017).

Figure 5.6 Dragging technique



Source: Adapted from "Protocol for cleaning and disinfection of surfaces and equipment". Quiroga, 2018

Figure 5.7 Grid technique.



Source: Adapted from "Protocol for cleaning and disinfection of surfaces and equipment". Quiroga, 2018

The techniques and activities related to cleaning and disinfection are focused on the most frequently identified critical points, in order to establish the following recommendations: Never sweep dry, use thebucket with wet syringe, to collect residues, start with clean areas and finish with the dirtiest areas, cleaning should not coincide with the distribution of food and clean clothes, the cleaning of areas with daily presence of patients, family members or health personnel should be carried out appropriately and permanently, in the same way on working days, as well as on holidays and weekends, do not create air currents that facilitate the movement of germs, cleaning will be performed in one direction only, from top to bottom or side to side, without backing up, cleaning will require friction to remove dirt and microorganisms, surfaces close to the patient should be the first to be cleaned, floors may be waxed, as long as a thorough previous cleaning has been performed (Pimienta, 2017).

5.2.7 Cleaning of hospital instruments

There are important aspects for this type of cleaning, such as the disinfectant for the processing of instruments and equipment used with the patient, disinfectants should be selected in each place considering the use, efficacy, acceptability, safety, and cost and should always be used in the dilution and manner recommended by the manufacturer (Quiroga, 2018).

The Spaulding classification of medical devices is currently accepted according to the degree of contactwith the patient, which will determine the risk of infection: a) Critical medical device: It is the material that is in contact with the vascular system and sterile areas of the organism in any intervention or hospitalization, requiring cleaning followed by sterilization. For example, surgical instruments, suction devices, nebulizers, comfort devices, among others. These instruments must be sterilized, which will bediscussed later. b) Semi-critical medical devices: These are materials that may encounter mucous membranes and non-intact skin. The processing of this material requires cleaning followed by high level disinfection. For example, Laryngoscope blade, otoscope, among others. These devices must undergo high-level disinfection and c) non-critical medical device: This material does not directly touch the patient; however, it requires cleaning processing followed by an intermediate or low level of disinfection. For example: thermometers, auxiliary tables and equipment such as electrocardiographs, ultrasound scanners, among others, should be cleaned-disinfected (Acosta-Andrade, 2008). In the case of incubators, they should be completely dismantled, washed, rinsed, dried and disinfected when disassembled and after assembly, they should be plugged in for complete drying (Quiroga, 2018).

For the cleaning and disinfection of surfaces of biomedical equipment that have electronic parts or that by manufacturer's recommendation should not be wetted with water, the detergent-disinfectant is used, the measures are as follows: Precautions should be taken such as turning off the equipment before cleaning and disinfection and not applying chemicals directly to the electrical part of the equipment and keyboards, the foam is applied directly on the surface of the biomedical equipment or a compress so that the surface of the equipment can be completely cleaned, it is allowed to dry (Acosta-Andrade, 2008).

5.3 Physical antimicrobial control

5.3.1 Sterilization

It is the set of operations intended to eliminate or kill all forms of living beings contained in an object or substance. Any material used in hospital areas that is called a critical article must undergo a sterilization process according to its properties. The standard establishes that the theoretical probability of a viable microorganism being present in the product must be equal to or less than 1 in 1,000,000. This expression is what is internationally known as SAL Level (Security Assurance Level) (Sanchez, 2017). But to rely on the sterility of a product, it is necessary to have internal control, which, is going to count on different factors, among which is the initial microbial load, in addition to the subsequent storage of the sterile product. As mentioned above, cleaning and disinfection (high, medium, or low level) must be carried out; to then move on to the sterilization process, although these decontamination processes are variable in terms of antimicrobial effectiveness (Sanchez, 2017).

Some factors affect the efficiency of these processes; therefore, some criteria have been implemented that must be followed to obtain adequate sterilization:

The number of microorganisms (Co). This is a fundamental factor since the sterilization effect of thematerials will depend on it. Organic matter (S) The presence of organic matter impedes the elimination of microorganisms, but it is one of the easily modifiable factors. These two factors Co and S highlight the importance of cleaning before sterilization. Time: An important factor for the correct sterilization process as it is taken as an indicative criterion. The F value is the time necessary for a suspension at a temperature of 121ºC to eliminate all bacterial spores. It is also used as a reference valuein the evaluation of sterilization methods (De Salud, 2010).

Temperature. The temperature is fundamental during this specific sterilization process because when thetemperature is higher than indicated, the better effect there is on the microorganisms and generally causestheir death. Relative humidity (RH). It is defined as the fraction of water vapor pressure in a system concerning another system with the maximum pressure (100% saturated) and at the same temperature. There is a direct relationship, higher relative humidity - higher water content in the cells or spores and better final sterilization result, which makes the process faster. Standardization of the load, the packages should have the measures (28 x 28 x 47 cm.) and wrappings internationally standardized. The load to be sterilized is very variable, it can change with respect to the number of instruments, load volume, size of the instruments and contents of the packages. It is important to standardize sterilization processes according to the different items in the load as the effectiveness of the method may vary depending on the items (De Salud, 2010).

5.3.2 Dry heat

It is characterized by the absence of water in the heating environment. This method has been less used due to the introduction of disposable materials such as needles or syringes, which are sterilized by radiation or ethylene oxide. However, the method is used to sterilize all materials that are reused, such as glass syringes, delicate cutting instruments, surgical instruments, stainless steel items, and oily materials, mostly dry sterilization is used in laboratories to sterilize glassware (Robilotti and Couso, 2011).

All microorganisms, according to their characteristics and components, are susceptible to the action of heat, this reaches the entire mass, even in places of the material that may be unreachable by other agents. The mechanism of heat as a sterilizing agent involves protein denaturation, fusion and disorganization of membranes and/or the occurrence of irreversible oxidative processes (Jimenez, 2018).

The duration of exposure to heat to saturate all the materials, due to the slowness of dry heat, was determined by experimental tests and it was concluded that: 1) The larger the volume of the material (flasks, instruments, etc.), the longer the pre-sterilization time, 2) The lower the conductivity of the material (talc, kaolin, oils, etc.) the longer the pre-sterilization time and 3) If the greater the thickness and lower the conductivity of the jar walls, the longer the pre-sterilization time (Jimenez, 2018).

The main variables of a heat sterilization process are temperature and exposure time, because the destruction of microorganisms by heat does not occur instantaneously, there is a time-temperature-general conditions relationship between the product to be sterilized, by the above criteria, microorganisms are classified into two groups, based on heat resistance: (a) Vegetative cells, whose thermal resistance is relatively small, being sufficient as a general rule to an exposure to 80°C for 1 minute for their destruction and (b) Sporulated bacteria, capable of surviving being subjected to temperatures of 100°C and higher for relatively long times. [29]

Depending on the amount of water contained in the microbial cell is its resistance to destruction by dry heat, although it was accepted that in states of extreme dryness microbial cells better-resisted inactivationby dry heat, later research showed that the influence of moisture on dry heating processes is much morecomplicated than previously anticipated (Jimenez, 2018).

The major advantage of this method is that it penetrates solids, non-aqueous liquids, and closed cavities.There is no corrosion of the materials involved and the disadvantages are that it requires high temperatures and very long periods. Dry heat is the method of choice for the sterilization of anhydrous oils and powders, due to its high penetrating power. There are two types of hot air sterilizers(as they are often called) commonly used in laboratories and hospitals: A) gravity convection and B) mechanical convection. For both types, the electrical heating method is used. The usual workingtemperature is 160ºC to 168ºC. The regulator is adjusted (Robilotti and Couso, 2011).

Gravity Convection

In the gravity convection sterilizer, the air circulates according to the temperature differences in the sterilizer chamber. The design of the gravity convection hot air sterilizer specifically influences its efficiency. The design features are not opposed to airflow with the flow to the corners of the chamber, bafflesor other places where obstacles to air circulation are encountered. The electric heater assembly will be placed at the bottom of the chamber, spaced by a perforated metal plate that is a uniform diffusing surfacefor the hot air over the entire vertical extent of the chamber (Robilotti and Couso, 2011).

In this type of sterilizer, there are factors such as the long heating time, therefore, more time is needed toreach the desired temperature and it is less uniform in the control of the chamber temperature, comparedto the mechanical convection sterilizer. Its use is recommended only for materials where heating is fast and precise, without the factors of chamber capacity restrictions and accelerated air circulation being decisive (Robilotti and Couso, 2011).

Mechanical Convection

The hot air mechanical convection sterilizer is of maximum functional efficiency at minimum cost. This instrument is equipped with a fan motor, which can move a large volume of hot air faster, transferring heat to the load, depending on the temperature control condition. In this case, the set of resistors is located directly in front of the fan motor, in a compartment separated from the chamber by a diffuser wall (Robilotti and Couso, 2011).

Factors such as air velocity, circulation direction, and heating intensity are moderated to achieve a stabletemperature inside the chamber, which is managed according to the type of load. It can reach temperatures of 160ºC in the chamber without a load in 30 minutes and with the chamber filledwith glassware it reaches 160ºC in 75 minutes. It has positive characteristics for the ventilation of gasesor humidity (steam) formed during the sterilization process (Pérez and Sosa, 2013).

In any procedure, there are parameters, which will vary according to the load, volume, weight, thermal resistance of the material (porcelain, glass, stainless steel, oils, among others), type and power of the stove. It is important to know A) Temperature. Sterilization temperatures fluctuate between 160ºC to 180ºC. The temperature used should not be below 160ºC for one hour, since it is the minimum condition, when the water content is 0%. Without exceeding the determined temperatures (above 180ºC), the material may be damaged. B) Time. The time needed for the air inside the chamber to reach the sterilization temperature (preheating time). 2. The time it takes for the materials to reach the sterilization temperature. 3. The sterilization time itself, as shown in Table 5.3 (Vásquez, 2001).

Table 5.4 Time-temperature relationship for dry heat sterilization

Temperature (°C)	Exposure time
180°C	30 minutes
170°C	1 hour
160°C	2 hours
150°C	2 hours and 30 minutes
140°C	3 hours
121°C	12 hours

Source: From "Manual de esterilización para centros de salud" Acosta-Andrade, 2008

Every sterilization procedure has advantages and disadvantages, among the advantages is that it does notcause corrosive effects for metals and instruments and allows the sterilization of powdery, viscous, non-aqueous (non-volatile) substances. (On the other hand, the disadvantage is that it requires a long time forsterilization, due to the low penetration of heat (Vasquez, 2001).

5.3.3 Moist heat

Steam sterilization is the most used sterilization procedure, and the equipment used is called an autoclave. The mechanism of action of moist heat is by denaturation of proteins (De Salud, 2010), it is the most widely used method, due to the much more penetrating action of moist heat, which facilitates the coagulation of bacterial proteins, coagulation that is directly related to the degree of hydration. Thismethod should be considered as the method of choice whenever the materials allow it (De Salud, 2010).The steam can sterilize the surfaces of the material, so that it establishes its action only on these surfaces.Therefore, the materials will be positioned in particular ways so that the action of the sterilizing agent iseffective, for example open tweezers, disassembled syringes, textile material, glass, rubber, Teflon, among others. Wet materials become better conductors of heat much faster than dry materials due to theenergy released during condensation. To achieve reliable sterilization, the standard method is saturated steam autoclaving, as shown in Table 5.4. This temperature is achieved by steam at one atmosphere above atmospheric pressure (Vasquez, 2001).

Table 5.4 Time-temperature relationship for moist heat sterilization

Temperature (°C)	Minimum exposure time (minutes)
134	3
121	15

Source: From "Guía de trabajos prácticos: Cátedra de Microbiología e Inmunología" 36. Medvedeff etal., 2009

The autoclave is mostly used in laboratories to sterilize cultures and solutions that do not form emulsionswith water and that do not denature at temperatures higher than 100°C. As mentioned above, it is used to sterilize textile material (provided that the autoclave is equipped with a vacuum drying system); for tubular elements, it is practiced moistening them with distilled water, so that the steam generated expelsthe air. Glassware can be sterilized in an autoclave but must then be dried in an oven (Vásquez, 2001). As any method, it has advantages and disadvantages, it has the advantage of raising its temperaturequickly without leaving toxic residues in the material (De Salud, 2010), on the other hand, thedisadvantage we can mention the long time in which the process is carried out and that it is not applicableto thermosensitive materials (Medvedeff et al., 2009).

The efficiency of steam as a sterilizing agent depends on: humidity, heat, penetration and the mixtureof steam and pure air (and other impurities it may contain) (De Salud, 2010).

5.3.4 Radiation

Radiation is the transport or propagation of energy in the form of particles or waves; if the radiation is due to electrical or magnetic forces, it is called electromagnetic radiation (Sanz, 2011). By their biological effect, radiation can be classified into two types:

a) Ionizing or high-energy radiation, they can beelectromagnetic or particle radiation, the former are short wavelength radiation, and the latter areconstituted by high-energy electrons produced by high-voltage generators (Barrera et al., 2005)

b) Non-ionizing or low-energy radiation, which are electromagnetic rays (i.e., not particles) with a wavelength longer than visible light and which are absorbed as heat in a large proportion (Sanz, 2011).

In sterilization, radiation is lethal to microbial cells as well as to other organisms. Of the various typesof radiation, those used for sterilization purposes are differentiated by their nature and energy. It is thephenomenon of emission and propagation of energy in space or through a material medium. Theradiations with a harmful action on microorganisms are ultraviolet rays, x-rays, gamma rays and cathoderays (Sanz, 2011). Their action depends on 1. type of radiation 2. exposure time 3. dose (Vásquez, 2001)

5.3.4.1 Ultraviolet rays

There are different types of ultraviolet radiation: 1) UV-A or long or near ultraviolet radiation, whose wavelength ranges from 380 to 320 nm (380 nm is the upper limit for the visual perception of violet color). This is the radiation that we know to be very intense, so much so that it reaches the Earth and can penetrate tissues. 2) UV-B or medium ultraviolet radiation, with a wavelength of 320 to 280 nm. It is used for the application of the photochemical effect (pigmentation or vitamin D formation). It is biologically harmful. 3) UV-C or far ultraviolet radiation, short or germicidal radiation, with a wavelength of 280 to 200 nm, presents the maximum energy (Barrera et al., 2005).

UV-C radiation or far ultraviolet radiation has a germicidal function, acting on the DNA molecules (deoxyribonucleic acid) of microorganisms, with wavelengths between 240 to 280 nm (nanometers), it mainly damages the nucleic acids of microorganisms, its main damage is in distorting the shape of DNAand interfering with normal base pairing, resulting in inhibition of DNA synthesis and therefore growthand respiration. Ultraviolet radiation can be produced artificially with mercury vapor lamps (Sanz, 2011).This sterilization method is used in the microbiology laboratory to disinfect surfaces, air, water, surgicalareas and sterile rooms (González, *et al*., 2011).

5.3.4.2 X-rays

X-rays were discovered in 1895 by Roëntgen and were the first known example of ionizing radiation of electromagnetic nature, they are produced by the collision against the matter of electrons accelerated at highspeed and in any X-ray apparatus, there is a cathode emitting electrons and an anode connected to a strongly positive potential to the cathode, which attracts the electrons and serves as a targetagainst which they collide. X-rays are short-wavelength electromagnetic radiation, which propagates in astraight line at the speed of light. They have great penetration capacity and are therefore used to obtain images for diagnosis (Barrera et al., 2005).

Their germicidal function is based on the ionization of molecules with which they cross, mainly affectingtheir nuclear DNA, which is vital in every cell (González, *et al*., 2011).

5.3.4.3 Gamma Rays

It is even more energetic than X-rays and more effective, although they act in the same way, they have a shorter wavelength (10-10 to 10-12 nm), attractive for sterilizing material of high thickness or volume, glass material tends to present a brownish color. Two sources are used to generate these rays: Cobalt-60 and Cesium-137 produced by atomic reactions. Gamma radiation can penetrate through products of great thickness, regardless of the type of product, density or packaging used, does not leave toxic residues in the product so it can be used without the need for quarantine and the only parameter that must becontrolled is the exposure time (Olmo, 2018).

Its mechanism of action is based on the production of ions and free radicals that attack the bases of nucleic acids, protein and lipid structures, and essential components for the viability of microorganisms. These rays have properties such as their high penetrability used for thermolabile materials such as disposable syringes, probes, needles, prostheses, catheters, etc. They are used on an industrial scale (antibiotics, vaccines, food, etc.), since complex and expensive installations are required. They are not used for culture media or protein solutions because they produce alterations in their components (Vásquez, 2001).

5.3.4.4 Cathode rays

Produced by an electron accelerator, which can be linear (classical) or circular (modern). This electron beam radiation is mostly used to sterilize surgical material, medicines and other materials; its main advantage is that the material can be sterilized already packaged (since these radiations penetrate the wrappings) and at an indeterminate or ambient temperature. Sterilization by accelerated electrons, developed in the 1950s from radiotherapy, has been shown in several studies to be the state-of-the-art method for sterilization of medical-surgical, laboratory and pharmaceutical materials (González, 2011). The treatment is based on the electron beam, which causes an ionizing effect, so energetically high that it can transform the electrons of the molecules into ions, i.e., they are electrically charged, without modifying the nuclei, i.e. without making them radioactive. This process has the disadvantage of causing physical effects on materials such as plastics, these changes include change in color or changes in mechanical resistance. This phenomenon, sought after in certain applications (cross-linking of plastic materials) could be limiting inthe sterilization of single-use medical products that use these materials. Radio-stable materials are therefore required. This is not a problem since there is a wide variety of polymers and other plastic materials on the market that do not modify their physical properties during ionization (González, 2011).It has notable advantages such as its excellent penetration and utilization performance, its availability and reliability, its treatment with unitary control, its high dose performance limiting degradation risks, its harmlessness on the product and its absence of environmental impact (Gonzalez, 2011).

5.3.4.5 Filtration

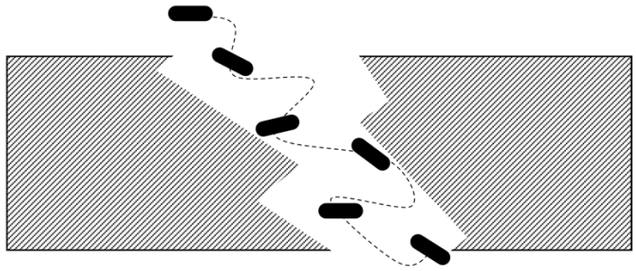
Sterilizing filtration is the process of complete removal of microorganisms (except viruses) from a fluid. A sterilizing grade filter must remove all microorganisms present in a fluid without adversely affecting the quality of the product (McBurnie, 2004). The materials that pass through this type must be fluids (gaseous or liquid), where mainly culture media are involved, in order to separate the existence of thermolabile substances, also used in vitamin solutions.

a) Operation of a sterilizing grade filter: Filters are composed of selective pores, and their mainfunction is to retain large particles that remain stagnant in the filter. This mechanism of particle arrestment or capture is known by different names, such as sieve retention, physical capture, direct interception, size exclusion, etc. Size exclusion is an important point, which is considered for sterilizingfilters the most reliable mechanism of filtering action, however, it is not the only one, as there is anothermechanism where particles small enough to enter and pass through the pores of a filter can be captured by adsorption to the pore walls. Regardless of particle size, a proper filtration process can capture even the smallest particle through the pores, as other conditions govern filtration, theseconditions are important if bacteria of smaller pore size are present (McBurnie, 2004).

As mentioned above, in the case of adsorption filtration, some conditions need to be controlled depending on the case, some of them are: applied pressure differential, flow rates, number of particles present, and the characteristics of the liquid in terms of surface tension, pH, ionic strength, among other factors. All must be monitored to have a good performance of the filtration process (Galeano, 2007).

b) Factors affecting microbial holding capacity for a sterilizing grade filter: 1) Bioburden - The total microbiological load present in a medium. In situations where not all contaminants are retained by size exclusion, the result can be said to be probabilistic. Figure 5.8 shows a bacterium (particle) smaller than the pore size entering the pore. The bacteria can either pass through the pore to exit with the flow, or stumble against the pore wall. Whichever situation prevails is the result of probabilities. Regardless of the level of challenge for a filter that is not clogged, the probability of any particle penetrating is the samefor any identical particle. The higher the number of particles (bioburden), i.e., the higher the challenge d ensity, the more likely it is that some particles will escape capture. The lower the bioburden versus thefilter, the better the filter performs (McBurnie, 2004).

Figure 5.8 Alternative pathway of bacteria through a pore



Source: Adapted from Technical Report No. 26 "Sterilizing Filtration of Liquids" PDA. 1998,

4) Pressure Differential - Another important aspect is the pressure differential, i.e., the pressure of both parts of the filtration site, where it is known that the lower the pressure differential, the higher the particlesequestration, due to the high residence time, which allows the absorption of the particle or microorganism in the pore; on the contrary, the higher the pressure differential, the lower the particle sequestration, due to the short residence time. There is, therefore, a direct relationship for adsorption capture, expressed by the inverse relationship of adsorption sequestration with the level of differential pressure (Galeano, 2007). 3) Other Affecting Factors - Several investigations have shown that propertiessuch as pH, surface tension, ionic strength and viscosity, can be factors of poor retention in filters, it is worth mentioning that filtration is also affected by the type of vehicle of the microorganisms present, bytheir change in size or morphology. Such changes can result in a non-sterile filtrate (McBurnie, 2004).

5.4 Conclusions

All the above exposed allows us to conclude that cleaning by physical methods isfundamental for intrahospital areas since everything is moderated through a cycle that has been constituted throughout the years by the sanitary emergencies that have been presented, which have given the guideline so that we can develop better control of diseases and thus improve those factors whether they are economic, infrastructure and attention.

The methods presented above have been updated and optimized for better use, through studies and research, which are important because thanks to them there is a better internal development; in the presentstudy, it was possible to compile information on the physical methods to be used in a hospital, some of them already updated, which already use computers for their management, new circuits for their operation, reduced size of the equipment and data such as time/temperature with a lower margin of error on sterilization.

The pandemic that has been experienced by COVID-19 has had a great impact on hospitals, mainly dueto the care of infected patients, but also due to the issue of ensuring the disinfection and cleaning of all areas, to take care of health personnel and other people involved.

It makes a difference to provide information in this study, which makes visible the importance of correctlyperforming the disinfection and cleaning processes concerning the material or surfaces in all hospitalareas that require it.

5.5 Appendix 1

Shared diagnostic equipment

Team	Description
Vital signs monitor	Vital signs display equipment.
Oximeter	Equipment for continuous measurement and recording of oxygen saturation in peripheralblood.
Fan	Equipment based on life support forventilatory support in adult and pediatric patients.
Nebulizer	Electric and pneumatic equipment, which generates vapor particles to provide air, under controlled humidity, temperature andoxygen conditions.
Defibrillator	Life support equipment, for electric shock and for monitoring the electrical activity ofthe heart.
Intensive care incubators	Equipment that provides life support in optimal conditions of temperature, humidity and oxygenation, in variable ranges and more similar to the intrauterineenvironment. For newborns
Stretchers	Bed specially designed for hospitalized patients or other people who need some kind of medical attention. Specialized stretchers are available by area.
Commode or urinal	The Comfortable is a medical accessorymade of very resistant plastic materials. It should be placed under the patient, at the level of his genitals to collect urine.
Table (Mayfield table, patient washingtable, surgical table)	Instrument holder, for bathing the patient and table to facilitate the surgical procedure.

Source: Adapted from "Medical equipment management glossary" by Secretary of Health. 2016

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Chapter 6 Use of power ultrasound, supercritical fluids and membrane technology to obtain and/or preserve biological products for clinical use

Capítulo 6 Empleo de ultrasonidos de potencia, fluidos supercríticos y tecnología de membranas para la obtención y/o conservación de productos biológicos de uso clínico

PANIAGUA-MARTÍNEZ, Ingrid†*, SÁNCHEZ-OTERO, María Guadalupe, CORRO-FERNÁNDEZ, Karina and ALEXANDER-AGUILERA, Alfonso

Universidad Veracruzana, Faculty of Bioanalysis, Iturbide s/n Esq. Carmen Serdán, Col.Centro, 91700 Veracruz, Veracruz, Mexico.

ID 1st Author: *Ingrid, Paniagua-Martínez* / **ORC ID:** 0000-0003-0955-941X, **CVU CONACYT ID:** 269088

ID 1st Co-author: *María Guadalupe, Sánchez-Otero* / **ORC ID:** 0000-0002-7916-7158, **CVU CONACYT ID:** 49392

ID 2nd Co-author: *Karina, Corro-Fernández* / **ORC ID:** 0000-0001-5090-1403

ID 3rd Co-author: *Alfonso, Alexander-Aguilera* / **ORC ID:** 0000-0001-8415-6923, **CVU CONACYT ID:** 123506

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* ipaniagua@uv.mx

A. Marroquín, J. Olivares, A. Dector L. Cruz. (Coord.) CIERMMI Women in Science TXIV Biology, Chemistry and Life Sciences. Handbooks-©ECORFAN-México, Querétaro, 2021.

Abstract

The awareness of the population to acquire products based on natural components that provide health care benefits has become a necessity nowadays. From this situation arises the initiative to look for new and better alternatives to replace synthetic active ingredients with components obtained from biological extracts. Bioactive compounds are molecules that contain a variety of beneficial properties for people as they contribute to the prevention and treatment of diseases; therefore, obtaining and preserving these components requires processes that guarantee their functionality. The implementation of emerging technologies that do not require the use of heat or require it at low temperatures during the processes of extraction and/or microbial inactivation of biological products, is a solution to the global problem of replacing conventional methods that affect the quality of the products, in addition to negatively impacting the environment. During this work, a vast compilation of information from articles, books and theses on non-thermal technologies such as Power Ultrasound, Supercritical Fluids and Membrane Technologies was carried out. As a result, it was found that these technologies are suitable for the extraction, separation and microbial inactivation of biological products for clinical use, since they represent multiple advantages, such as time and energy savings during the processes, reduction of chemical waste, easy reproducibility at industrial level, higher yields of extracts, cost reduction and they are environmentally friendly. It could be said that the use of these emerging technologies is still new, but the results they have offered to date are really promising.

Bioactive compounds, High power ultrasounds, Supercritical fluids, Membrane technology

Resumen

La concientización de la población por adquirir productos a base de componentes naturales que aporten beneficios para el cuidado de su salud se ha vuelto una necesidad hoy en día. A partir de esta situación surge la iniciativa por buscar nuevas y mejores alternativas que sustituyan los componentes activos sintéticos que pueden ser perjudiciales para la salud, por componentes obtenidos de extractos biológicos. Los bioactivos son moléculas que contienen variedad de propiedades benéficas en las personas ya que coadyuvan a la prevención y tratamiento de enfermedades; por lo que la obtención y conservación de estos componentes requieren de procesos que garanticen su funcionalidad. La implementación de tecnologías emergentes que no requieran el uso de calor o bien, lo requieran a bajas temperaturas durante los procesos de extracción y/o inactivación microbiana de productos biológicos, es una solución a la problemática de carácter global de sustituir los métodos convencionales que afectan la calidad de los productos, además de impactar negativamente al medio ambiente. Durante este trabajo se realizó una vasta recopilación de información procedente de artículos, libros, tesis sobre tecnologías no térmicas como lo son los Ultrasonidos de Potencia, Fluidos Supercríticos y Tecnologías de Membrana, como resultado se encontró que estas tecnologías son adecuadas para la extracción, separación e inactivación microbiana de productos biológicos de uso clínico, ya que representan múltiples ventajas, como un ahorro de tiempo y energía durante los procesos, disminución de desechos químicos, fácil reproducibilidad a nivel industrial, mayores rendimientos de extractos, reducción de costos y son amigables con el medio ambiente. Se podría decir que el uso de estas tecnologías emergentes aún es novedoso, pero los resultados que han ofrecido hasta el día de hoy son realmente prometedores.

Compuestos bioactivos, Ultrasonidos de potencia, Fluidos supercríticos, Tecnología de membranas

6.1 Introduction

The distribution of safe and quality products has an impact on the processing and manufacturing of these products, the ingredients of which must contain a high biological value. The safety of biological products is limited by the application of efficient preparation methods, e.g. extraction, separation, purification, viability of microorganisms, etc. (Castillo et al., 2004).

Obtaining and preserving biological products is an ancient practice and the methods that have been applied for this purpose have had the capacity to offer functional and safe products, but these methods can compromise their biological integrity and, as collateral damage, bring negative consequences that impact the environment.

Conventional methods of microbial extraction and inactivation are mostly based on the use of high temperatures, addition of aggressive solvents and destructive physical processes, which, although they achieve their purpose, are not the best processing option. For this reason, researchers were interested in finding technologies and mechanisms that do not rely on the use of heat, solvents or any other agent that compromises the safety of the final product. Among the objectives, it is also sought that these non-thermal technologies are environmentally friendly, require less processing energy and reflect a low cost for manufacturers and buyers (Barbosa and Bermudez, 2010).

This paper provides a literature review of three non-thermal technologies: Power Ultrasonics, Supercritical Fluids and Membrane Technology. Each of them is based on different mechanisms of action, but their similarity is based on extraction, separation and microbial inactivation that does not affect the quality of the bioactives, in addition to providing cost, energy and processing time savings (Barbosa and Bermudez, 2010).

Non-thermal technologies represent a new processing system, which in the last 30 years have been studied on a global scale with rapid growth in different industries, such as food, pharmaceutical, agronomy and cosmetics. Another advantage is the possibility of combining two technologies to intensify the extraction, separation and/or inactivation processes, or to seek synergism between emerging and conventional technologies to optimize the overall quality of the products (Barbosa and Bermudez, 2010).

6.2 Biological products for clinical use

There are many definitions of biological products used for therapeutic purposes, but most of them refer to products originating from any living being, i.e., from any biological source: plants, animals, microorganisms or human derivatives (Royal Decree, 2007). According to the World Health Organization (WHO), biological products are considered to be drugs obtained from microorganisms, blood or other tissues, whose manufacturing methods may include the growth of strains of microorganisms on different types of substrate, use of eukaryotic cells, extraction of substances from biological tissues, including human, animal and plant tissues, products obtained by recombinant DNA or hybridomas, propagation of microorganisms in embryos or animals, among others (Pombo, 2008).

The nature of the components of the biological product is what differentiates it from traditional chemical drugs, which are made from a combination of chemical ingredients5. Table 6.1 shows the main differences of bioproducts compared to synthetic small molecule drugs (Strohl and Strohl, 2012).

Table 6.1 Characteristics of biologic and small molecule drugs.

Property	**Biological (non-monoclonal antibody)**	**Monoclonal antibody**	**Small molecules**
Composition	Hormone or enzymes	Protein-Antibodies	Synthetic organic compounds
Molecular weight	>700 Daltons	150 000 Daltons	<700 Daltons
Mode of activity	Substitution of a lost or decreased activity of peptides, proteins or enzymes.	Binds to extracellulartargets to antagonize,agonize, or decrease activity	Binds to extracellulartargets to antagonize, agonize, or decrease activity
Production	By cells of bacterial, yeast or mammalian origin	Using cells of mammalian origin	Chemical synthesis
Product defined by	Biological activity; biochemical by the manufacturingprocess	Biological activity; biochemical analysis; by the manufacturing process	Chemically
Specificity	High	Very high	Tends to be low
Route of administration	Intravenous or subcutaneous injection	Intravenous or subcutaneous injection	Usually oral

Source: "Therapeutic Antibody Engineering: Current and Future Advances Driving the Strongest Growth Area in the Pharmaceutical Industry" by Strohl W, Strohl L. 2012.

The purpose of the use of this type of compounds lies in prevention to improve the quality of life and/or in the treatment of multiple types of ailments or diseases. The use of this technology started at the end of the 20th century, around the 1980s, and since then, they have become the main treatments for many diseases, such as carcinomas, diabetes, multiple sclerosis, heart attacks, strokes and autoimmune diseases (IAPO, 2013).

6.2.1 Types of biological products for clinical use

Biological products are characterized by being synthesized by living organisms, they are very large and complex molecules, they are compounds with a very labile structure, they present complex manufacturing processes, it is difficult to achieve stabilization in order to store them and maintain their structure and biological efficacy (Cuñetti, 2012). Table 6.2 briefly defines the products that are considered to be of biological origin (Pombo, 2008).

Table 6.2 Definition of the different products of biological origin

Biologicalproduct	Definition	Reference
Vaccine	Antigenic preparation that induces immunity against infections [9]	Abbas A, Lichtmann A, Pillai S. Basic immunology: functions and disorders of the immune system. 4th ed. Barcelona, Spain: Elsevier; 2014.
Allergen	Product that elicits an immune response to anallergenic agent [3]	Royal Decree 1345/2007 of October 11, 2007, which regulates the procedure for theauthorization, registration and conditions of dispensing of industrially manufactured medicines for human use. (Official State Gazette, number 267, of 11-10-2007).
Antigen	Product that elicits an immune response to aforeign agent [10]	Rojas W, Anaya JM, Cano LE, Aristizábal B,Gómez LM, Lopera D. Rojas Immunology. 17th ed. Medellín, Colombia: Fondo Editorial; 2015.
Hormone	Tissue-produced chemical that has a specific effect on a target tissue [11]	Bishop M. Clinical chemistry: principles, procedures and correlations. 5th ed. Mexico: Mc Graw Hill; 2007.
Cytokine	Proteins mediating inflammatory and immunereactions [9]	Abbas A, Lichtmann A, Pillai S. Basic immunology: functions and disorders of the immune system. 4th ed. Barcelona, Spain: Elsevier; 2014.
Enzyme	Molecules that catalyze the conversion of compounds to one or more different compounds increase the rates of the reaction [12].	Rodwell V, Bender D, Botham K, Kennelly P, Weil P. Harper's illustrated biochemistry. 30th ed. Mexico: Mc Graw Hill; 2015.
Human blood and plasma derivatives	Blood components for therapeutic, diagnostic,preventive or research applications [13].	Norma Oficial Mexicana del 26 de octubre de 2012, Para la disposición de sangre humana y suscomponentes con fines terapéuticos (Poder ejecutivo, Secretaría de Salud, del 26-10-2012).
Immune sera	Intended polyclonal antibody sera. They are used for passive immunization of certain diseases [14].	Linares J. Immunohematology and transfusion: principles and procedures. Venezuela, Caracas: Cromotip; 1986.
Immunoglobulins	Proteins capable of behaving as antibodies [14].	Linares J. Immunohematology and transfusion: principles and procedures. Venezuela, Caracas: Cromotip; 1986.
Antibody	A glycoprotein produced by B lymphocytes that binds to antigens, often with a high degree of specificity and affinity [9].	Abbas A, Lichtmann A, Pillai S. Basic immunology: functions and disorders of the immune system. 4th ed. Barcelona, Spain:Elsevier; 2014.
In vitro diagnosticreagents	Reagent product, calibrator, made in control material, intended for the study of samples from the human body [15].	Diaz Roa K. Application of reactivevigilance and its advances in Colombia. [IV Workshop on Strengthening and Continuous Quality Improvement for the National Laboratory Network]. Bogotá D.C.: INVIMA; 2018.

Source: "*Biological product" by Who 2008*

All these products are used in multiple circumstances; one of the practices employed daily around the world is the use of vaccines for disease prevention, also the use of blood and blood derivatives as treatment for multiple ailments, the supply of hormones, among many other cases that have the purpose of improving health.

The substances described above, which are used as the active fraction of the final product that is supplied to patients, are not originally found in pure form; they are a part of the total components of the raw material. This is why it is necessary to separate one or more components within a complex mixture (Pombo, 2008). In the following, different conventional extraction methods are described in contrast to the techniques under development.

6.2.2 Conventional methods for obtaining biological products

Extraction is the most important step to isolate different types of bioactive compounds (Yang et al., 2011). The desired compounds could be extracted by conventional methods, which are defined as classical techniques based on the extraction capacity of different solvents, application of heat and agitation (Viganó and Martínez, 2015).

The extraction technique is highly dependent on the type of solvents, energy input and agitation to improve chemical solubility and mass transfer efficiency (Yang et al., 2011). Some of the methods that have been used since ancient times are precipitation by addition of salts, by ionic potential (Harris, 1995), use of solvents (González et al., 2009), crushing and heat (Yang et al., 2007).

6.2.2.1 Precipitation by addition of salts or salting-out

Method applied to obtain products of protein origin. It is based on the decrease in solubility when the concentration of salts increases in excess, protein-protein interactions become stronger in comparison to protein-solvent interactions, so they precipitate (BQ Experimentals Blog, 2010). The salt mostly experimented for protein precipitation is ammonium sulfate, due to its high solubility and its stability at temperatures from 0 to 30°C (Rojas, 2009).

6.2.2.2 Isoelectric point precipitation

Method used for protein precipitation, in which the pH of the solution is adjusted to values close to or equal to the isoelectric point of the protein of interest (Rojas, 2009).

Proteins are made up of amino acids, which can be positively or negatively charged. If the isoelectric point is reached, these charges on the surface neutralize each other, preventing electrostatic repulsion with other molecules, and attraction between the dissolved proteins themselves, thus forming precipitates (Harris and Angal, 1995).

6.2.2.3 Addition of solvents

The use of solvents is mostly used to obtain lipid extracts (González et al., 2009). Extraction can be performed from a solid or liquid mixture to a liquid phase, where the solvents commonly used are organic solvents (UNP, 2020) such as hexane, ethanol, methanol (González et al., 2009).

The technique is based on the difference in solubility between the extraction solvent, the substance of interest and the other non-significant substances present in the initial compound. For a good choice of the solvent to be used, the desired product to be obtained, the volatility and toxicity of the solvent must be considered (UNP, 2020).

6.2.2.4 Crusher and heat

In addition to the addition of chemical products, mechanical procedures are necessary to assist in the extraction and obtaining of the product of interest, in order to cause mechanical damage to the raw material in which the compound to be isolated is present. Crushing as well as heat are intended to achieve the lysis of the starting material (Merino et al., 2019) in order to release the substances to be obtained through the different chemical methods mentioned above.

These are the mostly employed methods reported in the literature, but there is a diversity of techniques employed that aim to obtain bioproducts. These methods are widely used due to the advantages they offer, among them the easy handling, the acquisition costs of materials and reagents as well as the operating costs are economical (Yang et al., 2011). In contrast, conventional techniques also have disadvantages, mainly the treatment times are very long (Viganó and Martínez, 2015); large amounts of solvents are needed (Santos et al., 2013), which represents an environmental problem; another unfavorable point is the denaturation of the biological products themselves, due to the aggressiveness of the technologies used.

6.2.3 Conventional methods for the preservation of biological products

Throughout history, multiple techniques and procedures have been used to ensure the safety, quality and shelf life of products in multiple industries. In the present work we will be referring to products derived from biological sources and intended for clinical use, so it is understood the importance of complying with all the sanitation standards during all the processes and stages.

Temperature is one of the main elements that influence the viability and development of microorganisms; prolonged exposure to temperatures above the optimum produces structural changes, especially focused on proteins, enzymes, RNA, denaturation of membranes and/or nucleic acids (Hurst, 1977), in addition to functional alterations that end in a progressive decrease in the number of cells. (Perez et al., 2016).

Conventional preservation methods mainly include sterilization with the use of heat. Thermal processes are governed under two premises: the heat resistance of microorganisms for each specific product and the heating rate of the specific product (Awuah and Ramaswamy, 2006).

The use of thermal technologies for bacterial inactivation has proven to be effective in terms of product safety (Awuah and Ramaswamy, 2006), however, they are aggressive methods that lead to a decrease in the quality of the final product due to the denaturation of some biomolecules, affecting their initial functionality.

Currently, industries are opting for processing and preservation alternatives that are environmentally friendly, do not present health risks and provide high quality of the extracts used.

6.2.4 Non-thermal technologies for the production and preservation of biological products

The constant evolution of technology, the commitment to offer users better bioproducts and the awareness of reducing the environmental impact on our planet has led to the development and implementation of new and effective technologies for obtaining and/or preserving biological products. Thus, the so-called "emerging technologies" were born, which, although they do not completely replace conventional methods, complement each other to obtain more satisfactory results (Paniagua 2017).

The study of these new technologies has aroused particular interest thanks to all the benefits obtained in contrast to the use of conventional methods. And one of the main ones is that, by not requiring high temperatures during the processing of the raw material, many of the initial properties of the compounds remain unharmed; in turn, it significantly reduces costs, energy and production time, positively impacting the economic, social and environmental sphere (Bermúdez and Barbosa, 2010).

Within the group of these new technologies we can find High Intensity Pulsed Electric Fields, Light Pulses, Oscillating Magnetic Fields, Power Ultrasound (HPU), Supercritical Fluids (SF), Membrane Technology (MT), among others (Soleno, 2015). All of them are being tested and studied with the aim of being able to meet the constant demand for better products by consumers.

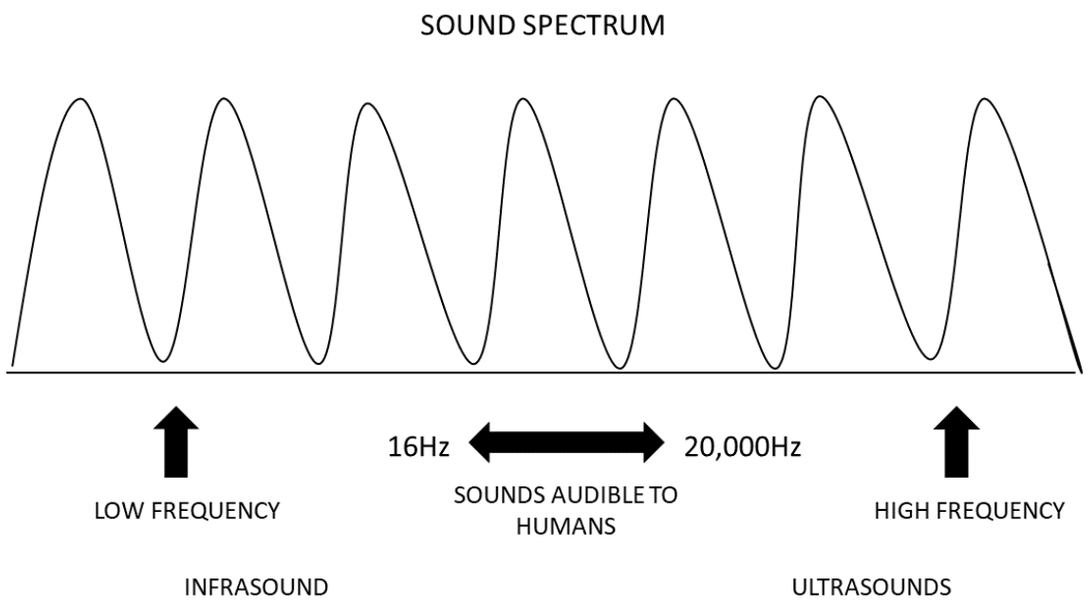
This paper reviews three technologies in particular: HPU, SF and MT.

6.3.1 Power Ultrasound

6.3.1.1 Ultrasound Generalities

We can begin by elucidating the term "ultrasound". It is made up of two words; -ultra: "beyond", "on the other side of"- (Real Academia Española, 2021), followed by -sound: "mechanical vibration transmitted by an elastic medium"- (Real Academia Española, 2021); being able to finally define the compound word as mechanical waves that propagate forcibly through an elastic medium longitudinally transmitting continuous vibrations between adjoining particles at a frequency above 20kHz up to 10MHz, thus exceeding the human audible spectrum. Figure 6.1 illustrates the boundaries between infrasound, audible range and ultrasound.

Figure 6.1 Sound spectrum



Source: (Morales and Martínez, 2015)

The range of the sound spectrum is determined by the frequency at which the mechanical waves that compose it oscillate, which are propagated longitudinally through a solid, liquid or gaseous medium. The wave frequency refers to the cycles completed per unit of time. This measurement is expressed in Hertz, where one Hertz corresponds to one complete cycle per second.

According to the frequency at which these acoustic waves vibrate, the sound spectrum is divided into three main groups: infrasound, sound and ultrasound.

Infrasound. They originate when the frequency of wave vibration is below 16 Hz below the threshold of human sensitivity. Although the infrasonic spectrum is imperceptible to the human ear, it does have an impact on the matter around us, producing contractions and dilations, including the particles of our body (Carcel et al., 2012). Thanks to the lower attenuation of infrasound these waves can travel a greater distance than sound or ultrasound, and this helps in the detection of large phenomena to prevent some natural disaster, such as earthquakes, earthquakes, volcanic explosions, turbulence, tornadoes Sound. Also called spectrum or human audible range. An individual is capable of perceiving an extensive range of sounds conditioned in a frequency range between 16 Hz to 20 KHz and, an amplitude range that goes from 0 to 140 dB. Although this hearing range varies depending on several factors, such as age. The perception of sound ranges from the threshold of audibility to the threshold of pain. The former, refers to the minimum signal pressure that is capable of stimulating an audible sensation to the human ear in the absence of noise.

Ultrasound. These are frequencies that exceed the human audible range, that is, higher than 20 KHz. However, ultrasound is perceived by some animals such as dogs. Depending on the frequency range and intensity, ultrasound can have applications such as in medicine, where one of the most common is ultrasound to observe different organs; or also used in multiple industrial areas.

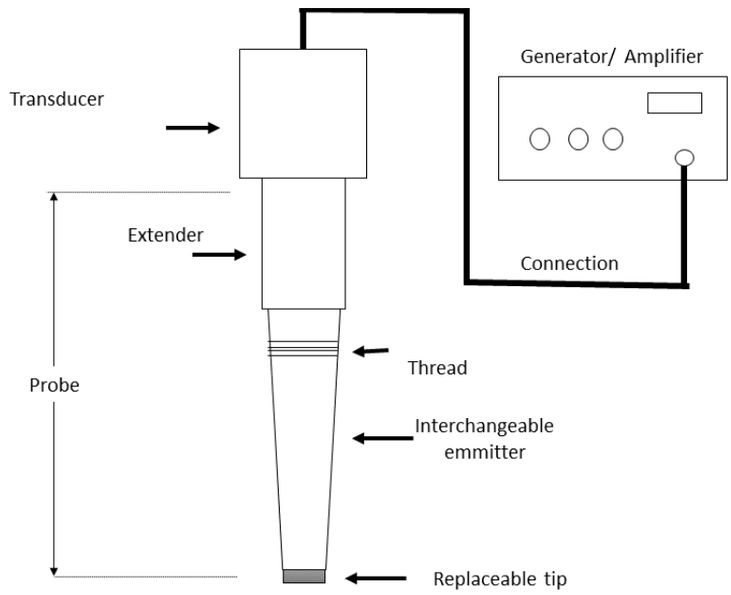
According to industrial applications, there are two classifications of ultrasounds, based on their frequency and intensity (McClements, 1997). The so-called signal ultrasounds are identified by being of low intensity (<1W/cm3), but high frequency (ranging between 100kHz and 20 MHz); because of these characteristics the power of this type of ultrasound is weak so it does not cause changes in the medium in which they expand, which is why they are used for non-invasive processes that require the destruction or transformation of components within the medium. On the contrary, there are power ultrasounds, which as its name refers, fluctuate at low frequencies (20 - 100kHz) and high intensities (higher than 1 W/cm^3) which gives them the property of making physical changes of some component suspended in the medium (Paniagua, 2017).

Due to the subject in question of this project, the present work focuses on power ultrasounds, since their previously mentioned qualities are necessary to meet the objective in the conservation and obtaining of bioproducts.

6.3.1.2 Power Ultrasonic Systems

Ultrasonic waves come from a source of acoustic energy, but initially this type of energy is not available, so its transformation is required. Ultrasound generation equipment is composed of three main elements, a generator, a transducer and an emitter (Robles and Ochoa, 2012). Broadly speaking, the generator is responsible for transforming the electrical signal from the network to the desired frequency; the transducer converts the high-frequency electrical signal into mechanical vibrations; and the emitter radiates the acoustic energy through the transducer to the medium to be treated (Cárcel, 2003). Figure 6.2 shows a schematic of the basic components of a probe-type ultrasound generator.

Figure 6.2 Schematic diagram of the ultrasonic generator equipment, probe type



Source: (Rayo, 2014)

Depending on the application needs required, the most appropriate ultrasonic system to be used will depend on the application requirements. Ultrasonic baths are the preferred systems to work with, due to their easy accessibility and low acquisition and processing costs (Povey and Mason, 1998). On the other hand, there are also probe systems, which must be manufactured with resistant materials to withstand the wear caused by cavitation generated in the medium. The probe systems are mostly used for research purposes, due to their convenience, and that is why we will be discussing experiments using this type of system.

The use of low frequency but high intensity ultrasound causes a transformation or damage of the matter in the medium that the ultrasonic waves pass through (Cárcel, 2003). These changes can be of a physical, chemical and biochemical nature that can be used in a number of applications in different industrial fields (Robles and Ochoa, 2012). One of the most studied and understood phenomena is cavitation.

6.3.1.3 Cavitation

In liquid media where mechanical sound waves propagate, microbubbles tend to be generated, and this is due to the fluctuation of the cycles giving rise to sudden pressure changes (Soria and Villamiel, 2010). During the low-pressure phase, gas spaces are created, which, in turn, grow as the pressure increases. This event results in continuous rarefaction and compression of the bubbles. During rarefaction the bubble increases in size, expands; while in compression it contracts and decreases in area, the amount of gas that the bubble gains in the expansion cycle is greater than that which it loses during the compression cycle. This means that in each pulse it will increase in size (Cárcel, 2003). Finally, the bubbles collapse and end up imploding, causing temperature rises of up to >4000°C and pressure of 1000 bar (Paniagua, 2017), although this environment has very short times of duration (fractions of a second). The intensity of cavitation and its effects will depend on the characteristics of the medium, such as its viscosity, process variables, ultrasonic intensity, frequency and pressure (Cárcel et al., 2012; Chandrapala et al., 2012). Cavitation is a mechanism that results in microbial inactivation due to high pressures and temperatures, inducing cell lysis.

6.3.2 Use of power ultrasound for obtaining and preserving biological products for clinical use

6.3.2.1 Obtaining biological products

In order to obtain the bioactives retained inside the cells, it is necessary to extract them by disrupting the cell wall. Generally, there are two mechanisms to achieve cell lysis: chemical and mechanical. The former usually consist of chemical treatment, osmotic shock and enzymatic treatment. While the latter, encompass other processes such as, high pressures, grinding, agitation, and ultrasonication (Lee et al., 2017). However, the advantage of ultrasound compared to traditional methods is the low energy consumption required, which, in turn, is reflected in decreased costs (Chemat et al., 2017) and decreased pollution to the environment.

Multiple studies have been conducted to test the efficiency of HPU as an extraction system for active compounds. Kuan et al. (2020), obtained the maximum values (recovery 95.08 ± 3.02%, extraction efficiency 99.74 ± 0.05% and partition coefficient 185.09 ± 4.78) of anthoxanthin, which is a potent antioxidant compared to other carotenoids, extracted from the microalgae *Haematococcus p*. It is worth mentioning, that for processing a HPU equipment was used (Bandelin Sonoplus UV2200, Germany), under conditions of 200 W/cm^2 and 20 kHz. After the sonication process, the cell morphology of the algae was observed, showing damage and rupture of the cell wall due to cavitation; at the same time, the rupture of the membranes favors the release of the anthoxanthin biomolecules. Therefore, it follows that ultrasonication is an effective technique for cell wall rupture of algae such as *Haematococcus p*. (Kuan et al., 2020).

One of the most promising sources for obtaining biomolecules that are intended for the manufacture of products with medicinal properties are plants. Medicinal plant extracts offer variety of biocompounds (Sun et al., 2019), such as alkaloids (Wang et al., 2018), anthraquinones (Jibril, et al., 2019), flavonoids (Zhou et al., 2019), glycosides (Dong et al., 2015), oils (Senrayan and Venkatachalam, 2019), pectins (Grassino et al., 2016), phenolic compounds (Um et al., 2018) and polysaccharides (Mandal et al., 2009). Lysis of the cell wall and membrane is necessary to release and obtain all these compounds (Sun et al., 2019). Traditionally, the mechanisms used have disadvantages, including long processing times, low bioactive purity and low efficiency (Mandal et al., 2009; Lu et al., 2012; Mura et al., 2015), which has led producers to search for new and better alternatives. As a solution to this problem, HPU was implemented as a technology that offers high efficiency, low energy consumption, higher quality and yield of extracts, as well as easy process automation (Wang et al., 2018).

In this context Sang et al. (2016), achieved the total extraction of anthocyanins (65.04 mg/100g) and polyphenols (947.39mg/100g), extracts with antioxidant, anti-inflammatory, anticancer and antimutagenic properties (Zhu et al.,2017; Huang et al., 2010); coming from Nitraria t. -raw material of active compounds and pigments- using HPU bath (TCX-600 S. Jiningtianyu Ultrasonic Instrument Co. Ltd., Shandong, China) at 30kHz and 300W/cm^2 conditions, also required a temperature of 70°C for 32 minutes by adding 51.15% ethanol (Sang et al., 2017).

In another assay by He et al. (2016), phenolic compounds and anthocyanins were extracted from Vaccinium a. employing HPU equipment (Hong Xiang Long Biotechnology Co., Ltd; Beijing, China) at 400W/cm^2, temperature of 61.03°C, processing time of 23.67 minutes and addition of 70% ethanol and 0.01% hydrochloric acid. As a result, total anthocyanins of 4.11±0.01mg/g and total phenolic of 16.01±0.03mg/g of extracts were obtained, demonstrating better compound recovery results unlike conventional solvent extraction (He et al., 2016).

Recovery of flavonoids is also of interest due to their biological importance and their various antitumor, hepatoprotective, antibacterial, anti-inflammatory properties, and antioxidant activity (Yang et al., 2013). Wei et al. (2013), recovered apigenin, baicalin, and luteolin, major flavonoids familiar for their effect against various human cancer cell lines. The system used consisted of HPU (Branson B-33510E-DTH, USA) at 40kHz and 185W/cm^2, heat reflux assisted, 50°C temperature, 30 min processing, plus addition of 60% ethanol. This synergy of technologies was compared with the use of heat reflux alone at 60°C, 85% ethanol and 60 minutes processing, showing that ultrasound-assisted extraction obtained better extraction yields. Associated with savings in time, energy, addition of organic solvents and obtaining analytes with higher purity (Wei et al., 2013).

6.3.2.2 Preservation of biological products - microbial inactivation

The main mechanism of microbial inactivation of HPU is due to cavitation. Continuous cycles of microbubble compression and decompression cause the abrupt collapse of the microbubbles, alternating the aqueous medium where the microorganisms are located due to the high temperatures and pressures reached during a fraction of time (Carcel et al., 2012).

Microorganisms are able to aggregate into communities that grow in a matrix of proteinaceous material (Costerton et al., 1995), in turn, these bacteria are released from the matrix to continue colonizing new surfaces (Lasa et al., 2005). Webber et al. (2015), used HPU at 40kHz frequency, power of 81W/cm^2 for 10 minutes for biofilm removal of three Salmonella species. They also used vortex method for 2 minutes to perform the comparison between both methods; the results showed no significant difference in log cycle reduction between Salmonella species (Webber et al., 2015).

Within the food industry, multiple studies have been conducted on the inactivation of microorganisms using HPU technology. Perez (2020), analyzed the effectiveness of using HPU as a treatment for inactivation of goat and maternal milk microbiota, for HPU treatment a continuous flow system was used; a digital ultrasonic device (Hielscher model UP400St) at conditions of 400W/cm^2 and 24kHz at 40°C. In the treatment of goat milk, a temperature of 53.2°C was achieved at the same frequency and intensity conditions. Regarding aerobic mesophilic microorganisms, a reduction of 0.50 logarithmic cycles was observed; while for enterobacteria, a reduction of 0.66 logarithmic cycles was achieved. On the other hand, for treatment with breast milk at the same conditions, but at a final temperature of 52.9°C, an inactivation of aerobic mesophiles of 0.69 logarithmic cycles was recorded (Pérez, 2020). As a second trial, the effect of HPU coupled with SC-CO_2 technology on goat milk alone was studied. The details of the processing are described in the section Combined technologies.

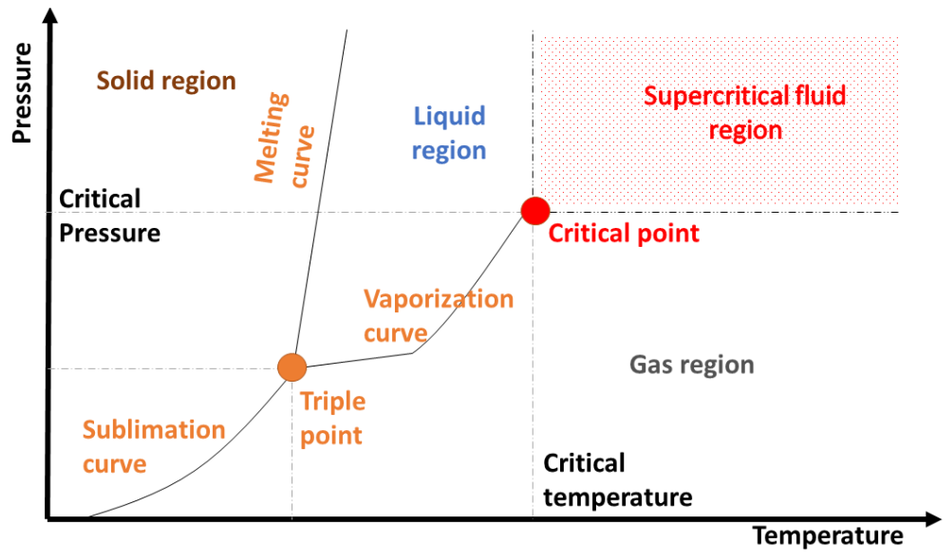
Likewise, other authors such as Gomez et al. (2020;2021) and Liao et al. (2018), have employed HPU-assisted microbial inactivation mechanism in combination with other technologies such as SF, Non-Thermal Plasma (NTP), etc. These, among other studies, have shown that HPU as the sole inactivation method does not achieve total inactivation of microorganisms (Li et al., 2016), however, the conjunction of this with other non-thermal technologies can obtain more effective results (Li et al., 2017). In the Combined Technologies section, the synergy of the use of HPU assisted by other methodologies can be analyzed.

6.3.3 Supercritical Fluids

As its name indicates, Supercritical Fluids (SF) are characterized by having pressure and temperature conditions beyond the critical point (McHug et al., 1994), being indistinguishable between a liquid and a gas, since some of its physicochemical properties oscillate between these two states of matter (Montañés 2009). Figure 6.3 shows the diagram of the different phases of a pure compound, visualizing the commonly known states of matter: solid, liquid and gas; it also shows a point where this triad converges, the so-called triple point (Montañés, 2009).

At the triple point all phases coexist. Scaling on the vaporization curve, there is a point where pressure and temperature are critical, thus modifying some of the physicochemical properties of the compounds, among the most important or of greatest interest are density, diffusivity and viscosity. The interest lies in the fact that they confer to this type of fluids the ability to be a better extraction solvent compared to if it were a simple liquid or gas (Paniagua, 2017).

Figure 6.3 Representative diagram of the different phases of a pure compound



Source: (Mendiola, 2008)

The use of Supercritical Carbon Dioxide (SC-CO_2) has generated great interest thanks to the pros resulting from its use as a solvent. SC-CO_2 is the ideal gas: it is inert, non-toxic and non-flammable; it is inexpensive and affordable, easily disposed of and reusable (Ortuño, 2014), it is also easy to control and is generally regarded as safe (Generally recognized as safe GRAS is a designation by the FDA that some chemical or substance added to food is safe).

Another advantage of SC-CO_2 is that both critical temperature (Tc) and critical pressure (Pc) are achieved without high energy demands. Pc is reached at 73.86 bar and Tc at only 31°C (Velasco et al., 2007). This property influences the preference of CO_2 over other solvents. In addition, many of the commonly used substances are more likely to leave product remnants, interfering with the purity of the desired extract.

Treatment with SC-CO_2 is revolutionizing the market in multiple industries, such as food, chemical, cleaning, pharmaceutical, agri-food, among others; this derived from the need to search for new and better methods of obtaining and/or preserving the active product of interest by manufacturers to consumers, who are increasingly aware and, therefore, demanding regarding the quality of the final product they purchase (Ozuna, 2014).

6.3.4 Use of supercritical CO2 in obtaining and preserving biological products for clinical use.

The characteristics of SC-CO_2 give it the potential to act as a solvent and extract active compounds or as an inactivator of the cell wall of microorganisms (Ortuño, 2014), contributing to the safety and preservation of the final product.

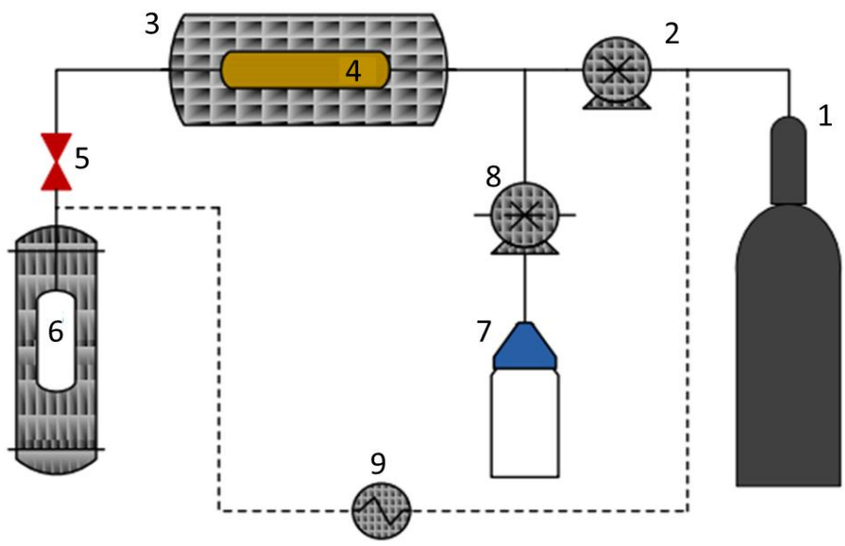
6.3.4.1 Obtaining bioproducts

The physicochemical properties of SC-CO_2 make it an effective extraction solvent. Its density, compared to that of a gas is 100 to 1000 times higher, but approaches liquid values (Paniagua et al., 2017), in addition to the fact that it can easily vary this parameter by increasing or decreasing pressure and temperature (Raventós et al., 2002).

This makes it a solvent of interest. On the other hand, the viscosity is 10 to 100 times lower than that of liquids, and conversely, the diffusivity 10 to 100 times higher compared also to liquids (Paniagua et al., 2017); viscosity and diffusivity being approximate to the values of gases. In addition, the surface tension of SFs is equal to zero, facilitates the extraction of substances contained in solid matrices. These properties (density, diffusivity and viscosity) cause an increase in the mass transfer rate of the solute in supercritical fluids (Brunner, 2005).

The use of SF extraction technology is relatively inexpensive when applied at an industrial level; Figure 6.4 shows the basic components of an SF extraction system (Montañes, 2009). According to Figure 6.4, the CO_2 coming from the bottle is pumped by the pump to the extraction cell where the raw material to be extracted is located, which is usually inside an oven that controls the temperature to be reached during extraction above the critical point. The components of the raw material dissolved or entrained by the CO_2 precipitate in the separator due to the decrease in the solvent power of the CO_2 as the pressure is reduced. If the addition of modifiers is necessary, they are usually mixed with the CO_2 stream by means of a pump before entering the extract. In addition, if CO_2 recirculation is required, it would be necessary to liquefy the CO_2 at the outlet of the separators by means of a cooling stage with a heat exchanger (Montañés, 2009).

Figure 6.4 Basic diagram of a supercritical fluid extractor



1.CO_2 container; 2. Pump; 3. Oven; 4. Extraction cell; 5; 7. Modifier container; 8. Pump; 9. Heat exchanger. (Montañés 2009)

It is worth mentioning that in most cases the feedstock requires grinding as a pretreatment to increase the extraction rate.

One of the major advantages of using this technology is that when the SC-CO_2 extraction and separation cycle is completed, it is reused, reducing waste generation, lowering production costs and environmental impact, considering the use of SC-CO_2 as a green solvent.

The recovery of active bioproducts is of great interest due to their development potential for the manufacture of functional compounds (Essien et al., 2020), which serve as replacements for active components of synthetic origin. These compounds are extracted from natural sources of animal or plant origin. The extraction of bioactives is a complex process, because other structures are protecting the desired functional moiety. In the case of plants, bioactives are surrounded by insoluble structures, such as vacuoles and lipoproteins, complicating extraction (Corrales et al., 2008).

The healing properties of plant extracts have been known for centuries: they work as treatment for infections, fever, repel insects, as food additives (Ragab and Raafat, 2016). Such is the case of Pulicaria j., which has been shown to possess antimicrobial and antifungal properties and has even been used as an anti-malaria and anti-insect treatment (Algabr et al., 2012; Fawzy et al., 2013; Ragab and Raafat, 2016).

Al-Maqtari et al. (2020), evaluated the total phenolic content, antioxidant activity by DPPH-, antioxidant capacity by ABTS-+ and antimicrobial activity of *Pulicaria j*. extract, using SC-CO_2 extraction and 10% ethanol (EtOH10%) as cosolvent; the addition of a cosolvent such as ethanol is necessary to increase the solubility of polar organic components, because the solubility of CO_2 alone is low. The equipment used for the assay was an SF extractor (Waters, Milford, MA), at temperature conditions of 40°C, 300 bar for 120 minutes. Extraction with SC-CO_2 - EtOH10% was compared with conventional extraction methods, such as stirring supported with two solvents (ethanol and water) at different concentrations (100, 70, 50, 50, 30 and 0% v/v). The results of this experiment showed that the best extraction yield was obtained with the shaking-EtOH50% method, followed to the shaking-EtOH70%, while the SC-CO_2 - EtOH10% treatment obtained the lowest extraction (Al-Maqtari et al., 2020). The authors conclude that the results could be due to the fact that SC-CO_2 extraction is not suitable for obtaining sugars, water-soluble compounds and some phenolic compounds (Roseiro et al., 2013).

In 2021, Al-Maqtari et al. performed another assay on extraction of four aromatic herbs (*Artemisia arborescens, Artemisia abyssinica, Pulicaria j*., and *Pulicaria p*.) to evaluate the antioxidant activity by DPPH-, antioxidant capacity by ABTS-+ and antimicrobial activity of the extract of these plants by SC-CO_2. The procedure was carried out in an SF extractor (Waters, Milford, MA); at 40°C temperature, 300 bar pressure and 120 min processing time, with addition of a cosolvent (ethanol 10%). The highest extraction yield (at 8.92±0.41) corresponds to *Artemisia abyssinica*., showing a significant difference compared to the other three plants (Al-Maqtari et al., 2020). This variation could be attributed to the difference in abundance of polar compounds between one species and another (Mustapa et al., 2015). However, as many other factors are also involved in the extraction yield rate of herbs, such as the difference in plant structures, size of leaves and stems, moisture, essential oils and volatile compounds contained. Despite the aforementioned data, when evaluating the antioxidant activity by DPPH-, antioxidant capacity by ABTS-+ and antimicrobial activity it was revealed that the extract coming from Pulicaria j. was significantly superior than that of the remaining three plants (Al-Maqtari et al., 2020).

On the other hand, one of the major applications in the use of SC-CO_2 has been the extraction and separation of lipids from multiple biological sources, such as plants and animals (Sohran et al., 2021). Lipids have diverse industrial and technological applications, such as polymers, coatings, pharmaceuticals, cosmetics, solvents, surfactants, lubricants, pesticides, biofuel, among others (Tao, 2007).

Olive oil, which is mostly known for its nutritional use, contains properties that also include it as an adjuvant in physiotherapy. Conventionally, prior to oil extraction, olive trees are crushed to reduce their size and to obtain the oil in a more efficient way; however, extraction by SC-CO_2 offers the possibility of obtaining the oil without previous crushing. Al-Otoom et al. (2014), studied the propensity of olive oil extraction with SC-CO_2 technology by testing different parameters regarding pressure, temperature and operation time. Subsequent to the tests they were able to evidence that, the maximum yield of 12.3% oil with respect to olive tree weight, was obtained at 2400 bar, 60°C and 150 minutes of treatment, thus demonstrating that the most effective parameter is extraction time, followed by temperature and, finally, operating pressure (Al-Otoom et al., 2014).

Although the main sources for obtaining lipids are of animal and plant origin, they can also be extracted from other microorganisms, such as bacteria or algae (Valenzuela, 2009). The extraction of lipids from microalgae is generally performed with the addition of organic solvents, however, this process cannot be replicated on an industrial scale because of the pollution it represents for the environment and the high toxicity for humans (Obeid et al., 2018); in addition to the fact that the organic solvent does not have good diffusivity on the penetration of the cell wall of microalgae, making diffusion into the interior of the cells difficult for a successful extraction of lipids. Obeid et al. (2018) studied the selective extraction of neutral lipids in freeze-dried *Nannochloropsis o*. and *Chlorella v*. microalgae.

The methodology was evaluated under different operating parameters (use of co-solvent, pressure and time), with a constant temperature of 50°C. The highest neutral lipid extraction (97%) observed for Chlorella v. was with the use of 10% ethanol as co-solvent, at 250 bar and 230 minutes of treatment; on the other hand, the maximum extraction recorded for *Nannochloropsis o.* was 83% under the same pressure conditions and 230 minutes of treatment. Microscopic observation showed that cell wall integrity was maintained during the extraction process (Obeid et al., 2018).

In addition to lipid extraction by microalgae, it is also possible to obtain other bioactive products, such as antioxidants (β-carotene) (Bhat and Madyastha, 2000), sulfated polysaccharides (antiviral) and sterols (antimicrobial) (Otles and Pire, 2001). Chatterje and Bhattacharjee, 2013 applied SC-CO_2 technology for the extraction of antioxidants from *Phormidium v*. The SC-CO2 extraction equipment was SPE-ED SFE 2 model of M/s Applied Separations, Pennsylvania, USA, at temperature of 50°C, 500 bar and 90 minutes processing time. As a result, it was observed that in 10g of *Phormidium v*. biomass, the best combination of phytochemical properties was extracted with a 93% reduction of anatoxin-a. It is worth mentioning, that the assay was also performed at 40°C, 350 bar and 90 min, and at these conditions the highest extract yield was seen, however, the desired properties were obtained at the above mentioned parameters (Chatterjee and Bhattacharjee, 2014).

6.3.4.2 Microbial inactivation

The inactivation of microorganisms by applying SC-CO_2 technology has been widely used within the food industry, where satisfactory results have been obtained. From the first time that SF was used as a non-thermal preservation technology by Fraser, (1951); Foster et al. (1962), to the present, a variety of research has been published on the inhibitory effect of SC-CO_2 on microorganisms such as viruses, bacteria, yeasts, fungi (Corwin and Shellhammer, 1992; Gasperi et a., 2009; Facroniet al., 2010).

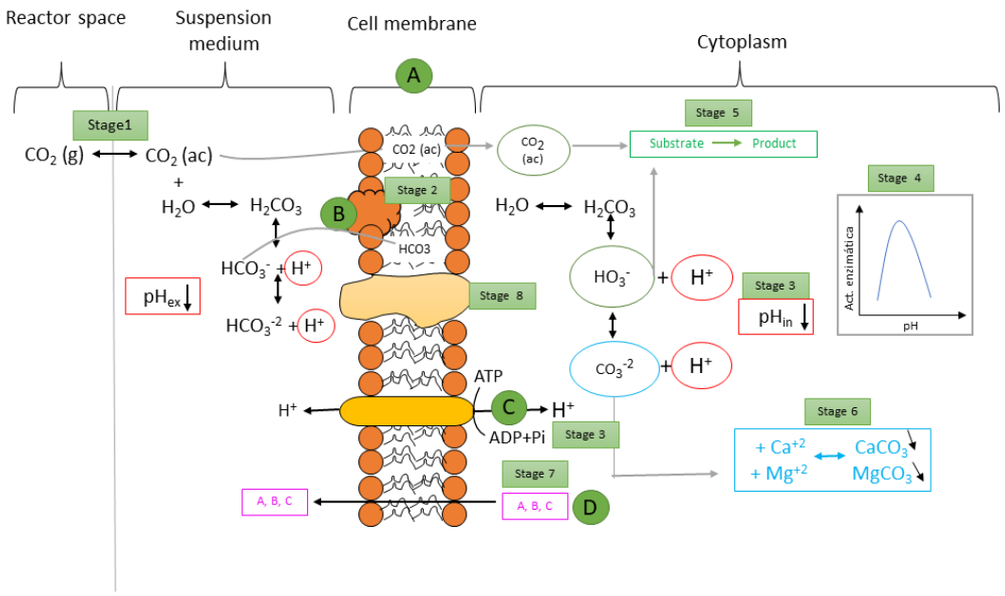
Dillow et al. (1999) subjected Gram-negative bacteria (*Salmonella s*., *Pseudomonas a*., *Escherichia c*., *Proteus v*. and *Legionella d*.) and Gram-positive bacteria (*Listeria i*. and *Staphylococcus a*.) to treatment with SC-CO_2 (205 bar at 34°C); resulting in higher resistance by Gram-positive bacteria compared to Gram-negative bacteria. This is due to the membrane composition of both classes of bacteria. Between the bilipid membrane of Gram-negative bacteria there is a thin layer of peptidoglycan (10%); in contrast, Gram-positive bacteria have a much thicker cell wall with respect to the percentage of peptidoglycan (90%), this property gives them greater resistance and lower permeability to external agents in advantage with Gram-negative bacteria that will be more susceptible due to their thin layer of peptidoglycan (Erkmen, 2012).

The rate of microbial inactivation depends on multiple factors, such as pressure, temperature, time and treatment medium, the nature of the microorganism, among others (Ortuño et al., 2014).

The model of the SC-CO_2 inactivation mechanism was proposed by Daniels et al. in 1985 and have been accepted by authors who have focused their research in this field of study (Dillow et al., 1999; Spilimbergo- Bertucco, 2003). Nowadays, these theories have been reinforced and better detailed thanks to technologies such as scanning electron microscopy and transmission electron microscopy, where it is possible to evaluate the condition of cells before and after treatment (Oulé et al., 2006). Thanks to previous research it is possible to compile the mechanisms of SC-CO_2 inactivation in a series of eight steps: (1, Figure 6.5) dissolution of pressurized CO_2 into the liquid phase where the cells are suspended and decrease in pH; (2, Figure 6.5) diffusion of CO_2 and modification of the cell membrane; (3, Figure 6.5) penetration of CO_2 into the cell interior and decrease in intracellular pH; (4, Figure 6.5) inhibition of cell metabolism due to inactivation of key enzymes; (5, Figure 6.5) direct inhibition of cell metabolism in response to the presence of CO_2 and formation of HCO^{3-}; (6, Figure 6.5) imbalance of intracellular electrolytes; (7, Figure 6.5) removal of intracellular components; (8, Figure 6.5) cell rupture (Damar-Balaban, 2006; Garcia-Gonzalez et al. , 2010).

Figure 6.5 schematizes the mechanism of microbial inactivation of SC-CO_2, emphasizing that this series of steps do not occur in a concatenated manner, but occur simultaneously.

Figure 6.5 Scheme of the stages of the inactivation mechanism by SC-CO_2: A, phospholipid bilayer; B, protein membrane; C, membrane; D, intracellular components



Source: (García González et al. 2007)

Qing et al, 2009 worked with a porcine acellular dermal matrix which was inoculated with a series of microorganisms, such as bacteria (*Enterobacter aerogenes*, *Staphylococcus cohnii*, *S. haemolyticus*, *Bacillus atrophaeus*), viruses [porcine encephalomyocarditis virus (EMC), porcine parvovirus (PPV), porcine pseudorabies virus (PRV) and murine leukemia retrovirus (LRV)], fungi (*Penicillium sp*, *Aspergillus sp*, *Verticillium sp*) and yeasts (*Debaryomyces hansenii*); all samples were treated with SC-CO_2 with the addition of a sterilizing agent such as paracetic acid (PAA). The different matrix samples inoculated with the different microorganisms were packed in a Tyvek® bag and treated in SC-CO_2 equipment (NovaSterilis, NY) with addition of PAA at temperature of 35-41°C, pressure of 94.1-100.3 bar, during variations from 1 to 30 minutes. At the end of sterilization, the biochemical and physical properties of the matrix were evaluated by enzymatic digestion and physical tests. The results showed that after 1 minute of treatment the vegetative forms of bacteria and yeast were reduced by 7 logarithmic cycles, while *Bacillus atrophaeus* was more resistant to the same treatment by reducing only 3 logarithmic cycles. For the sterilization of fungal forms, 5 minutes of treatment with SC-CO_2-PAA was used, obtaining a reduction of 6 logarithmic cycles, showing greater resistance than bacteria and yeast despite the longer sterilization time; however, fungi were more sensitive to the treatment compared to *Bacillus atrophaeus* due to a reduction of 2 logarithmic cycles. Post-sterilization, evaluations through enzymatic digestion and physical properties showed that SC-CO_2-PAA treatment does not cause significant changes in the structure and functionability of porcine acellular dermal acellular matrix (Qing et al., 2009).

6.3.5 Combined technologies

Techniques based on power ultrasound and supercritical carbon dioxide are useful for the extraction of substrates or for microbial inactivation; however, there are occasions in which they do not achieve a complete or optimal extraction or inactivation, without obtaining the desired results or prolonged process times are required to obtain satisfactory results.

Not obtaining the expected result on inactivation of aerobic mesophiles and enterobacteria in goat milk and human milk, Perez, (2020), attended HPU with SC-CO_2 equipment operating at 50W±5W/cm^2, at frequencies of 30±2kHz, at different variations of pressure and temperature, studying on the one hand the inactivation of aerobic mesophiles and on the other hand, the inactivation of enterobacteria. For the first class of microorganisms, in the treatment at low pressure and temperature (150 bar and 35°C), the aerobic mesophiles underwent a total inactivation of 4.72 logarithmic cycles in five minutes.

At high pressure and low temperature conditions (350 bar and 35°C) total inactivation of 4.39 logarithmic cycles was achieved in four minutes. Finally, in the last test, by increasing the temperature to 50°C and lowering the pressure to 150 bar again, total inactivation of 4.35 logarithmic cycles was observed to occur in only two minutes. This shows that at higher temperatures and lower pressures, in the case of mesophilic microorganisms, total inactivation is achieved in a shorter period of time compared to the previous conditions. In the case of Enterobacteriaceae, under conditions of low pressure and temperature (150 bar and 35°C) total inactivation of 3.3 logarithmic cycles was achieved in minute two; at conditions of 350 bar and 35°C total inactivation of 2.66 logarithmic cycles was witnessed at one minute of treatment, at 150 bar and 50°C a reduction of 3.29 logarithmic cycles was achieved in 2 minutes of treatment. Contrary to what happened with mesophilic microorganisms, enterobacteria achieved inactivation in less time using the parameters of low temperature at high pressures (Perez, 2020).

According to the results obtained through the previous work, it is possible to show that the HPU treatment does not show much effectiveness on its own; however, the HPU-SC-CO_2 combination shows a synergistic effect, achieving total inactivation of microorganisms.

Gomez-Gomez et al., (2020) tested the combined HPU-SC-CO_2 technology for pasteurization of lipid emulsions for clinical use on *B. diminuta*. and *E. coli* Lipid emulsions are a fatty acid preparation administered venously for those individuals who cannot obtain them orally through diet. The ultrasound system (Model WT210, Yogogawa, Japan) was programmed at power of 50 ±5 W/cm2 and frequency of 30 ±2 kHz. In conjunction, for the SC-CO_2 equipment (LDB, LEWA, Japan), a parameter variation of 100 and 350 bar and temperature of 35 and 50°C was used for 50 minutes of treatment. The experiment was also performed testing conventional thermal inactivation, which was carried out at the same 50°C and 50 minutes of treatment; this system only managed to obtain a reduction of 0.4 logarithmic cycles in E. coli and 0.9 logarithmic cycles in *B. diminuta*. The maximum reduction for E. coli using the combined HPU-SC-CO_2 technology at 350 bar, 50°C and 50 min processing conditions was about 7.5 logarithmic cycles.

In contrast, under the same conditions, *B. diminuta* was more sensitive to treatment, having a maximum reduction of about 8.4 log cycles. As a result of the multiple trials carried out, tested at different pressures and temperatures, it was evident that pressure had a significant effect on the inactivation of both genera of bacteria (Gómez-Gómez et al., 2020).

The synergistic bacterial inactivation power of HPU-SC-CO_2 could be essential not only for vegetative forms, but also for microorganisms with more resistant, complex and reinforced structures, such as spores (Dong et al., 2016). Bacteria form spores as a mechanism of resistance to extreme chemical and physical environments (Spilimbergo-Bertuco., 2003); therefore, processes are required that can achieve the necessary conditions for spore inactivation without compromising product safety. The use of HPU alone has proven to be ineffective for total spore inactivation (Mondal et al., 2015), however, the combination with SC-CO_2 improves the process of mass and heat transfer, together with the cavitation mechanism produced by HPU that would cause the degradation of the outer layers of the spore structure128, facilitating the penetration of SC-CO_2 inside the cells.

Gómez-Gómez. et al. 2021 evaluated the combination of these two technologies for the inactivation of spores from *Bacillus s.*, *Bacillus p*. and *Geobacillus s*. The system consisted of a SC-CO_2 extractor (LDB, LEWA, Japan) and an ultrasound system (WT210, Yokogawa Electric Corporation, Tokyo, Japan) at conditions of 35±5 W/cm^2 power, frequency of 30±2 kHz. For *Bacillus s*. the treatment was carried out at conditions of 85-95°C, 350 bar and 20 minutes of processing. On the other hand, for *Bacillus p*. and *Geobacillus s*. the temperature was set at 95°C, also changing the pressure to 550 bar for *Geobacillus s*. The results obtained were compared with the conventional thermal treatment, showing that inactivation with HPU-SC-CO_2 was higher by 2.5 and two for Bacillus s and Bacillus p., respectively. On the other hand, the same system was not effective for *Geobacillus s*., as there was no significant difference between the use of HPU-SC-CO_2 versus conventional heat treatment (Gómez-Gómez et al., 2021).

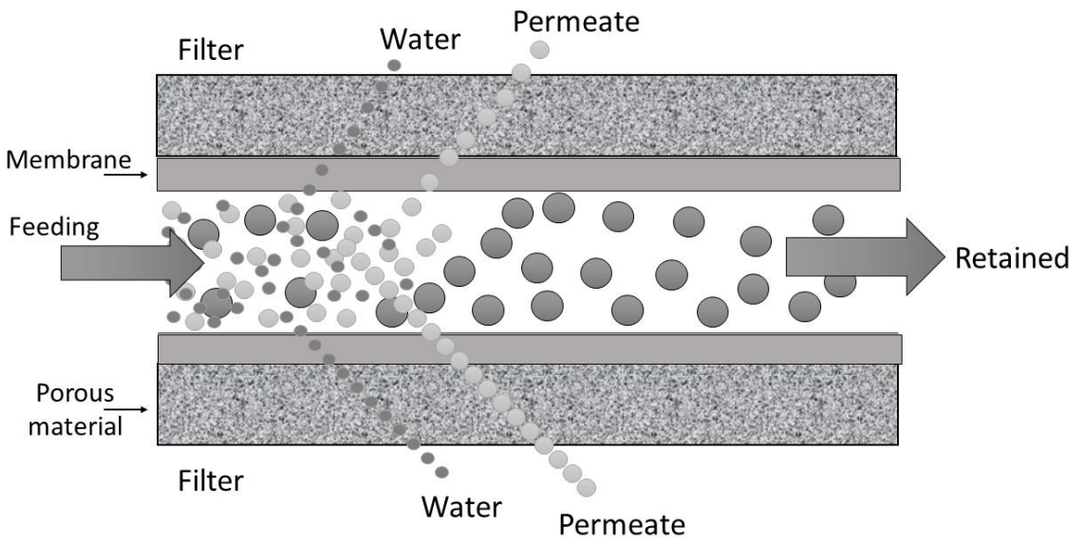
In addition to the combination with SC-CO_2, other technologies that intensify the power of HPU have been experimented. One example is the use of non-thermal plasma (NTP). Liao X. et al., 2018 employed this mechanism in conjunction NTP-HPU for the inactivation of *Staphylococcus a*. After several trials with different methodologies the highest reduction of 4.04 log cycles of *Staphylococcus a*. was obtained at 5 minutes of pretreatment with NTP followed by 10 minutes of HPU at 20 kHz and 200W/cm^2. In contrast, the use of HPU without pretreatment achieved only a 0.55 log cycle reduction in 20 minutes of treatment (Liao et al., 2018).

Reviewing the HPU and SF sections alone and, subsequently, comparing them with the combined technologies section, it can be analyzed that the use of the latter technologies represents better results than the former. The synergy of their mechanisms achieves good yields for both extraction and microbial inactivation.

6.3.6 Membrane Technology Processes

Membrane Technology (MT) is a method that consists of the separation or filtration of solutes present in a liquid; this separation is based on the difference in size and molecular weight between the particles suspended in the fluid (Sastre et al., 2009). The classification of MT processes is defined on the basis of the diameter of the pores of the membrane, which allow the concentration by retention of the larger components with respect to the pore itself, this product is called retained; on the other hand, substances smaller than the diameter of the pore, which are able to pass through it, are called permeate (Peña, 2006). Figure 6.6 shows an example of the membrane technology process.

Figure 6.6 Separation scheme with semi-permeable membranes



Source: (Solís et al., 2016)

The membrane process to be applied depends on the product to be obtained or, alternatively, the product to be removed. Table 6.3 summarizes the types of processes, pore diameter and examples of products to be separated (Pandolfi, 2008).

Table 6.3 Membrane processes

Type of process	Pore diameter	Examples
Microfiltration	10 - 0.1µm	Microbial cells, large colloids, small particles, etc.
Ultrafiltration	<0.1µm - 5nm	Proteins, emulsions, macromolecular colloids
Nanofiltration	Approx. 1nm	Organic compounds and dissolved salts
Reverse osmosis	<1nm	Small organic compounds, dissolved salts
Electrodialysis	<5nm	Dissolved salts
Dialysis	<5nm	Clinical treatment of renal failure

Source: "Membrane processes" by Pandolfi, 2008

6.3.6.1 Use of membrane technology to obtain biological products for clinical use

Separation processes are used in many industries to purify a compound of interest from the rest of the mixture, or otherwise to eliminate that component in order to increase the purity of the original solution. Whatever the case, the separation is based on the differences in the physical and chemical properties of these components (Mohsenin, 1980).

The obtaining of natural products has been of great relevance during the last few years, and this is due to the fact that consumers demand better quality products, which maintain their biological properties intact without changing their physical form. Researchers have opted to try new natural resources from which these bioactives can be obtained, sources that until a few years ago had not been exploited. Such is the case of algae, capable of producing various types of biomolecules such as carotenoids, lipids, polysaccharides, proteins, amino acids and carbohydrates (Khoo et al., 2020).

Denis et al. (2009) tested the feasibility of implementing ultrafiltration as the only separation step. For this, they used a tubular polyethersulfone membrane (PCI-MT600), surface area of 0.033 m^2 and molecular weight cut-off of 25-30 kDa. The permeate flux obtained was 35.1 Lh-1 m-2 at 7°C and 4 bar, allowing the retention of 100% of R-phycoerythrin pigment from the macroalgae *Grateloupia t*. Many times, obtaining and separating the desired product requires more than one process to achieve good purity, Safi C et al. (2014), obtained different biomolecules from *Tetraselmis s*. microalgae extract by two consecutive stages of ultrafiltration treatment. The test was performed with two membranes (TFF system Millipore, USA) of different molecular weight cut-off, during the first ultrafiltration it was possible to separate the starch present in the mixture with a cut-off of 100kDa, pressure of 2.07 bar for 30 minutes allowing a flux of 47.83kg h-1 m-2. In a second continuous stage, retention of proteins and sugars of interest was possible under the same pressure and time conditions, with a membrane cut-off of 10kDa, allowing a flux of 42.8±1.3kg h-1 m-2(Safi et al., 2014).

The recovery of value-added compounds involves different stages: macroscopic extraction, separation of macro- and micromolecules, extraction, purification and the final product (Galanakis, 2013). For the second and fourth stage processes, mainly microfiltration, ultrafiltration and nanofiltration techniques have been preferred, because their membrane characteristics allow for easy removal of suspended solids, concentration of molecules and clarification of smaller analytes178. Particularly, the MT of choice is UF, as it requires very low transmembrane pressure, inferring in better performance, lower energy expenditure and costs (Li et al., 2011). UF is regularly used for the separation of protein compounds. Modi et al., (2019) achieved separation of lysosome, trypsin, pepsin, human serum albumin, gamma globulin and fibrinogen with rejection values of 92.9±1.3%, 94.5±1.1%, 96.9±1.2%, 99.5±0.5%, 100% and 100%, respectively. Separation was performed with a polyethersulfone hollow fiber ultrafiltration membrane with a flux 110.0±3.8 L/m^2h, with a high flux recovery of 97.8%. UF was assisted by iron oxide nanoparticles-nanosheets decorated with carboxylated graphene oxide, this synergy proved to be efficient for the separation of biomolecules, especially proteins (Modi et al., 2019).

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Membrane Technology (MT) is a separation process that has the advantage of low temperature and energy requirements, it is considered a cheap system since it does not require many solvents and chemicals, and those used can be instantly recycled, it also achieves a clean separation, keeping the components in their native state, and it does not require precipitation, centrifugation and dialysis stages that can dilute the samples (Marella et al., 2013).

6.4 Conclusion

According to the literature review analyzed throughout this work, it can be observed that non-thermal technologies, such as Power Ultrasound, Supercritical Fluids, in particular Supercritical Carbon Dioxide, and Membrane Technologies are tools that, compared to conventional extraction methods, have marked advantages, among which are lower energy consumption, shorter extraction time, less damage to the active components and high extraction yields.

The synergy of the technologies not only showed good results during the extraction of extracts; it also favored the logarithmic reductions of the different microorganisms subjected to sterilization, marking a gap between the use of a particular technology and the combination between them.

The study of these new technologies is relatively new and in spite of this it has had an accelerated growth in many parts of the world due to the satisfactory results that have been reported by multiple researchers from different industrial areas, however, more research is required to take these technologies to a large scale, since many experiments have only been carried out at laboratory level. Even so, the future of non-thermal technologies seems to be very promising and beneficial in every sense.

6.5 Glossary

ABTS-. 2,2 azino bis(3-ethylbenzo thiazolin-6 sulfonic acid), a chemical compound involved in redox reactions.

Nucleic acids. Structurally composed of a nitrogenous base, a pentose and a phosphate group, they constitute the genetic material of organisms, storing their genetic information.

Human serum albumin. Main protein present in plasma with multiple functions, where the main one is to transport or inactivate substances such as heavy metals, drugs, fatty acids, hormones and enzymes, besides being an excellent expander of plasma volume.

Alkaloid. Nitrogenous organic compound of vegetable origin with good pharmacological activity at low doses, since they have a high toxicity.

Allergen. A product that elicits an immunological response to an allergenic agent.

Antibody. A glycoprotein produced by B-lymphocytes that binds to antigens, often with a high degree of specificity and affinity.

Antigen. A product that elicits an immune response to a foreign agent.

Anthocyanin. A plant pigment belonging to the flavonoid group that produces the red to blue colors in fruits and vegetables. It is attributed with a reduction in coronary heart disease, anticancer, anti-inflammatory and antidiabetic effects, improvement of visual acuity and cognitive behavior.

Anthoxanthin. A colorless, astringent compound derived from the flavonoid group with antioxidant properties.

Anthraquinone. Main group of natural quinones. Aromatic polyhydroxylated compound, if it has OH in positions 1 and 2 it presents coloring properties, on the other hand, if the OH are in positions 1 and 8 the effect is laxative.

Apigenin. A type of flavonoid that reduces oxidative stress, has sedative, anxiolytic, antimutagenic, antitumor, antiallergic and anti-inflammatory effects, as well as regulating different signaling pathways.

Baicalin. A type of flavonoid with diverse pharmacological activities, such as antitumor, antimicrobial and antioxidant.

Carotenoid. Tetraterpenes that leak as natural liposoluble pigments synthesized by plants, algae and photosynthetic bacteria, with antioxidant properties.

Cytokine. Proteins that mediate inflammatory and immune reactions.

Derivatives of human blood and plasma. Blood components for therapeutic, diagnostic, preventive or research applications.

DPPH-. 2,2-diphenyl-1-picrylhydrazyl is a free radical that determines the antioxidant capacity of foods and synthetic compounds characterized by the yielding of a hydrogen provided by the antioxidant agent.

Lipid emulsions. fatty acid preparation administered venously for those individuals who cannot obtain them orally through the diet.

Enzyme. Molecules that catalyze the conversion of compounds to one or more different compounds by increasing the rates of the reaction.

Sterols. Derivatives of steroids with a cyclopentanohydrophenanthrene base nucleus, an OH group at carbon 3, and most, a side chain of 8 or more carbons at carbon 17. Compounds of interest for the production of steroid drugs.

Fibrinogen. Protein that functions as factor I of the coagulation cascade and the final substrate from which the clot is produced.

Flavonoids. The most abundant group of polyphenols, derived from phenylalanine and tyrosine. They decrease the incidence of cardiovascular diseases.

Gamma globulin. A positively charged globular protein present in blood serum. The main gamma globulins are immunoglobulins or antibodies.

Glycoside. Each of the organic substances, existing in many vegetables, which by hydrolysis produced by the action of dilute acids give, as products of decomposition, glucose and other bodies, many of which are energetic poisons and, in very small doses, are used as drugs.

Hormone. A chemical produced by tissues that has a specific effect on a target tissue.

Immunoglobulins. Proteins capable of behaving as an antibody.

Lysosome. Cellular organelles containing digestive enzymes, which aid in the elimination of viruses and bacteria. They are also responsible for recycling cellular waste and participate in apoptosis.

Luteolin. A type of flavonoid produced by various medicinal plants, with antioxidant, free radical scavenger, anti-inflammatory, neuroprotective, anticarcinogenic and antiallergic properties.

Pectin. Vegetable heteropolysaccharide contained mainly by D-galacturonic acid; purifying properties are attributed to it as it eliminates residues and toxins from the organism.

Pepsin. Enzyme produced by the stomach that breaks down food proteins during digestion.

Polyphenols. Compounds made up of one or more aromatic rings with at least one hydroxyl group linked, originated by the secondary metabolism of plants with diverse functions, such as nutrient assimilation, protein synthesis, enzymatic activity, formation of structural components and marked antioxidant activity; in addition to anti-inflammatory, antiallergic, antimicrobial, antineoplastic and anticarcinogenic properties.

Reagents for in vitro diagnostics. Reagent product, calibrator, made in control material, intended for the study of samples from the human body.

R-phycoerythrin. A class of phycobilin contained in microalgae are used as cell markers, cell analysis and immunoassays due to their fluorescence emission properties.

RNA. Ribonucleic acid, interferes with the transfer of information contained in DNA into cellular compartments.

Immune sera. Serum containing polyclonal antibodies. They are used for passive immunization of certain diseases.

Trypsin. A proteolytic enzyme produced by the pancreas and secreted into the duodenum, it cleaves peptide bonds of proteins to obtain small peptides and amino acids.

Vaccine. Antigenic preparation that induces immunity against infections.

β-carotene. A type of carotenoid with provitamin A activity.

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Chapter 7 Obtaining and characterization of the ethanolic extract of the leaves of the *Tradescantia Spathacea* SW

Capítulo 7 Obtención y caracterización del extracto etanólico de las hojas de *Tradescantia Spathacea* SW

ESTRADA-HERNÁNDEZ, Gladys Vianey†´, FIGUEROA-RAMÍREZ, Sandra Jazmín*´, SIERRA-GRAJEDA, Juan Manuel Tadeo´ and AGUILAR-UCÁN, Claudia Alejandra´´

´Universidad Autónoma del Carmen, Faculty of Engineering, Campus III, Avenida Central S/N, Esq. con Fracc. Mundo Maya, C.P. 24115, Ciudad del Carmen, Campeche, Mexico.

´´Universidad Autónoma del Carmen, Faculty of Chemistry, Main Campus, Calle 56 No. 4 Av. Concordia, C.P. 24180, Ciudad del Carmen, Campeche, Mexico.

ID 1st Author: *Gladys Vianey, Estrada-Hernández* / **ORC ID**: 0000-0003-0238-2324, **CVU CONACYT ID**: 1007995

ID 1st Co-author: *Sandra Jazmín, Figueroa-Ramírez* / **ORC ID**: 0000-0003-1968-1741, **CVU CONACYT ID**: 164797

ID 2nd Co-author: *Juan Manuel Tadeo, Sierra-Grajeda* / **ORC ID**: 0000-0002-0565-6450, **CVU CONACYT ID**: 219284

ID 3rd Co-author: *Claudia Alejandra, Aguilar-Ucán* / **ORC ID**: 0000-0002-1733-2867, **CVU CONACYT ID**: 93717

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G. Estrada, S. Figueroa, J. Sierra and A. Aguilar

* sfigueroa@pampano.unacar.mx

A. Marroquín, J. Olivares, A. Dector L. Cruz. (Coord.) CIERMMI Women in Science TXIV Biology, Chemistry and Life Sciences. Handbooks-©ECORFAN-México, Querétaro, 2021.

Abstract

In the last 20 years, chemical studies of plants in México have increased notably, intending to provide society with alternative mechanisms without damaging the atmosphere and the environment, ensure effectiveness and efficiency. There is a great diversity of native plants in the Mexican southeast, such as *Tradescantia Spathacea SW*, which has antioxidant and antibacterial properties. For this reason, this work presents the obtaining and characterization of the ethanolic extract of the leaves of the Tradescantia *Spathacea SW* (Purple Maguey) plant. The leaves were obtained from Nayarit Castellot, Champotón Campeche, and the ethanolic extract was obtained by the traditional method using purification processes. As a result, the ethanolic extract was obtained without purification, which was characterized by phytochemical and spectroscopic techniques. Phytochemical tests and thin layer chromatography showed polyphenols, and UV-VIS and FTIR spectroscopy showed the presence of the phenol group. The extract obtained in this work will be subsequently evaluated as a corrosion inhibitor in API 5L-X52 steel.

Extract, Purple Maguey, Maceration, *Tradescantia spathacea* SW

Resumen

En los últimos 20 años, los estudios químicos de las plantas en México han incrementado notoriamente, con el objetivo de proporcionar a la sociedad mecanismos alternos sin la nocividad a la atmósfera y el medio ambiente asegurando efectividad y eficiencia. En el sureste mexicano se tiene una gran diversidad de plantas nativas, como es la *Tradescantia Spathacea SW,* la cual tiene propiedades antioxidantes, y antibacterianas. Por esta razón, en este trabajo se presenta la obtención y caracterización del extracto etanólico de las hojas de la planta *Tradescantia Spathacea SW* (Maguey morado). Las hojas se obtuvieron en la localidad Nayarit Castellot, Champotón Campeche y el extracto etanólico se obtuvo mediante el método tradicional empleando procesos de purificación. Como resultado, se obtuvo el extracto etanólico sin purificación, el cual se caracterizó mediante técnicas fitoquímicas y espectroscópicas. Las pruebas fitoquímicas y cromatografía en capa fina mostraron la presencia de polifenoles y la espectroscopia UV-VIS y FTIR mostraron la presencia de grupo fenol. El extracto obtenido en el presente trabajo será evaluado posteriormente como inhibidor de corrosión en el acero API 5L-X52.

Extracto, Maguey Morado, Maceración, *Tradescantia spathacea* SW

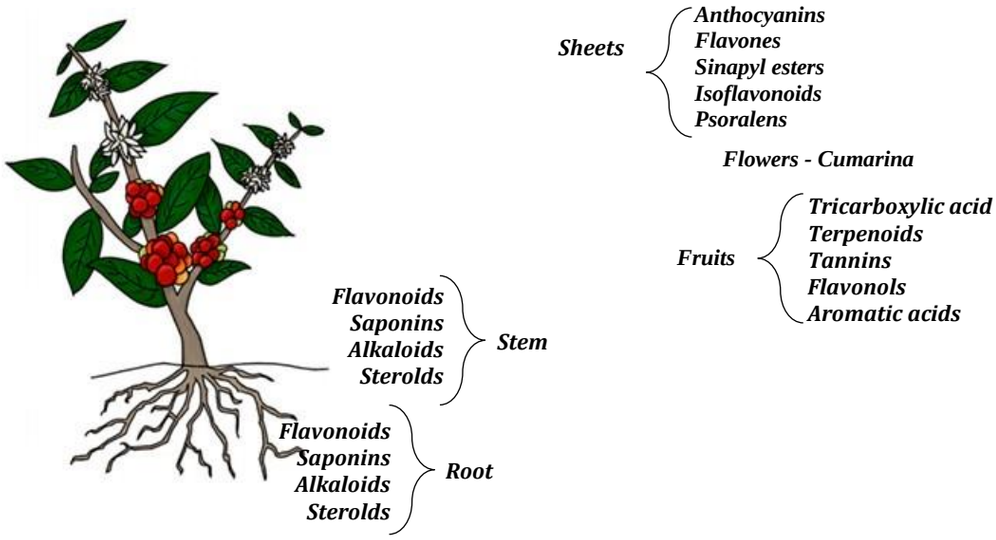
7.1 Introduction

In order to find practical and sustainable solutions to everyday problems in the industrial sector, such as internal and external corrosion problems in the oil industry (offshore platforms). Corrosion inhibitors have been used as an internal protection measure for metals.

The inhibitor is a chemical substance added in small concentrations to a corrosive electrolyte whose function is to slow down the corrosion rate of the exposed metal (Umoren et al., 2019). Commercial inhibitors used in practice are expensive, highly toxic and environmentally damaging. For this reason, scientists have focused their research on the search for natural or green inhibitors. Novel green inhibitors have been synthesised from a variety of natural resources, mainly using plants, as they are easy to obtain and non-toxic.

Plants are composed of leaves, fruits, flowers, stems and roots, so they can contain different organic compounds such as flavonoids, polyphenols and polysaccharides that act as inhibitors (see figure 7.1). These have been obtained from flowers, leaves and roots of different plants showing the ability to inhibit the corrosion process (Kesavan et al., 2012; Mazumder & Abstract, 2013; Miralrio & Vázquez, 2020a; Tejeda Benítez et al., 2014).

Figure 7.1 Basic parts of a plant and their common organic compounds



Source:(Miralrio & Vázquez, 2020b)

Particularly, *Tradescantia spathacea SW* also known as Rhoeo discolor, *Rhoeo spathacea (Sw.)*, *Tradescantia discolor* and commonly called maguey morado, is a herbaceous species belonging to the commelinaceae family and native to Belize, Guatemala, Gulf of Mexico and Southeast Mexico (see figure 7.2). Its study has focused on medicinal, food and, to a lesser extent, energy and textiles (Lai et al., 2008; Reyes-Munguía et al., 2009).

However, its main use has been in the field of medicine, as it has properties to inhibit the growth of cancer cells, antibacterial, stomach pain, headache, anti-inflammatory, asthma and cough, among others (Prakash & Rajesh, 2014). It is important to mention, Tradescantia Spathacea SW is very abundant in the southeastern region of Mexico, it is easy to reproduce due to the environmental conditions. It has also been reported to contain phenolic compounds and flavonoids showing antioxidant activities (Tan et al., 2015).

Figure 6.2 *Tradescatia Spathacea* SW plant

Source: (Own elaboration)

For this reason, the present work presents the extraction of Tradescantia Spathacea SW plant leaves by the maceration method and identifies the organic compounds it contains by means of phytochemical tests, thin layer chromatography and spectroscopic techniques such as ultraviolet-visible spectroscopy (UV-Vis) and Fourier transform infrared spectroscopy (FTIR).

7.2 Methodology to be developed

7.2.1 Harvesting, cleaning and drying of leaves

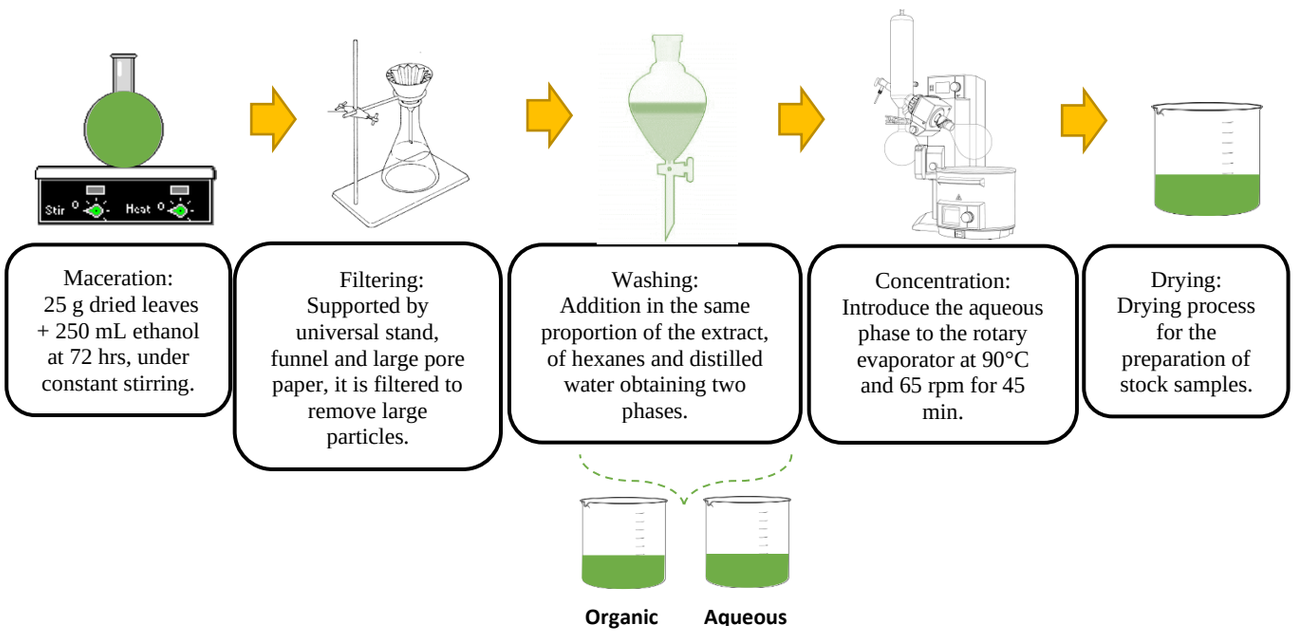
The leaves of the Tradescatia Spathacea SW plant were obtained on 27 November 2020 in the locality of Nayarit Castellot (Xnoha) located in the municipality of Champotón in the state of Campeche, a place that according to the literature has optimal conditions such as: soil type, temperature, pH, humidity, altitude, etc., which guarantee proper development and growth as well as quality in all components of the plant (Villarreal-Ibarra et al., 2015). Subsequently, the leaves obtained were washed with water and taken to the oven to dry for 4 days at 68 °C.

7.2.2 Preparation of the extract

The leaves of the dried Tradescantia spathacea SW plant were weighed to obtain the decrease in grammage. The extract was obtained by the maceration method. In a round flask equipped with a Vigraux column and magnetic stirrer, 25 g of dried leaves of the Tradescantia spathacea SW. plant and 250 mL of 95 % ethanol were added to the mixture, which was left at room temperature (25C°), with constant stirring for approximately 72 hours. At the end of this time, the mixture was filtered using large pore filter paper to obtain the ethanolic extract (EA). Finally, the EA is placed in the rotary evaporator to concentrate the extract.

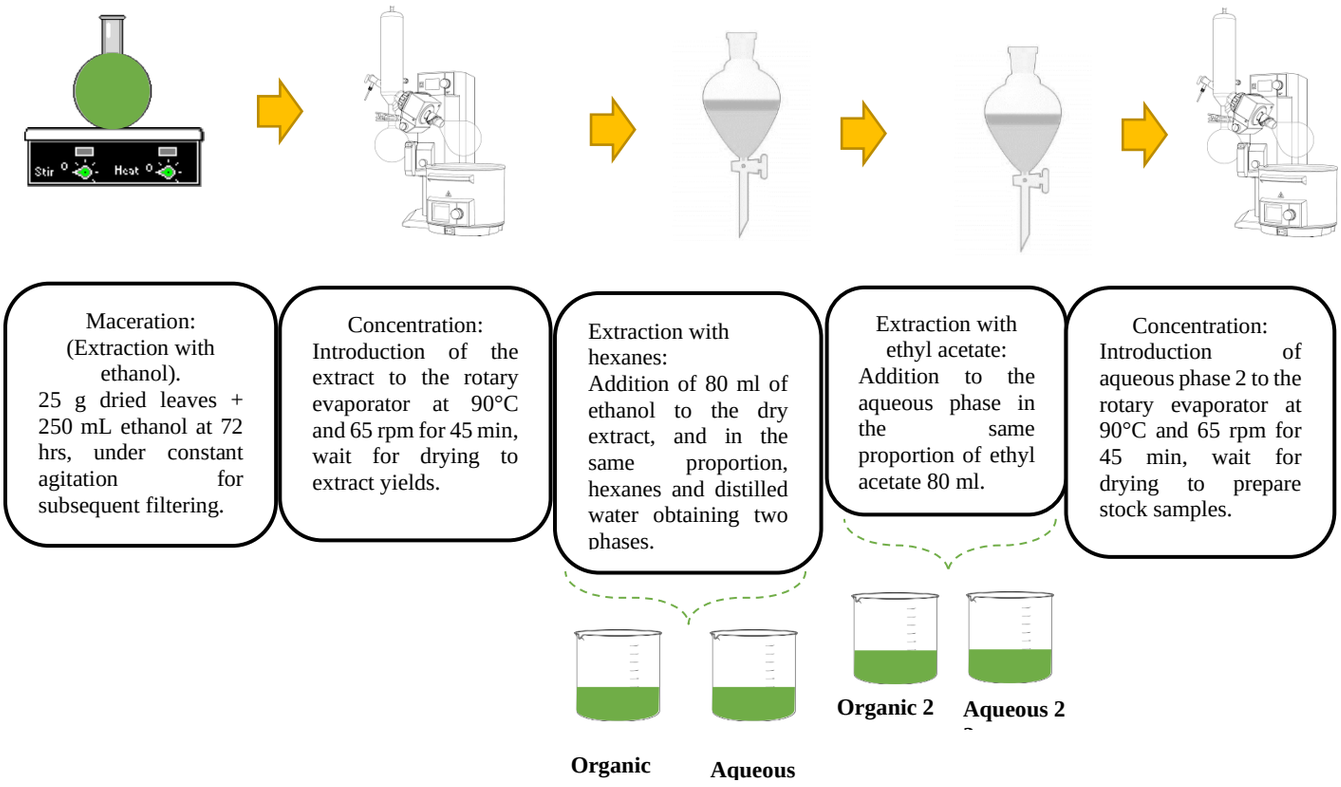
As a complement to the previous process, it was proposed to include the purification of the extract through successive washes with hexanes and ethyl acetate, in this way 3 different extracts are obtained for subsequent analysis, the description of the procedure is shown in figures 7.3 and 7.4.

Figure 7.3 Hexane purification process



Source of consultation: (Own elaboration)

Figure 7.4 Purification procedure with hexanes and ethyl acetate



Source of consultation: (Own elaboration)

7.2.3 Characterisation of the extract

In order to identify the organic compounds present in the extract, different analyses were carried out, which are described below:

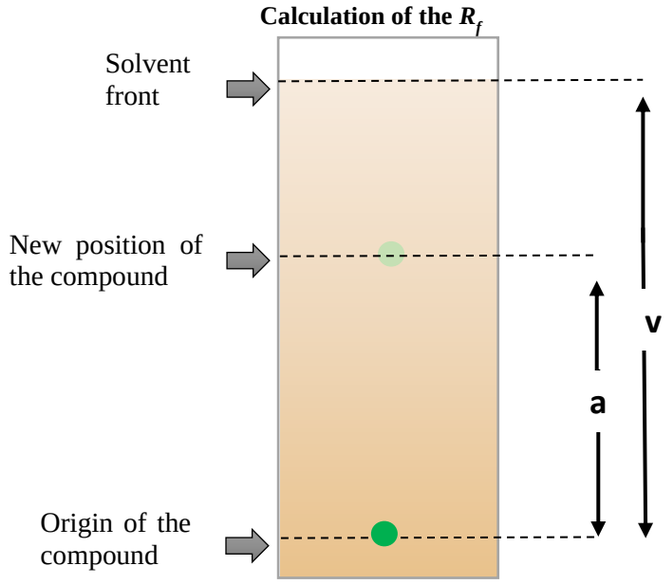
- Thin layer chromatography

A large pore filter paper, 9 cm long and 3 cm wide, was used, the upper end (in front of the solvent) and the lower end (origin of the compound) are marked, see Figure 7.5. Subsequently, the filter paper is placed in a flask containing the eluent (iodine solution dissolved in ethanol), taking care that the solution does not touch the lower mark, and left to act for 10 minutes. Once the eluent rises by capillary action until it reaches the upper edge, it is removed from the flask. Finally, the filter paper is allowed to dry and the sample is revealed by forming coloured complexes. The retention factor (Rf) is used to determine the presence of the compound of interest. The Rf is calculated by the following equation:

$$R_f = \frac{a}{v} \quad (1)$$

Where a is the distance travelled from the origin of the compound to the new position and v is the distance from the origin along the eluent front.

Figure 7.5 Image of the filter paper used for the thin layer chromatography test



Source of consultation: (Own elaboration)

R_f is a physical constant for a given compound, assuming that the chromatographic conditions (e.g. solvent, temperature and nature of the stationary phase) are specified to a dimensionless value between the length of the plate and the extent of the major components, the certain organic compounds contained in the sample can be determined.

- Phytochemical tests

They consist of colouring tests that will allow the identification of the chemical compounds in the extract, and the yield of the extracts is evaluated.

a) **Identification of flavonoids:** Dissolve 0.5 mL of the total extract (ethanolic extract) in 2 mL of absolute ethanol and divide into three test tubes. The third test tube is taken as the control and the following tests are then carried out:

- Shinoda reaction: 2 drops of concentrated hydrochloric acid are added to the first test tube, then a piece of magnesium metal is placed in the test tube for 5 minutes at room temperature between 25 and 26 C°.

- 10% sodium hydroxide reaction: 3 drops of sodium hydroxide are added to the second test tube and observations are noted.

b) **Identification of Saponins:** To identify this compound, the foam height and stability test is carried out, which consists of placing 1 mL of total ethanolic extract in a test tube, shaking it vigorously, waiting 15 minutes, and measuring the height of the foam that appears (the height of the foam obtained after 15 minutes is measured).

c) **Identification of Tannins:** Take 1 mL of the ethanolic extract in a flask and add 2 mL of distilled water and 3 drops of 2% sodium chloride, heat to boiling for one minute, cool and filter. The filtrate is divided into three test tubes, the third test tube is used as a control. To identify tannins, the following tests are carried out:

- Gelatine reaction: 2 drops of gelatine reagent (0.5 g of pure grenetin gauged to 50 mL of distilled water) are added, if no changes are observed, no tannins are present.

- Ferric chloride reaction (gallic acid derivatives or catechol); to the second test tube is added a drop of 1% ferric chloride (0.5 g of ferric chloride to 50 mL of distilled water), the sample tends to have a strong blue colour in the presence of polyphenols.

d) **Lead acetate reaction**: In order to identify the presence of phenols in the extract, this test is carried out. To 1 ml of ethanolic solution, 1 mL of 10% lead acetate solution was added.

- Spectroscopic techniques

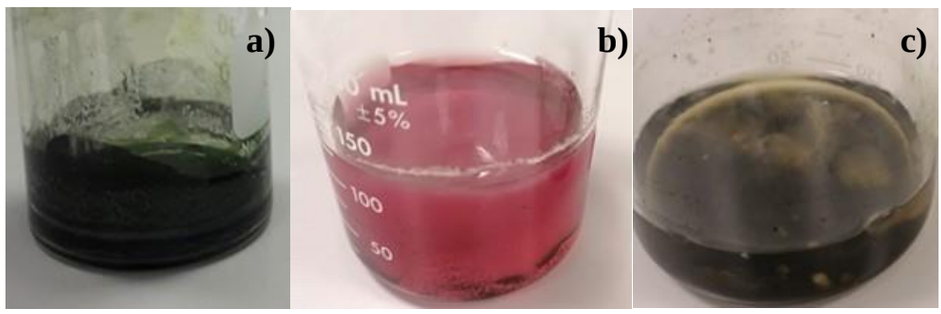
The extract was analysed by Ultraviolet-Visible Spectroscopy (UV-Vis) to identify the organic compounds contained in the ethanolic extract and Fourier Transform Infrared Spectroscopy (FTIR) was used to identify the functional groups present in the extract.

7.3 Results

In order to determine the optimal conditions for obtaining organic compounds from Tradescantia spathacea Sw leaves, three procedures were considered: 1) extraction of the leaves using the maceration process without a purification process, 2) maceration process with hexane purification and 3) maceration process with hexane and ethyl acetate purification (see figure 7.6).

Figure 7.6a shows the extract obtained without a purification process. As can be seen, the dry extract 22 days after leaving the rotary evaporator shows a good appearance with a characteristic odour of the plant. On the other hand, the extracts obtained with hexane and hexane purification with ethyl acetate that were left to dryness after being concentrated in the rotary evaporator and taken to dryness show after 20 days the presence of fungi due to the decomposition of the sample (figure 7.5b). Similarly, fungi and bacteria were observed in the extract purified with hexanes and ethyl acetate (figure 7.5c). At the end of the process of obtaining the extract using different purification processes, only the extract obtained by maceration without purification was considered for subsequent characterisation.

Figure 7.6 Image of the extract obtained by maceration process: a) without purification process, b) purification with hexanes and c) purification with hexanes and ethyl acetate



Source of consultation: (Own elaboration)

7.3.1 Extract yield

Table 7.1 shows data obtained before and after the process of obtaining the ethanolic extract from the leaves of Tradescantia Spathacea SW. With respect to the volume measured, 40% of the volume recovered was obtained. On the other hand, the yield of the ethanolic extract was 26%, showing that a small amount of dry extract is obtained after concentration at the rotary evaporator.

Table 7.1 Recording of the extraction conditions of the extract from the leaves of the plant *Tradescantia spathacea* SW

Data	Value
Weight of dried leaves	25 g
Volume of solvent	250 mL
Temperature	25 °C
Volume of extract recovered	100 mL
Weight of the extract after the concentration process using the rotary evaporator	6.5 g

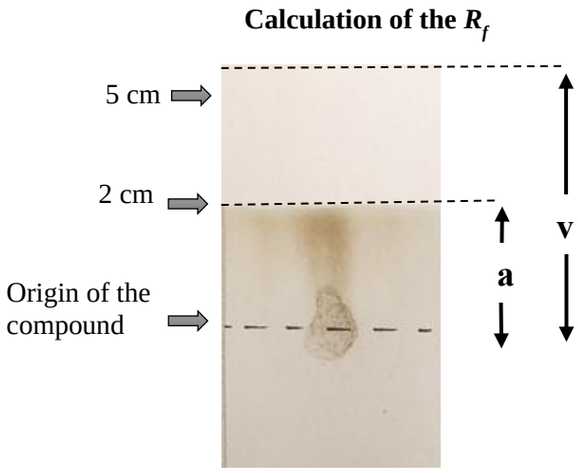
Source of consultation: (Own elaboration)

Plants contain primary metabolites (lipids and gradates, proteins, nitrogenous compounds, etc.) and secondary metabolites (alkaloids, isoprenoids and phenolic derivatives) (Auwal et al., 2014). In order to identify the presence of polyphenols, the evaluation of the extract by phytochemical tests and spectroscopic techniques is presented.

7.3.2 Thin layer chromatography (CCF)

In order to identify the major compound in the Tradescantia Spathacea SW extract, thin layer chromatography was performed. Figure 7.7 shows the evidence of the test. A retention factor (Rf) of 0.40 was determined. This technique allows revealing the amount of polyphenols present in the sample, however, to identify which polyphenols are present, other techniques are required, which are shown below.

Figure 7.7 Thin layer chromatography of the ethanolic extract

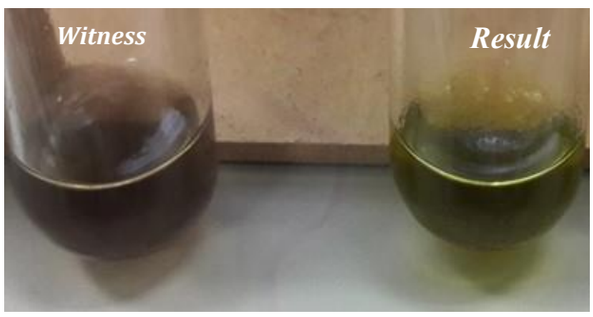


Source: (Own elaboration)

7.3.3 Phytochemical characterisation

Phytochemical tests are used to identify organic compounds, in this case, they were used to identify the polyphenolic compounds present in the extract. The Shinoda reaction allows the identification of flavonoids, *i.e.* the presence of polyphenolic compounds such as flavonols, flavones, flavonones, chalcones and aurones in the extract. Some flavonoids called anthocyanidins react with concentrated hydrochloric acid at boiling temperature and show a reddish colour change as shown in figure 7.8.

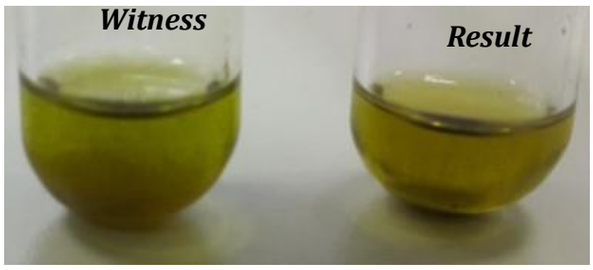
Figure 7.8 Shinoda reaction



Source: (Own elaboration)

Another specific reaction of phenols is their acidic capacity, which was demonstrated by reacting the polyphenolic compounds with a basic NaOH solution, where the presence of polyphenolic compounds was revealed when adding the solution to the ethanolic extract showed turbidity and suspension of particles (see figure 7.9).

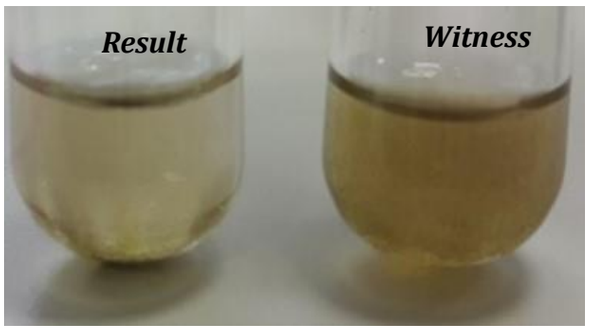
Figure 7.9 Reaction with NaOH



Source: (Own elaboration)

The grenetin test was positive and showed the existence of tannins (polymeric structures of flavones or flavonoids). When these compounds reacted with grenetin, they showed precipitate in solution and white colouring as shown in figure 7.10.

Figure 7.10 Reaction with grenetin



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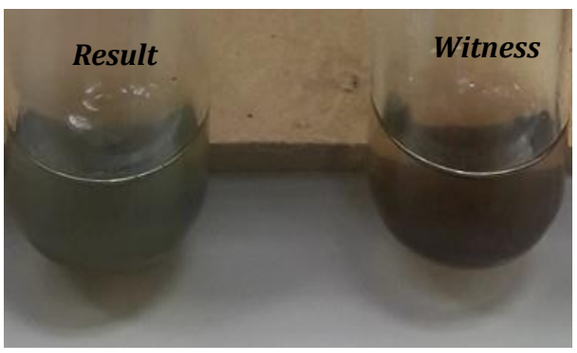
The ferric chloride reaction with phenols is a general test that allowed to identify the presence of polyphenols, by colour changes to strong blue as shown in figure 7.11.

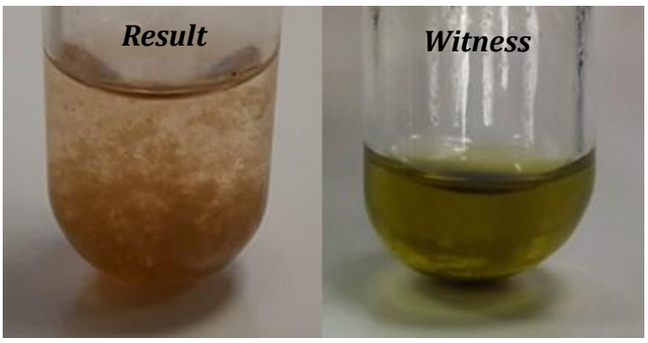
Figure 7.11 Ferric chloride reaction



Source: (Own elaboration)

In the reaction with lead acetate, the presence of polyphenols was identified, ranging from large size such as tannins; medium structure such as flavones, flavonols or as small as caffeic acid, where the increase in particle size was observed (see figure 7.12).

Figure 7.12 Lead acetate reaction



Source: (Own elaboration)

Table 2 shows a summary of the results obtained from the ethanolic extract by different phytochemical tests.

Table 7.2 Phytochemical tests on ethanolic extract

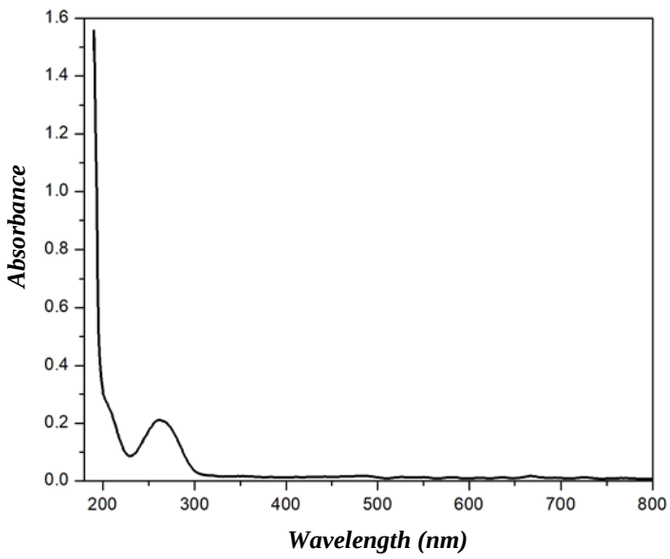
Phytochemical tests	Resulted	
Shinoda reaction	Positive	Negative
10% sodium hydroxide	X	
1% ferric chloride	X	
Reaction with grenetin	X	
Foam height test		X

Source: (Own elaboration)

Ultraviolet-Visible Spectroscopy (UV-Vis)

UV-Vis spectroscopy is widely used to identify the characteristic bands associated with organic compounds present in the range of 100-400 nm (UV) and the visible spectrum of 400-700 nm. Figure 7.13 shows the UV-Vis absorption spectrum of the total ethanolic extract. A maximum peak is observed at 270 nm corresponding to flavonoid and phenolic group structures (Porras & López, 2009).

Figure 7.13 UV-Vis spectrum of the extract

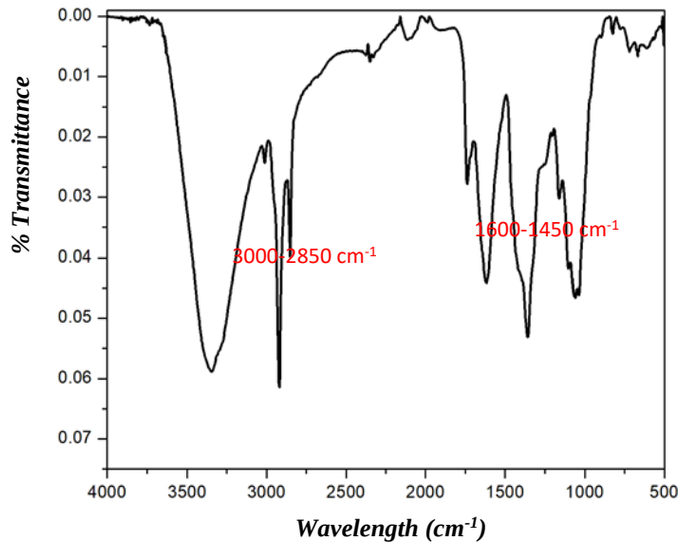


Source: (Own elaboration)

Fourier Transform Infrared Spectroscopy (FTIR)

Figure 7.14 shows the response of the ethanolic extract obtained by infrared spectroscopy. A maximum peak at 3344 cm^{-1} associated with the O-H band characteristic of the presence of an alcohol, phenol, polyphenols is observed in the range 3700-3600 cm-1, if hydrogen bridge bonds are present there is a broadening of the band and a slight decrease in the absorption frequency (3600-3000 cm^{-1}). The vibrations corresponding to the C-H stretching of the methyl and methylene groups appear between 3000-2850 cm-1. Bands corresponding to the carbonyl C=O group appear in the range (1830-1650 cm^{-1}), possibly corresponding to flavonoids and/or coumarins and the aromatic C=C double bond, occurring in pairs at 1600 cm-1 and 1450 cm^{-1}. (Interaction & Sales, 2010).

Figure 7.14 FTIR spectrum obtained from ethanolic extract *Tradescantia*



Source of consultation: (Own elaboration)

7.4 Acknowledgement

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7.5 Conclusions

The ethanolic extract of the leaves of the Tradescantia Spathacea SW plant was obtained by the maceration process. The purification of the extract with hexanes and hexanes with ethylene could not be obtained because the samples showed decomposition before the natural drying process for the production of a concentrate. On the other hand, the ethanolic extract without purification was able to evaluate the compounds it contains. The results of the phytochemical tests showed the presence of polyphenols in the extract obtained from the leaves of the Tradescantia Spathacea SW plant. Finally, the UV-Vis spectroscopy test confirmed the presence of polyphenolic groups with flavonoid-type structures and the FTIR results showed the presence of different functional groups, mainly the characteristic bands of the phenol group. It is important to mention that this study has an impact on a current topic and opens up a wide research panorama, promoting the study and use of native resources.

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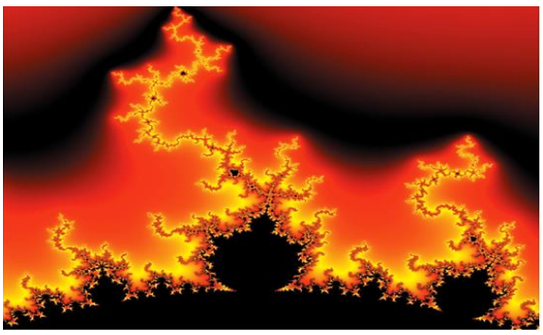

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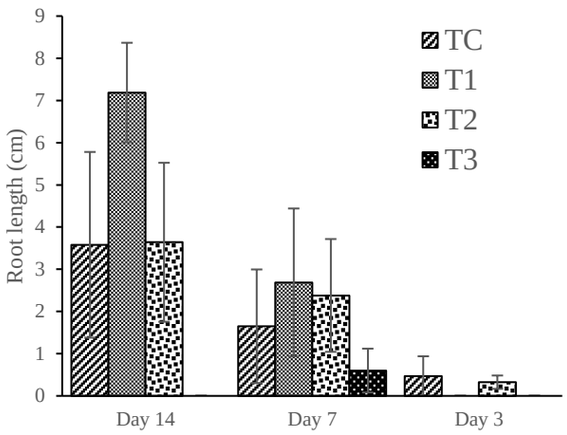

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