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In the first chapter we present, *Hypolipidemic activity of Phaseolus vulgaris (Fabaceae) in mice*, by MEX-ALVAREZ, Rafael Manuel de Jesús, GUILLEN-MORALES, María Magali, GARMA-QUEN, Patricia Margarita and RAMOS-GOMEZ, Lázaro Guadalupe, with ascription in the Universidad Autónoma de Campeche, as a second article we present, *Design and determination of double emulsions ($W_1/O/W_2$) for the trapping of antioxidant compounds sensitive to thermal processes*, by MONTER-JUÁREZ, Francisco, ROMERO-LÓPEZ, María del Rosario, MENDOZA-MENDOZA, Bethsua and ESTRADA-FERNÁNDEZ, Ana Guadalupe, with secondment in the Instituto Tecnológico Superior del Oriente del Estado de Hidalgo, as the following article we present, *Oxidative stress in the central nervous system of iron-deficient females*, by VIEYRA-REYES, Patricia & BLANCAS-CASTILLO, Sergio E., with affiliation at the Universidad Autónoma del Estado de México, as next article we present, *In vitro study of the film thickness of six resin cements*, by ROESCH-RAMOS, Laura, MORA-SÁNCHEZ, Aura Leonora, MORENO-MARÍN, Flora and MANTILLA-RUIZ, Manuel, with affiliation at the Universidad Veracruzana.

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Hypolipidemic activity of *Phaseolus vulgaris* (Fabaceae) in mice

Actividad hipolipemiante de *Phaseolus vulgaris* (Fabaceae) en ratones

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Abstract

Objective: To evaluate the lipid-lowering activity of an aqueous extract obtained from the seeds of *Phaseolus vulgaris* in male mice using the hyperlipidemia induction model with Triton X-100. Methods: the chemical quality of the extract obtained was characterized by quantifying the total polyphenols (Folin Ciocalteu method) and total anthocyanins (colorimetric method) as well as their antioxidant activity by their ferric ion reducing capacity (FRAP, TPTZ method). Results: The data obtained show that the aqueous extract contains a large amount of total polyphenols (415 mg EAG / 100g of seed) and total anthocyanins (43EMG / 100 g of seed) and significant antioxidant activity (11.080.83 of Fex / g of seed). Administration of the aqueous extract to hyperlipidemic mice improved their lipid profile, especially by reducing the serum value of total cholesterol (144 mg/dL) and triglycerides (147 mg/dL) and increasing HDL values (67 mg/dL) in the group that received a dose of 300 mg of extract / kg of weight. Conclusion: These results show that the aqueous extract of *P. vulgaris* exerts an antioxidant activity in vitro and a lipid-lowering effect in mice.

Dyslipidemia, Antioxidant, Anthocyanins

Resumen

Objetivo: Evaluar la actividad hipolipemiante un extracto acuoso obtenido a partir de las semillas de *Phaseolus vulgaris* en ratones macho usando el modelo de inducción de hiperlipidemia con Tritón X-100. Métodos: se caracterizó la calidad química del extracto obtenido mediante la cuantificación de los polifenoles totales (método de Folin Ciocalteu) y antocianinas totales (método colorimétrico) así como su actividad antioxidante por su capacidad reductora de iones férrico (FRAP, método de TPTZ). Resultados: Los datos obtenidos demuestran que el extracto acuoso contiene una gran cantidad de polifenoles totales (415±mg EAG/ 100g de semilla) y de antocianinas totales (43±EMG/ 100 g de semilla) y una actividad antioxidante significativa (11.08±0.83 de Fex/ g de semilla). La administración del extracto acuoso a los ratones hiperlipidémicos mejoró su perfil lipídico, especialmente al reducir el valor sérico del colesterol total (144 mg/dL) y trigicéridos (147 mg/dL) e incrementar los valores de HDL (67 mg/dL) en el grupo que recibió una dosis de 300 mg de extracto/ kg de peso. Conclusión: Estos resultados demuestran que el extracto acuoso de *P. vulgaris* ejerce una actividad antioxidante in vitro y un efecto hipolipemiante en ratones.

Dislipidemia, antioxidante, antocianinas

Citation: MEX-ALVAREZ, Rafael Manuel de Jesús, GUILLEN-MORALES, María Magali, GARMA-QUEN, Patricia Margarita and RAMOS-GOMEZ, Lázaro Guadalupe. Hypolipidemic activity of *Phaseolus vulgaris* (Fabaceae) in mice. ECORFAN Journal-Bolivia. 2022. 9-17:1-9.

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Introduction

Obesity is a risk factor for contracting various diseases that compromise the patient's life; the higher the overweight index, the higher the amount of fat in the body and the higher the dyslipidaemia, characterised by an increase in triglycerides, production of low-density lipoprotein (LDL) particles and a reduction in high-density cholesterol (HDL), increasing the risk of vascular accidents; In addition, there is an increase in blood glucose because fat provides energy to muscle to the detriment of glucose, this state causes the pancreas to secrete excess insulin in an attempt to reduce hyperglycaemia but fails to compensate for this balance causing peripheral insulin resistance which can lead to the development of diabetes[1-4].

Hyperlipidaemia has a high predictive value as a risk factor for atherosclerosis and cardiovascular and cerebrovascular accidents, characterised by elevated serum levels of total cholesterol and LDL and decreased serum levels of HDL; treatment of hyperlipidaemia is therefore one of the best therapeutic models for slowing down the process of atherogenesis [5-7]. One measure taken to control the imbalance present in dyslipidaemias, in addition to pharmacotherapy and physical exercise, is a healthy, balanced diet with functional foods that help to improve the patient's metabolic status [5, 8, 9]. Beans (also known as beans in other regions of the Americas), whose scientific name is *Phaseolus vulgaris*, in addition to providing macronutrients to the diet, the whole bean, and nutrients such as non-heme iron, fibre, folic acid, thiamine, potassium, magnesium and zinc, provide substances beneficial to the health of the consumer such as various phenolic acids (ferulic, p-coumaric and gallic acid), flavonoids and anthocyanins 10-13. Previous studies have shown that aqueous extracts of *P. vulgaris* exert beneficial biological activity by stabilising blood levels of glucose and lipids, because polyphenolic compounds facilitate the binding of insulin to its receptors, affect the digestibility of carbohydrates, for example by inhibiting amylase, among other proposed mechanisms [10, 14, 15].

However, there are not enough studies demonstrating the effectiveness of *P. vulgaris* in the treatment of dyslipidaemia, so the aim of the present study was to determine the lipid-lowering activity of the aqueous extract of lyophilised *P. vulgaris* seed powder in hyperlipaemic mice treated with Triton X-100 to estimate the pharmacological and biotechnological potential of the plant.

Material and Methods

Black bean (*Phaseolus vulgaris*) samples were collected in the region of Hopelchén, Campeche State (Mexico) and their taxonomic identity was corroborated; the seeds obtained were dried at room temperature and stored in plastic containers refrigerated at 4 °C; prior to extraction, the seeds were ground to obtain a powder that was subjected to an extraction process by static maceration, for which 100 g of powder were deposited in 2. 0 L beakers and 1.0 L of sterile distilled water was added, left to stand for 8 h and the supernatant was separated by filtration. For quantification, an aliquot of 100 mL was taken for the determination of total polyphenolic compounds by the Folin Ciocalteu method, anthocyanin quantification and ferric ion reducing power by the TPTZ method. The rest of the extract was subjected to a freeze-drying process (13.3 Pa for 72 h) to obtain the dry extract for bioassays, the freeze-dried extract was kept refrigerated at 4 °C in amber vials until evaluation [29-31].

Determination of Total Polyphenolic Compounds [29-31]

100L of the extract was added to 500L of water in a test tube and then 100L of Folin-Ciocalteu's reagent was added, left to react for 30 min and then 500L of Na_2CO_3 20% was added, incubated at room temperature for 30 min, and finally read in a spectrophotometer at 760 nm. A calibration curve was performed with a standard solution of gallic acid 250 ppm to determine the concentration of polyphenols present in the extract, the test was performed in triplicate.

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Quantification of anthocyanins [29,30]

The test was performed in triplicate by acidifying the solution with 0.1 M HCl and finally filtering the solution to recover the supernatant. The absorbance of the acid solution was measured at 540 nm and the anthocyanin concentration was estimated by the following formula (proposed by Di Stefano):

$$Anthocyanins\ \frac{mg}{L} = A_{540\ nm} * 16.7 \qquad (1)$$

Where is the absorbance of the acid solution at 540 nm and 16.7 is the conversion factor considering the absorbance of malvidin-3-glucoside.

Determination of Ferric Ion Reductive Antioxidant Power (FRAP) [30,31,38]

The test was performed using TPTZ (2,4,6-tripyridyl-S-triazine) reagent as a ferrous ion complexing agent; first the FRAP reagent was prepared by mixing 25 mL of 300 mM sodium acetate buffer, 2.5 mL of 10 mM TPTZ (2,4,6-tripyridyl-S-triazine) solution and 2.5 mL of 20 mM ferric chloride solution. Subsequently, 100 µL of the bean extract was added to 1000 µL of a freshly prepared solution of FRAP reagent, mixed thoroughly, allowed to react for 60 min and the ferrous-tripyridyltriazine complex formed was measured at 590 nm in a spectrophotometer; a calibration curve was performed with ferrous sulphate as a standard.

Lipid-lowering activity in albino mice [32-35]

Ten-week-old male albino mice (Mus musculus) weighing more than 25 g (range 25-30 g) and pre-conditioned for one week, maintained at 30°C and 50% relative humidity, with water and purina® food ad libitum, with 12-hour light-dark cycles, were used. Following the standard indications of mouse caretakers and breeders and the instructions of the Mexican Official Standard NOM-062-ZOO-1999 that dictates the technical specifications for the reproduction, care and use of laboratory animals.

Hyperlipidaemia was induced by intraperitoneal administration of the surfactant Triton X-100 (a non-ionic detergent) in freshly prepared saline (100 mg/kg) to mice, after an overnight 18-hour fast, to cause elevation of plasma cholesterol and triglycerides (1).

After 72 hours of triton administration, the animals were randomly divided into groups of six and treatments were initiated as shown in table 1, the substances were administered orally for 7 days.

Group	Name	Induction Hyper-lipidemia	Treatment
1	Witness	No	Saline solution
2	Control Negative	Yes	Saline solution
3	PV1	Yes	Aqueous extract of *P. vulgaris*, dosage 100 mg/kg
4	PV2	Yes	Aqueous extract of *P. vulgaris*, dose 200 mg/kg
5	PV3	Yes	Aqueous extract of *P. vulgaris*, dose 300 mg/kg
6	Positive Control	Yes	Atorvastatin in 0.5% aqueous methylcellulose, dose 10 mg/kg

Table 1 Experimental protocol used to determine the hypolipidemic activity of *P. vulgaris*, hyperlipidaemia was induced with Triton X-100 (i.p.) and after 72 hours the treatments were started for 7 days, orally
Source: Own elaboration

On the eighth day of treatment and after an 18 h fasting period, the animals were anaesthetised to obtain blood samples by intracardiac puncture. Sera were separated by centrifugation at 3000 rpm for 10 min for determination of blood parameters (total cholesterol, triglycerides, HDL) using commercial enzyme kits. Cholesterol was determined by the enzymatic method of the enzyme cholesterol ester hydrolase which hydrolyses all serum cholesterol esters present in the sample and then using the enzyme cholesterol oxidase which oxidises the free cholesterol generating hydrogen peroxide, which by the action of peroxidase reacts with chromogen to produce a coloured compound. The experimental procedure for the determination of total cholesterol was done with 1.0 mL of commercial total cholesterol reagent and 10 L of serum, mixed well and incubated for 10 minutes at 37°C, after which the absorbances were read at a length of 505 nm in a spectrophotometer. A calibration curve was also determined with the cholesterol standard provided by the Bayer® commercial kit (1).

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For the determination of triglyceride concentration, the enzymatic method was used which hydrolyses serum triglycerides to glycerol and fatty acids, the glycerol produced is phosphorylated by the action of the enzyme glycerol kinase and the glycerol-1-phosphate generated is oxidised by the enzyme glycerol phosphate oxidase which produces hydrogen peroxide, which in the presence of peroxidase and chromogens produces a coloured compound. The experimental procedure was carried out with 1.0 mL of the working reagent and 10 L of serum was added, mixed well and incubated for 10 minutes at 37ºC, finally, the absorbance was measured at 505 nm in a spectrophotometer. A calibration curve was performed with the standard provided in the commercial kit (1).

For the determination of HDL cholesterol, the method based on the precipitation of VLDL and LDL lipoproteins with phosphotungstate in the presence of magnesium ion was used, then the remaining cholesterol in the serum was determined with the total cholesterol enzyme reagent. LDL-cholesterol concentration was estimated by the Friedelwlad equation:

$$LDL = CT - \left(HDL + \frac{TG}{5}\right) \quad (2)$$

Where LDL is the concentration of LDL cholesterol, TC is the concentration of total cholesterol, HDL is the concentration of HDL cholesterol and TG is the concentration of triglycerides (all expressed in mg/dL).

On the other hand, the atherogenic index expresses at a mathematical level the ratio or proportion between total cholesterol levels and high-density lipoprotein levels and serves to find the risk of atherosclerosis, a value equal to or lower than 3.5 represents a low risk, an atherogenic index between 3.5 and 4.5 implies a moderate risk and a value higher than 4.5 means a maximum risk of atherosclerosis. The Castelli formula was used to calculate the atherogenic index:

$$IA = \frac{CT}{HDL} \quad (3)$$

Where AI is Castelli's atherogenic index, TC is total cholesterol concentration and HDL is HDL cholesterol concentration, expressed in mg/dL.

Statistical analysis

Statistical analysis of the data was performed using SPSS V25.0 statistical software, the descriptive statistics with which the values are reported are the mean and one standard deviation; the results of each test were analysed for significant statistical differences by a one-way Analysis of Variance (ANOVA), followed by a multiple range test employing Tukey's multiple comparison of means method by the least significant difference LSD procedure, with a confidence level of 95% (=0.05).

Results

The chemical characterisation of the aqueous extract of Phaseolus vulgaris is shown in table 2, these results reveal that the extract contains a high concentration of polyphenolic compounds and anthocyanins, in addition the extract presents a good ferric ion reducing capacity.

Total polyphenols	415±15 mg EAG/ 100g of seed.
Anthocyanins	43±1 EMG/ 100g of seed.
FRAP	11.08±0.83 of Fe^{2+}/ g of seed.

Table 2 Polyphenol and anthocyanin content and ferric iron reducing activity of Hopelchén bean seed acidic aqueous extracts. EAG= gallic acid equivalents, EMG= malvidin-3-glucoside equivalents, results are shown as X SD, n=3.
Source: Own elaboration

The values of cholesterolemia and triglyceremia were elevated with Triton X-100 treatment, if the results of the control group (not treated with Triton) are compared with those of the negative control group (who received Triton and saline); however, the individuals treated with the aqueous extract of *Phaseolus vulgaris* had a lower serum concentration of cholesterol and triglycerides than the negative control group, thus observing the hypolipidemic action of the extract of *P. vulgaris* (Graphic 1).

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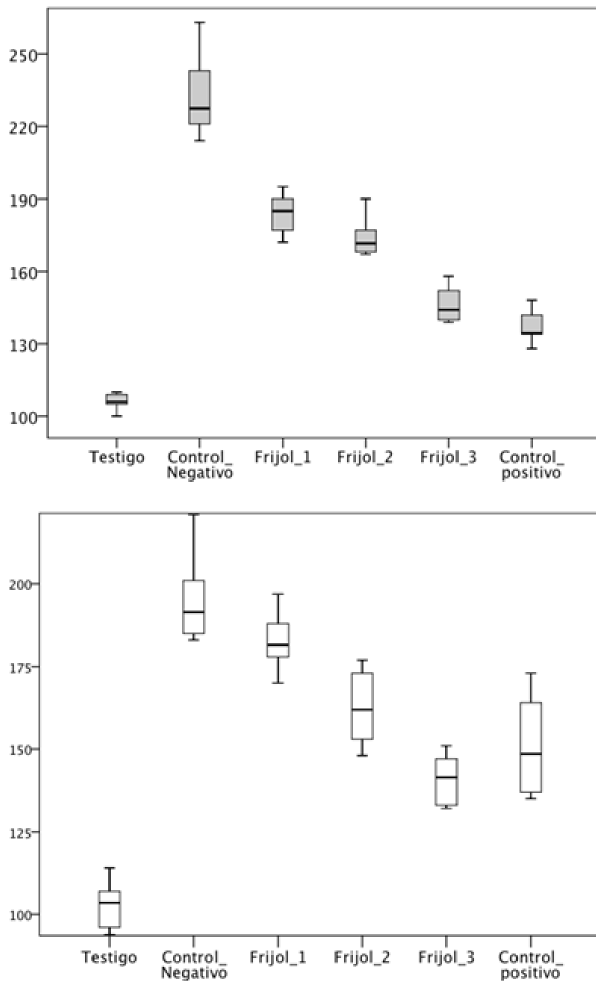

Graphic 1 On the left, the serum cholesterol values of the groups studied. On the right, serum triglyceride values (n=6), different letters in each group indicate significant differences. In both graphs the y-axis indicates the concentration expressed in mg/dL
Source: Own elaboration

Likewise, the other lipid profile data are shown in table 3, together with the atherogenic index; these results show that the administration of the aqueous extract of *P. vulgaris* increased HDL values in contrast to the negative control group. LDL values and the atherogenic index decreased in the mice treated with the extract, which is beneficial because it decreases the risk of vascular accidents. It can be It can be seen that the increase in HDL and decrease in LDL was a dose-dependent effect.

Group	HDL	LDL	Atherogenic index
Control	$49 \pm 7^{a,c,d}$	36 ± 5^{a}	2.19 ± 0.27^{a}
Negative control	36 ± 4^{b}	157 ± 18^{b}	6.51 ± 0.92^{b}
PV 1	40 ± 3^{a}	107 ± 8^{c}	4.58 ± 0.38^{c}
PV 2	$57 \pm 8^{c,d}$	84 ± 10^{s}	3.08 ± 0.38^{d}
PV 3	67 ± 3^{c}	51 ± 9^{e}	2.19 ± 0.16^{e}
Positive control	55 ± 4^{d}	52 ± 6^{e}	2.51 ± 0.14^{e}

Table 3 Serum HDL and LDL values and atherogenic index of the different study groups. Results are presented as X SD, n=6, different letters in the same column represent differences (<0.005) statistic
Source: Own elaboration

Discussion

The model used in this research to induce hyperlipidaemia was the administration of Triton X-100 which causes elevation of serum total cholesterol, LDL and triglyceride values, as well as a reduction in HDL levels, which is a major risk because it increases the atherogenic index 5,[22-24]. The mechanism by which non-ionic surfactants produce hyperlipidaemia in mice appears to be the accelerated induction of hepatic cholesterol synthesis, post-administration of the chemical compound which increases the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA) which is the enzyme involved in cholesterol biosynthesis. In addition, triton inhibits lipoprotein lipase, an enzyme that hydrolyses triglycerides to fatty acids and glycerol, thereby increasing plasma triglyceride levels[36,37].

In this study, an increase in lipidaemia levels was observed in the negative control group compared to the control group, corroborating the success of the model used to induce hyperlipidaemia [32,35,37]; in the positive control group (to which the lipid-lowering drug was administered) a decrease in serum lipid values (cholesterol and triglycerides) was observed and in the problem groups the lipidaemia levels were significantly lower than in the negative control group, which demonstrated that the aqueous extract of *P. vulgaris* aqueous extract exerted a dose-dependent lipid-lowering effect.

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Since the proposed mechanism of action for the induction of hyperlipidaemia by triton X-100 is the induction of lipid biosynthesis, it can be assumed that if a plant extract exerts a lipid-lowering effect it may be because it contains metabolites that interfere with cholesterol biosynthesis; Furthermore, the drug used as a reference treatment in this research was atorvastatin, a member of the statin group that acts as a competitive inhibitor of the enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCoA), which is responsible for hepatic cholesterol biosynthesis [25-28]; the group treated with the drug showed a decrease in serum cholesterol and triglyceride levels. Accordingly, the atherogenic index in the mice treated with aqueous bean seed extract decreased with respect to the negative control group, as there was an increase in HDL levels and a decrease in LDL concentration in the problem group mice; The decrease in the atherogenic index is beneficial because values lower than 4 do not represent a cardiovascular compromise or risk, and when it is higher than 4 (as in the negative control group) there is a greater probability that atheroma plaque will form in the arteries and cause atherosclerosis [4-6, 22-24]. In addition to inducing hyperlipidaemia by increasing HMG-CoA reductase activity and inhibiting lipoprotein lipase, triton increases the production of free radicals, causing oxidative damage, especially in liver tissue; this oxidative damage is also observed in patients with dyslipidaemia [35-37].

Therefore, the content of polyphenolic compounds in the extract could exert a synergistic action with its lipid-lowering activity, as the antioxidant activity of the bean extract correlates with its high polyphenol content and may be beneficial in dyslipidaemias because these clinical conditions are associated with oxidative stress, which is implicated in the aetiopathogenesis of cardiovascular diseases [18, 19]; Although the present study did not evaluate the antioxidant activity in vivo, these results could support future research to show whether there is a protective association due to *P. vulgaris* extract against oxidative damage in vivo under this same model because it has been established that Triton causes lipoperoxidation and that malonylaldehyde levels increase and endogenous antioxidant levels decrease, compromising the health status of the individual and increasing the risk of vascular accidents [18-21, 39].

The presence of polyphenolic compounds, especially anthocyanidins, in the aqueous extract of these results are in agreement with the values expected for *P. vulgaris* according to different studies published in the scientific literature [13, 16, 17]; phenolic compounds are excellent transition metal chelators, which play a fundamental role in the formation of radicals and influence lipid peroxidation40. In addition, the mechanism by which polyphenols exert their action is based on modulation of enzyme activity; it has been shown that they can inhibit enzymes related to inflammatory or oxidative activity [13-15, 40]. Consequently, the ferric iron reducing action of the aqueous extract of *P. vulgaris* could be beneficial in vivo in individuals with hyperlipidaemia because hyperlipidaemia generally causes oxidative stress and in obese patients it is an intermediary in the development of metabolic syndrome, and it has also been observed that adipose tissue is the main source of reactive oxygen species [9, 5, 18, 19, 41].

The advantage of bean consumption in the diet is based on the contribution of polyphenolic compounds with antioxidant activity and its beneficial effect on the consumer's serum biochemistry due to its hypoglycaemic and lipid-lowering properties, which give it its nutraceutical character [9, 16, 25, 39, 41]; it would be necessary to study whether its pharmacological activity is preserved in decoction (bean broth).

Conclusions

Phaseolus vulgaris extract showed a dose-dependent lipid-lowering activity by reducing total cholesterol and triglycaemia levels, as well as a protective action by increasing HDL cholesterol concentration and decreasing the atherogenic index in the groups treated with the extract.

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Article
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Design and determination of double emulsions ($W_1/O/W_2$) for the trapping of antioxidant compounds sensitive to thermal processes

Diseño y estabilización de emulsiones dobles ($W_1/O/W_2$) para el atrapamiento de compuestos antioxidantes sensibles a procesos térmicos

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Abstract

Currently, there are several advances in the development of functional foods, since consumers are increasingly aware of the close relationship between food and the health of the body. Thus, this research proposes the encapsulation of bioactive compounds, extracted from the xoconostle fruit using micro emulsions for its protection at high temperatures in the jam making process. For the extraction, three treatments were proposed, with different means of extraction (EX); EX1: water, EX2: ethanol and EX3: ethanol-water mixture (1:1). Statistical analysis revealed that the extraction medium did not have an effect on antioxidant capacity. The emulsion was made with the EX1 extract, forming a multiple water-in-oil-in-water emulsion (W1/O/W2), using three biopolymers whey protein concentrate (WPC), whey protein isolate (WPI) and gum arabic (GA) in concentrations of 6.0%, 7.5%, and 9.0%, in which physical stability and stability to creaming were determined. The 7.5% WPI emulsion presented greater stability. The antioxidant capacity tests in the jam showed that the emulsion is efficient to preserve the antioxidant capacity, observing an increase in this in EXC/EM, compared to the EX and EXS/EM treatment.

Xoconostle, Aguamiel, Antioxidants

Resumen

Actualmente, existen diversos avances en desarrollo de alimentos funcionales, ya que los consumidores son cada vez más conscientes de la estrecha relación entre alimentación y salud del organismo. Es así, que la presente investigación, propone la encapsulación de compuestos bioactivos, extraídos del fruto xoconostle utilizando microemulsiones para su protección a las altas temperaturas del proceso de elaboración de mermelada. Para la extracción, se plantearon tres tratamientos, con diferentes medios de extracción (EX); EX1: agua, EX2: etanol y EX3: mezcla etanol-agua (1:1). El análisis estadístico reveló que el medio de extracción no tiene un efecto en la capacidad antioxidante. La emulsión se realizó con el extracto EX1, formando una emulsión múltiple agua-en-aceite-en-agua (W1/O/W2), empleando tres biopolímeros concentrado de proteína de suero (WPC), aislado de proteína de suero (WPI) y goma arábiga (GA) en concentraciones de 6.0%, 7.5 %, y 9.0%, en las cuales se determinó la estabilidad física y la estabilidad al cremado. La emulsión de WPI 7.5 %, presentó mayor estabilidad. Las pruebas de capacidad antioxidante en la mermelada mostraron que la emulsión es eficiente para conservar la capacidad antioxidante observando un incremento de esta en EXC/EM, comparado con el tratamiento EX y EXS/EM.

Xoconostle, Aguamiel, Antioxidantes

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Introduction

The world's population is considering important changes in their eating habits, due to the influence that scientific progress has had in demonstrating the effects of functional foods or bioactive compounds on certain functions of the organism, obtaining as a benefit the well-being and health of the consumer. Therefore, a functional food can be designed according to two aspects, one where the presence of compounds that can be harmful to health can be limited, or increasing the proportion of those that have a positive influence on the consumer (Jiménez-Colmenero, 2013), due to this it is necessary to incorporate new technologies for the optimisation of the extraction processes of bioactive components, to strategies that involve their protection during their inclusion in different food matrices, to increase their stability and bioavailability.

However, several investigations have focused on the use of agro-industrial by-products of plant origin as a source of bioactive compounds, especially those with antioxidant activity, so that extracts are produced with quantities that have this property (Castromonte et al., 2020). Among these antioxidant components are polyphenols, which are a group of secondary metabolites in plants that have gained commercial importance; polyphenols are compounds that differ in their structure, which determines their biological functions and which, when incorporated into foods, confer functional properties that provide practical applications (Ríos-Aguirre and Gil-Garzón, 2021). However, it is important to take into account that these compounds, being outside their source of origin, are susceptible to environmental factors such as light, oxygen and pH, causing their degradation and therefore a decrease or loss of their functionality, so encapsulation becomes more relevant, since it is a micro-packaging technology (Castromonte et al., 2020), which involves the coating or entrapment of a pure material or a mixture inside another material forming a capsule of between 5 to 300 microns in diameter (Esquivel-González, Ochoa Martínez and Rutiaga-Quiñones, 2015).

The main function of these capsules is to protect the material both from adverse environmental conditions and during its passage through the gastrointestinal tract, thereby promoting a controlled release of the bioactive component, increasing its shelf life and bioavailability (Castromonte, Wacyk, Valenzuela, 2020). Therefore, food production continuously seeks strategies for the integration of these compounds without losing their integrity and therefore without compromising the sensory quality of food products (Buyukkestelli and Sedef Nehir, 2021).

Water-in-oil-in-water (W1/O/W2) double emulsions are one of the carrier systems used for encapsulation, protection, and delivery of hydrophilic and hydrophobic active components. Double emulsions are complex multiphase systems in which small water droplets (inner water phase W1) are trapped inside larger oil droplets (W1/O), which are subsequently dispersed in a continuous water phase (outer water phase W2). Recently, double emulsions have been studied in the release of hydrophilic bioactives in the food and pharmaceutical industries. Several parameters have been shown to affect the droplet stability of double emulsions, e.g. the phase mass fraction and the type of emulsifier. However, double emulsions are thermodynamically and kinetically unstable systems with a strong tendency towards coalescence, flocculation and creaminess due to the presence of two interfaces with opposite curves in a single structure. The proximity of these interfaces also induces diffusion from one interface to the opposite interface. Subsequently, this destabilises the structure to form a single emulsion. Furthermore, the stability of double emulsions after heat treatment can be important for improving the safety and shelf life of many food products containing active components in the food industry (Jo and van der Schaaf, 2022).

Therefore, the present research proposes the design of multiple emulsions for the protection of antioxidant compounds extracted from xoconostle fruit for incorporation into a jam.

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Materials and methods

Conditioning of the raw material

The xoconostle was harvested from the plot of Mr. Silvestre Jiménez Montiel, located in the municipality of Tlanalapa, State of Hidalgo, Mexico, whose coordinates are Latitude 19°48'41.33 "N, Longitude 98°35'28.65 "W, at an altitude of 2460 m above sea level. The fruits were selected according to their optimum ripeness, working with fruits in which pink colour was predominant (Pimienta-Barrios et al., 2008), in addition to verifying that there were no perforations or presence of pests that could cause any alteration in the fruit.

The total weight of the xoconostles was obtained and then they were washed and disinfected with a solution of sodium hypochlorite in water (5 ppm). Subsequently, the epidermis, mesocarp and pulp or seeds (endocarp) were separated. Each fraction was weighed to calculate the yield. Finally, they were vacuum packed and stored frozen, protected from light until further analysis.

Extraction of bioactive compounds from xoconostle pulp

Anthocyanins were extracted from the pulp with seeds. The process consisted of an initial grinding for three minutes in an industrial blender (Oster brand), followed by filtering through an organza cloth to remove larger particles. The treatments were established according to the experimental design shown in Table 1. Three treatments were proposed, in which the extraction medium was changed: 1) distilled water (EX1), 2) ethanol (EX2) and 3) a 1:1 mixture of ethanol and water (EX3), these three treatments with and without the application of temperature and a maceration time of 2 hours (Romero-López, 2015).

Treatment	Extraction medium	Temperature (°C)	Time (hours)
EX_1	Water	25	2
EX_2	Ethanol	45	2
EX_3	Water-ethanol	25	2

Table 1 Experimental design for the extraction of xoconostle pigments with different solvents

After the extraction time, the samples were centrifuged at 6000 rpm for 20 minutes (Thermo Scientific Medialite 6 PL Centrifuge), the supernatant was decanted and the antioxidant activity was determined by the ABTS method to corroborate that there was no decrease in this parameter. The extracts were kept refrigerated (4°C), protected from light until further use and analysis.

Emulsion design

The methodology proposed by Rodríguez-Hueso et al. (2014) was applied, forming a multiple water-in-oil-in-water (W1/O/W2) emulsion in two stages. In the first stage, a W1/O primary emulsion was formed, with a 70:30 ratio, 70 % aqueous phase (xoconostle pulp extract) and 30 % oil phase with canola oil (Capullo®, Unilever de México, S.A. de C.V., Tultitlán Edo. de México, Mexico) at a total emulsifier concentration of 8 % w/w. The hydrophilic emulsifier used was Panodam SDK (monoglyceride and diglyceride esters of diacetyl tartaric acid) and the hydrophobic emulsifier was Grindsted PGRR 90 (fatty acid esters of polyglycerol and polyricinoleate), both from Danisco México, S. A. de C. V. The emulsification process was carried out with a Wiggen-Hauser D-500 Disperser homogeniser at 10,000 rpm for five minutes.

For the second stage, 30 mL of the primary emulsion W1/O was reemulsified in 70 mL of aqueous medium, testing different biopolymers; Whey Protein Concentrate (WPC), Whey Protein Isolate (WPI) and Gum Arabic (GA) at concentrations of 6. 0%, 7.5 % and 9.0% as external aqueous phase (W2) in a Wiggen-Hauser D-500 Disperser homogeniser at 4500 rpm for five minutes, producing double emulsions (W1/O/W2).

Physical stability of the emulsion

Particle size analysis of the W1/O/W2 double emulsions was carried out under an optical microscope (VELAB model VE L5MCD digital) and applying Pixemetre image analyser software (version 5.1) to measure the length of the average diameter of the oil globules of the double emulsion.

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The diameter of 30 randomly selected globules was determined to determine the measurement of the diameters of the primary and double emulsion, immediately after their realisation and subsequently over a 14-day time. Taking into account what is reported by Nava, (2019) the droplet size was determined, as it depends on the strength with which the emulsion is made and is responsible for the water solubility of the ingredients.

Stability of the emulsion upon creaming

Determined by observing a creaming layer at the top and the presence of a clear water phase at the bottom of the tube containing the double emulsion, the creaming index (CI) was calculated by applying the following formula:

$$CI(\%) = \frac{hW}{hT}\, x\, 100 \quad (1)$$

Where hW is the height of the clear water phase at the bottom and hT is the total height of the double emulsion. The accumulation of the phase with a higher proportion of oil at the top is similar to the formation of cream, but with a less clear phase at the bottom (Yesiltas et al., 2021).

Antioxidant activity analysis

ABTS-+2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) method.

A solution of ABTS-+ radical cations at a concentration of 7 mM (ABTS-++) was prepared and activated with 2.45 mM potassium persulphate for 12 h under dark conditions at room temperature prior to analysis. The ABTS-++ radical is diluted in potassium phosphate buffered saline (PBS) to adjust the absorbance to 0.700 ± 0.020 at a wavelength of 734 nm. To measure the antioxidant capacity, 10 µL of sample was mixed with 990 µL of radical solution and the absorbance at 734 nm was recorded on a UV-Vis Digital spectrophotometer (FIAXA 325-1000 nm-722G), readings were taken at time zero and 6 min after sample addition to determine the percentage inhibition of antioxidant activity (Re et al., 1999; Guzmán-Maldonado, 2010).

Antioxidant activity assay. DPPH method (1,1-diphenyl-2-picrylhydrazine)
A solution was prepared from 7.4 mg of DPPH (SIGMA-ALDRICH) by gauging to 100 mL with absolute ethanol and shaken until complete homogenisation. For the standard calibration curve, concentrations of 300, 200, 100, 100, 50 and 0 mmol/L Trolox were used as standard solution. From each dilution or sample, 200 µL were taken, 1000 µL of DPPH were added and finally left to stand for one hour until subsequent reading at 520 nm, using a UV-Vis Digital spectrophotometer (FIAXA 325-1000 nm-722G) (Llica, 2008).

Determination of total phenols

It was determined using Folin-Ciocalteu reagent (SIGMA ALDRICH), at 10% (solution A), sodium carbonate at 7. 5 % (solution B), and for the standard curve 100 mg of gallic acid in 10 mL of deionised water, from which concentrations of 0, 10, 50, 100, 100, 300, 500, 700, 900 and 1000 mg/mL were prepared, of each dilution 100 µL were mixed with 500 µL of solution A and 400 µL of solution B, after 30 minutes of rest the absorbance at 760 nm was obtained. This procedure was applied for each sample analysed (Ordoñez et al, 2020).

Jam production

According to the NMX-131-1982 standard; and according to Paltrinieri, (1997); Jiménez and Bonlla, (2012); Makanjuola, (2019) the characteristics that a jam should have are 64 -65° Brix, pH of 3.4, acidity of 0.4282 g of citric acid and 6 % of pectin. Table 2 shows the proposed formulations. Adjustments were made to the amount of pectin, so that the minimum amount necessary for gel formation could be determined. Subsequently, the xoconostle jam was made by substituting the sugar with mead honey (MA) and adding the bioactive compounds extracted from the xoconostle pulp; table 3 shows the different treatments. The antioxidant activity and total phenol content were determined for each jam produced.

*Formulation	Xoconostle (g)	MA (g)	Pectin (g)
1	145.79	125	1.25
2	145.79	125	2.50
3	145.79	125	3.75
*The amount of final product was 225 g.			

Table 2 Treatments to determine the amount of pectin suitable for gel formation

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Statistical analysis

All determinations were performed in triplicate, reporting the mean and standard deviation. Additionally, a one-way analysis of variance was used, followed by a Tukey mean comparison test with a 95 % confidence level, the analysis was performed using the statistical programme Sigma Plot 12.0 (Systat Software, Inc. SigmaPlot for Windows).

Jam	Xoconostles (g)	MA (g)	Pectin (g)	Pulp extract (mL)
1	140.0	225	7.3	20
2	140.0	225	7.3	20*

Table 3 Formulations for jam production with pulp extract

*Microencapsulated pulp extract.

Results

Extraction of bioactive compounds

For the selection of the extraction process, the percentage inhibition of the ABTS-+ radical was determined for each treatment. These measurements revealed that the antioxidant capacity is not affected by the extraction method, since the statistical analysis revealed that there are no significant differences between the treatments (EX1: 1.78, EX2: 1.51, EX3: 1.89 µmol ET /g of extract), so it was decided to use the EX1 treatment, since this refers to the use of water as the extraction medium and no temperature. The results are related to those obtained by Hernández-Fuentes et al., (2015) in xoconostle pulp, who indicate values of 1.10 to 1.22 µmol ET /g. Sánchez-González, (2016) recommends extraction with aqueous ethanol solutions from 20 to 50% to achieve complete extraction. In this case, as there were no significant differences, the treatment with water without solvents was applied due to the fact that the product in which the extract will be used is intended for a foodstuff. Soto and Rosales, (2016), report greater extraction efficiency using 80% ethanol in aqueous solution, as it favours obtaining phenolic compounds and total antioxidant activity; however, these extracts are for medicinal use, Bustos (2012) mentions that the extraction process used depends directly on the type of compound and the food matrix from which the extraction is carried out.

This author reports that the best extraction of vitamin C, flavonoids and anthocyanins from apple peel is achieved with boiling water, even compared to ethanolic or methanolic solutions.

Physical stability of the emulsion

Different treatments of double emulsions were carried out, using different W2 aqueous phases formed with three biopolymers (WPC, WPI and GA) and with a concentration variation of 6.0, 7.5 and 9.0 %, respectively. In the emulsions formed by WPC at a concentration of 6.0 and 7.5 %, the same trend was presented, increasing the droplet size of the double emulsion from 7.92 ± 0.68 and 9.07 ± 1.28 µm at day 1 to 20.09 ± 1. 88 and 20.97 ± 2.23 µm at day 14, respectively, being the most stable compared to the 9.0 % concentration that presented a diameter increase of 23.85 ± 2.76 at day 1 and 33.57 ± 2.58 µm at day 14 (Figure 1a). This behaviour may be due to the fact that the stabilisation of the oil-water interfaces with a higher percentage of biopolymer is presenting a drastic increase in size indicating an instability system in which the emulsion droplets are growing. The same behaviour is observed in the WPI emulsions (Figure 1b), where the double emulsions similarly do not show a drastic change in size; stabilised with WPI6.0% and WPI 7.5% from 11.41 ± 2.97 at day 1 and 17.95 ± 1.72 µm at day 1 to 23.09 ± 2.84 and 20.21 ± 3.99 µm at day 14, respectively, not so for the emulsion stabilised with WPI9.0%. In the GA emulsions at 6.0% concentrations a peculiar behaviour was presented (Figure 3c), from a large double emulsion droplet size of 48.46 ± 2.57 µm there was a decrease to 28.75 ± 1.89 µm. This same behaviour was observed for the 7.5% concentration. However, at the 9.0% concentration the opposite occurs as there is a drastic increase in emulsion size from 15.09 ± 2.05 at day 1 to 51.56 ± 2.49 µm at day 14 (Figure 1c), indicating that an instability system is occurring possibly due to saturation of the biopolymer.

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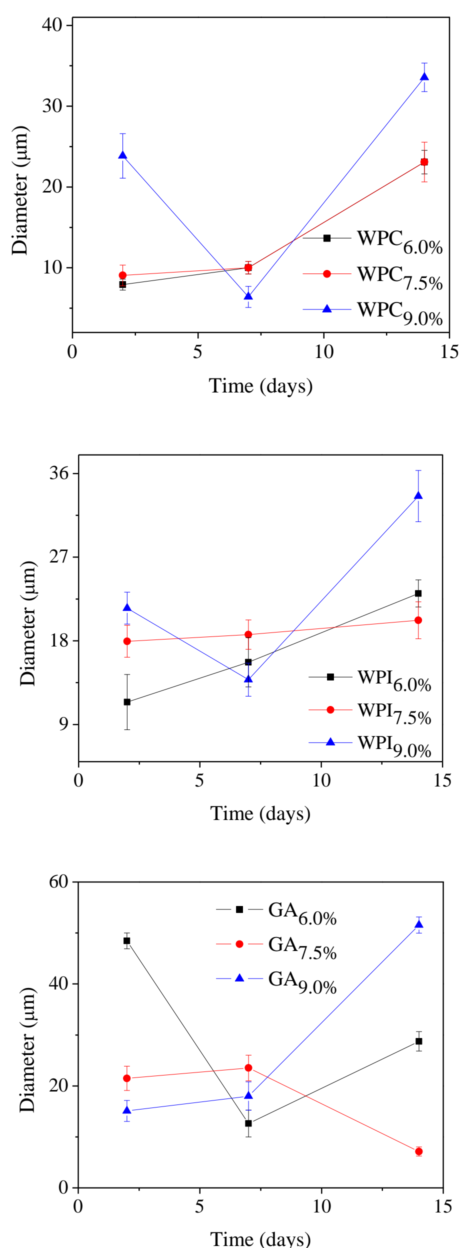

Figure 1 Change in diameter of the double emulsions. Where (a); WPC for concentrations of 6.0%, 7.5% and 9.0%; (b) WPI for concentrations of 6.0%, 7.5% and 9.0%; and (c) GA for concentrations of 6.0%, 7.5% and 9.0%

Derived from the above results, it was decided to make only the comparison study of the double emulsions stabilised with the three biopolymers at the concentrations of 6.0 and 7.5% as can be seen in figures 2a and 2b. In the double emulsions stabilised with 6.0%, it can be seen in figure 2a, the change in diameter of the double emulsions according to the biopolymer used, presenting a smaller diameter and better stability over time in the double emulsion stabilised with WPC 6.0%. As for the 7.5% concentration, the WPI biopolymer is the one that presents better stability compared to the 6.0% concentration over time, because its increase is not significant (Figure 2b).

According to McIntyre et al., (2018), the formation of a stable emulsion will depend on the ability of the proteins or biopolymers to migrate to the oil-water interface and reduce the interfacial tension, and for this to be achieved, the proteins must be sufficiently soluble. Therefore, the main form of instability in the emulsions formed is coalescence that arises due to the inability of the biopolymers to adequately stabilise the oil surface area generated during homogenisation. The biopolymers are absorbed at the oil-water interface, where they decrease the free energy of the emulsion system allowing stabilisation. However, only a proportion of the biopolymer is actually at the interface; an unadsorbed fraction is present in the aqueous phase as an aggregate. Higher levels of biopolymers not adsorbed in the aqueous phase may result in the biopolymer concentration not being sufficient to allow adequate coverage at the oil-water interface. If such a case occurs, biopolymer aggregates could be shared between adjacent emulsion droplets, resulting in flocculation and, consequently, a marked increase in droplet size (McIntyre et al., 2018). This theory agrees with the results reported in the present work, as the droplet size of the emulsions was larger when a higher concentration of biopolymers (9.0%) was used.

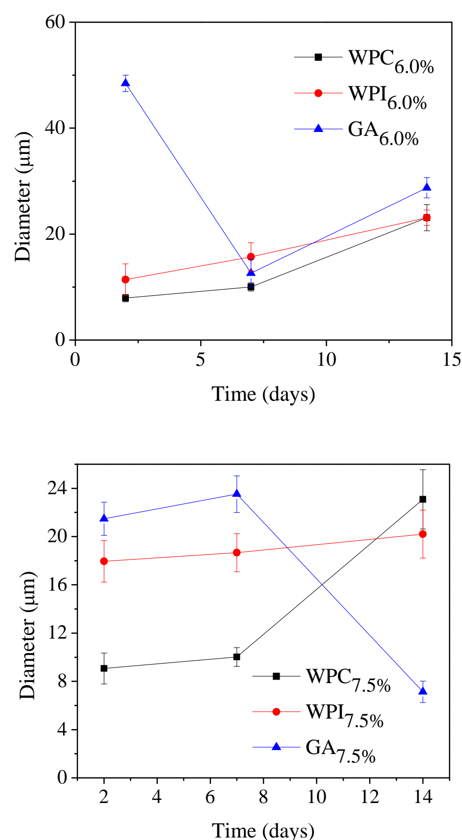

Figure 2 Change in diameter of the double emulsions (a) concentration 6% and (b) concentration 7.5%

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Emulsion stability at creaming

The creaming index (CI) was applied to further evaluate the emulsion stability for different storage periods (1, 7 and 14 days). One of the main indications of physical instability of the emulsions was creaming (CI). Creaming describes the upward movement of oil droplets due to gravitational separation that leaves a clear water phase at the bottom (Yesiltas et al., 2021). Lipid droplets in emulsions stabilised by different biopolymers and in their different concentrations tend to move upwards in the upper layer, while water-soluble substances may move downwards due to gravity (Chen et al., 2020). A lower CI value corresponds to a smaller emulsion separation, representing a more stable system with less separation. These results can be seen in Table 4. All double emulsion treatments tested showed an increase in CI as storage time increased. The double emulsions stabilised in the external aqueous phase with GA 6.0% at day 1 showed the lowest CI value, but after 14 days of storage it increased drastically. The 7.5% biopolymer concentration, stabilised with 7.5% WPI, had the lowest creaming rate after 14 days of storage, from 25.0 ± 2.23% at day 1 to 29.0 ± 0.23% at day 14. In contrast, the 9.0% concentration of biopolymers showed lower CI at day 1 but with a large increase at day 14 of storage. Therefore, in figure 2 it can be observed that, with increasing concentration of the biopolymers, the lowest CI values occurred at the shortest storage time (1 day), but as the storage time increased, the value increased. Chen et al., (2020), report stability of nano-emulsions and evaluated the creaming index only for a storage time of 48 hours, which indicates that the systems evaluated in the present work are more stable over time.

Analysis of antioxidant activity and phenols in marmalade

In addition to the treatments shown in table 3, a control sample was made for the production of jam, which consisted of jam to which no xoconostle pulp extract (Ms/E) was added. For each treatment, the percentage of ABTS-+ radical inhibition, DPPH and total phenol concentration were determined (Table 5).

Treatment	**CI (%)**		
	1 day	**7 days**	**14 days**
$WPC_{6.0\%}$	38.0± 0.23	48.0 ± 1.32	51.0 ± 1.99
$WPI_{6.0\%}$	25.0 ± 1.45	38.5 ± 2.78	41.0 ± 2.01
$GA_{6.0\%}$	13.0± 2.31	56.5 ± 1.45	57.0 ± 1.99
$WPC_{7.5\%}$	42.5 ± 1.83	54.0 ± 3.01	56.0 ± 2.99
$WPI_{7.5\%}$	25.0 ± 2.23	26.5 ± 2.41	29.0 ± 0.23
$GA_{7.5\%}$	15.0 ± 1.56	62.5 ± 1.12	65.0 ± 1.25
$WPC_{9.0\%}$	25.0 ± 2.78	51.0 ± 0.89	57.5 ± 2.03
$WPI_{9.0\%}$	15.0 ± 0.89	49.0 ± 0.99	50.5 ± 1.78
$GA_{9.0\%}$	15.0 ± 0.28	60.0 ± 3.09	75.0 ± 0.78

Table 4 Creaming index of double emulsions stabilised in the external aqueous phase (W_1) with different biopolymers and concentrations

The results show that the antioxidant capacity of the jam increases with the addition of pulp extract (table 5). Additionally, when comparing the percentage of inhibition of the ABTS radical in the extract (10.2 %) with that of Ms/E (10.7 %), an increase in this inhibition was observed, which may be due to the addition of the fruit pulp. In general, the highest percentage of inhibition, as well as of total phenols is presented in EXC/EM, revealing that the encapsulation process is efficient in the conservation of the functional properties of the extracted bioactive compounds by protecting them from the high temperatures that occur in the elaboration of the jam.

Treatment	**ABTS-+ (% inhibition)**	**DPPH (mg TE/100mL)**	**Total phenols (mg/100g)**
EX	$10.2±4.2^a$	$2.05±0.03^a$	$2.3±3.6^a$
$M_{S/E}$	$19.7±2.8^b$	$2.21±0.72^a$	$4.55±1.7^b$
$EX_{S/EM}$	$29.1±5.8^c$	$2.83±0.7^a$	$5.3±5.0^b$
$EX_{C/EM}$	$36.1±1.6^d$	$3.11±0.1^b$	$12.3±3.6^c$

Table 5 Results of antioxidant activity of xoconostle extract applied to a food system (jam)

Average of three determinations ± Standard deviation. Where: EX: Xoconostle extract, Ms/E: Jam without extract, EXS/EM: Extract without encapsulation applied in jam and EXC/EM: Encapsulated extract applied in jam. a, b, c different letters in superscripts indicate significant differences ($P<0.05$).

The results obtained are in agreement with other research. Šaponjac et al., (2016) and Davidov et al., (2012) mention that the incorporation of encapsulated bitter cherry pomace and grape seed extracts respectively in biscuits positively influences their functional characteristics and even the shelf life of the product.

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This research, together with the present report, shows that the incorporation of encapsulated antioxidant compounds in different types of food matrices improves the stability of the active ingredients and provides functional properties to foods, thus opening up possibilities of application in other types of products in the food industry.

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Conclusions

The microencapsulation carried out with double emulsion was able to provide protection to the bioactive compounds of the xoconostle pulp extract, as they resisted the heat treatment during the jam making process. In this way, according to the analyses carried out, it can be guaranteed that this product has properties that allow it to be a functional food for daily consumption.

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Oxidative stress in the central nervous system of iron-deficient females

Estrés oxidativo en sistema nervioso central de hembras deficientes de hierro

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Abstract

Iron deficiency is a worldwide public health problem associated with behavioral and cognitive disturbances. These disturbances are irreversible if not treated during the perinatal period. Iron is a structural part of several proteins, such as the antioxidant enzyme catalase. It has been shown that females are less susceptible to oxidative stress. However, the levels of oxidative stress at the central system in the presence of chronic iron deficiency or post-weaning supplementation are unknown. Objective: to determine the levels of oxidative stress and antioxidant defense in females with chronic iron deficiency, untreated or treated with iron supplementation. Methodology: female Wistar rats with chronic iron deficiency and rats supplemented from weaning to adulthood (70 postnatal days) were euthanized to analyze brain tissue and determine oxidative stress through lipid peroxidation; and antioxidant effect by superoxide dismutase, catalase and total proteins. Contribution: in the presence of chronic iron deficiency, lipid peroxidation levels at the central system are so high that they cannot be counteracted by superoxide dismutase or catalase. However, postnatal supplementation prevents lipid peroxidation from being altered due to the high production of iron-induced antioxidant defense.

Iron, Iron deficiency, Sex

Resumen

La deficiencia de hierro es un problema mundial de salud pública asociado con perturbaciones conductuales y cognitivas. Dichas alteraciones son irreversibles si no son tratadas durante el periodo perinatal. El hierro es parte estructural de diversas proteínas, como la enzima antioxidante catalasa. Se ha demostrado que hembras son menos susceptibles a estrés oxidativo, sin embargo, se desconocen los niveles de estrés oxidativo a nivel central ante deficiencia de hierro crónica o suplementación posdestete. Objetivo: determinar los niveles de estrés oxidativo y defensa antioxidante en hembras con deficiencia de hierro crónica no tratada o tratada con suplemento férrico. Metodología: ratas Wistar hembras con deficiencia de hierro crónica y ratas suplementadas desde el destete hasta la edad adulta (70 días-posnatales) fueron eutanasiadas para analizar tejido cerebral y determinar estrés oxidativo a través de peroxidación lipídica; y efecto antioxidante por superóxido dismutasa, catalasa y proteínas totales. Contribución: ante deficiencia de hierro crónica, los niveles de peroxidación lipídica cerebral son tan elevados que no pueden ser contrarrestados por superóxido dismutasa o catalasa. Sin embargo, suplementar posnatalmente, impide que la peroxidación lipídica se altere debido a la alta producción de defensa antioxidante inducida gracias al hierro.

Hierro, Deficiencia de hierro, Sexo

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Introduction

Iron is a trace element involved in various functions such as: erythropoiesis, oxygen transport and storage, mitochondrial functions, synthesis and degradation of proteins, lipids and ribonucleic acids, cardiac and skeletal muscle metabolism, thyroid gland functions, central nervous system and immune system (Andrews & Schmidt, 2007). This micronutrient in its divalent or ferrous form (Fe^{2+}) can donate electrons, while in its trivalent or ferric form (Fe^{3+}) it can accept them. This is an essential characteristic for its function as an oxygen carrier, however, it can also have toxigenic potential by generating free radicals (Ganz & Nemeth, 2006). Iron is a structural part of antioxidant enzymes such as: cytochromes, catalases, peroxidases and oxygenases (Bresgen & Eckl, 2015; Casanueva & Viteri, 2003; Forrelat B M, 2000; MacKenzie *et al.*, 2008; Toxqui *et al.*, 2010), therefore, its deficiency can also lead to oxidative stress (Askar *et al.*, 2017a).

Previous research shows that females are less susceptible to oxidative stress than males (Austad, 2006; Kander *et al.*, 2017; Vina *et al.*, 2011). This may be due to oestrogenic factors, including the antioxidant effect (Badeau *et al.*, 2005; Kagan *et al.*, 1992; Kagan & Tyurina, 1998; Packer *et al.*, 1979) and the regulator of body iron levels (Borras, 1998) that favour splenic Fe stores and their serum levels (Haouari *et al.*, 1993; Haouari *et al.*, 1994).

When the amount of iron absorbed from the diet exceeds the demand required by the body, iron deficiency (DFe) occurs (Tussing-Humphreys et al., 2012). It particularly affects infants, young children, adolescents, older adults, those with chronic inflammatory diseases, and women; in the latter, menstruation and pregnancy are additional risk factors. Approximately 30-40% of women and preschool children in industrialised countries have DFe, while almost all individuals in these groups are affected in developing countries (Lundqvist & Sjoberg, 2007).

There are sex-specific and life-stage-specific increases in iron nutritional requirements. In the absence of dietary supplementation, DFe is reported in about 40% of preschool children, 30% of menstruating women and girls, and 38% of pregnant women (Kassebaum *et al.*, 2014; Pasricha *et al.*, 2013; Stevens *et al.*, 2013). The causes of DFe in developing countries are typically: insufficient dietary intake and/or intestinal blood loss due to parasite colonisation. In contrast, in high-income countries, the main causes are: certain dietary habits (vegan diet or not consuming red meat) and certain pathological conditions (chronic blood loss or malabsorption) (Kassebaum *et al.*, 2014).

During gestation, iron is obtained by the foetus through the placenta, with 80% of the transfer occurring during the third trimester of pregnancy. It is essential that the foetus acquires adequate iron stores from the mother to maintain its growth during the first 6 months of life, as the iron provided by breastfeeding is very low (Widdowson & Spray, 1951). Throughout pregnancy the prevalence of DFe is high; 43% of pregnant women worldwide are anaemic, with DFe being the cause in 50-75% of cases (Di Renzo et al., 2015). Even worldwide, the most common cause of DFe during the gestational and early postnatal period is maternal DFe (Lozoff *et al.*, 1996). Other causes of DFe during the foetal and neonatal period are preterm birth and gestational complications (maternal diabetes, intrauterine growth restriction, maternal smoking, maternal obesity and inflammation) (Chang *et al.*, 2011; Lukowski *et al.*, 2010; Murray-Kolb & Beard, 2007).

In infants there are 3 dietary sources of iron: breast milk (with iron bound to lactoferrin), heme iron and non-heme iron. For neonates and young infants their only source of iron is in breast milk and/or formula (Siimes et al., 1979). At birth most term infants have normal to high Hb concentrations (15-17 g/dL) and thus remain iron saturated until 6 months of age. Infants born to mothers with DFe are at high risk of developing DFe at approximately 4-6 months of age, without being manifested at birth (Mills & Davies, 2012). During this stage iron is acquired entirely from the diet, in contrast to adults, for whom the diet provides only 5% of the daily requirement (DH, 2011).

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One of the major non-hematological problems associated with DFe is behavioural and cognitive disturbances, the underlying mechanisms of which involve dysfunctional myelination, altered neurotransmission and impaired brain development. Infants with DFe are particularly prone to cognitive problems, as well as auditory and visual dysfunction (Algarin *et al.*, 2003). Unfortunately, the above problems appear in early childhood, when dopamine is the main neurotransmitter, so prevention of these impairments by late iron supplementation is uncertain (Algarin *et al.*, 2013; Algarin *et al.*, 2003). The strongest evidence for neurological impairment comes from studies on cognition in school-aged children and adolescents with DFe and DFe anaemia (Low *et al.*, 2013). However, it is not known whether females suffering from chronic iron deficiency from foetal to adult age, as occurs in many people worldwide, or in their case, treated with iron supplementation during childhood, have altered levels of oxidative stress and antioxidant defence in the central nervous system, which would be related to behavioural and cognitive alterations such as those mentioned above, the reason for the present research.

Methodology

Ethical declaration

All studies were conducted according to approved institutional protocols in accordance with the Principles and Procedures outlined by the National Institutes of Health, National Institutes of Health Guide for the Care and Use of Laboratory Animals, in agreement with the Local Ethics Committee. For sample collection, sacrifice was induced with CO_2 to minimise distress.

Animals and diet

Wistar rats were used and maintained under standard vivarium conditions: a 12:12 light/dark cycle was used (light on at 5 o'clock), with a controlled temperature of approximately 22 ± 2°C and free access to food and water.

The study was conducted on female broods. Parents or offspring were subjected to the following conditions: 14 days prior to mating and during 25 days of gestation, 20 female rats (3 months old or 250 g) were fed an iron-deficient diet (10 ppm $FeSO_4$, Lab Diets AIN-76W / 10), "DFe group". Another 10 female rats received control diet (100 ppm FeSO4, Lab diets AIN-76W/100) "control group". 21 days after birth (DPN), the pups were weaned. Only females were selected for the present experiment, males were used in other projects. Female offspring were maintained on the same type of diet offered to their mothers until 70 DPN; with the exception of the "DFe+S" supplemented group, a set of ID female offspring, which received from 21 to 70 DPN control diet.

Sample collection

At 70 PND, study subjects were euthanised in a 100% saturated CO2 chamber in order to extract brain tissue and a blood sample for determination of haemoglobin-bound iron (Fe-Hb).

After collection, the brain was washed and immediately placed in PBS (pH 7.4) at a ratio of 1ml/3g sample weight. 1 mL of blood was obtained in test tubes with heparin.

The brain tissue was cut into small pieces and homogenised in a cold mortar and pestle and then in a Potter homogeniser, using a total volume of 4 mL of PBS (pH = 7.4). It was centrifuged at 12,000 rpm for 15 minutes at -4°C. The supernatant was taken and stored at -70°C until analysis.

To determine oxidative stress, the following markers were analysed: lipid peroxidation levels (LPOx), total protein (TP); and antioxidant activity of superoxide dismutase (SOD) and catalase (CAT).

Iron bound to haemoglobin (Fe-Hb)

Hb concentration was determined in triplicate by the cyanomethaemoglobin method using Drabkin's solution (Randox Mexico SA de CV) (Prohaska & Gybina, 2005; Unger *et al.*, 2007).

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Total body Fe-Hb concentrations were determined indirectly using the following formula (Hernandez *et al.*, 2006; Wienk *et al.*, 1999):

$$Fe - Hb(mg) = \frac{\left(\left(\frac{Hb}{L}\right)*(body\ weight)*6.7*0.335\right)}{10000} \quad (1)$$

Where Hb (g) contains 0.335% iron. The blood volume in growing rats is 6.7% of body weight (g).

For the statistical analysis, SPSS 22® statistical software was used; initially a descriptive statistical analysis was performed for each variable considered. The results were presented as means ± standard deviation (mean±SD). To compare differences between two groups (e.g., "Control" and "DFe" or "DFe+S" groups), the Mann-Whitney U-test was used. Results of $p<0.05$ were considered statistically significant, with a 95 % confidence interval.%.

Determination of oxidative stress

Levels of lipid peroxidation

LPO levels were determined by the method of Buege and Aust (1978), where thiobarbituric acid reactive substances (TBARS) are quantified. The decomposition of unstable hydrogen peroxides derived from polyunsaturated fatty acids results in the formation of malondialdehyde which reacts with 2-thiobarbituric acid, giving a pink colour absorbing at 535 nm. The concentration of malondialdehyde was calculated with its molar extinction coefficient: $1.56x10\text{-}5cm^{-1}/M^{-1}$.

Determination of the antioxidant effect

Super oxide dismutase "SOD" activity

SOD activity was determined by the method of Misra and Friodovich (1972) which is based on the measurement of the kinetics of oxidation of adrenaline by the superoxide radical in 5 minutes, the absorbance was measured at the wavelength of 480 nm in a Thermo Scientific Genesys 10S UV-Vis spectrophotometer with quartz cell. SOD activity was calculated using the molar extinction coefficient of epinephrine ($0.021\ mM^{-1}\ cm^{-1}$).

'CAT' catalase activity

CAT activity was determined by the method of Radi *et al.*, (1991), which is based on the measurement of the kinetics of hydrogen peroxide degradation over 2 min by CAT at a wavelength of 240 nm. The enzyme activity was calculated with the molar extinction coefficient of H2O2 ($0.043\ mM^{-1}\ cm^{-1}$).

Total proteins

To specifically report enzyme activity, total proteins were measured by the method of Bradford (1976). Proteins bind to the dye Chromassie Blue G-250 in acidic medium achieving a blue colour, which has an absorbance at 595 nm wavelength. The concentration was calculated using a standard curve obtained with bovine serum albumin in the range of 50 to 500 ug/mL.

All experiments were performed in triplicate.

Results

Determination of iron bound to haemoglobin

When studying the ID group with respect to the control group, it was found that the former had 10.9% less Fe-Hb and 3.8% less Fe-Hb than the ID+S group, see Table 1.

Group	Fe - Hb
Control	3.71±0.11
Iron deficient+supplementation	3.47±0.11
Iron deficient	3.18±0.23*
* vs. Female control group (p≤0.05).	

Table 1 Haemoglobin-bound iron levels "Fe-Hb"

Determination of oxidative stress

Lipid peroxidation: As shown in figure 1a, DFe females presented higher levels of peroxidation, 17.6% higher in relation to control females and 14.1% to DFe+S.

Determination of antioxidant effect

Super oxide dismutase: Figure 1b shows that among the study groups, ID+S subjects reported the highest SOD levels, 253% higher than control females. In ID subjects, SOD levels are 116% higher than in control females and 81.2% lower than in ID+S subjects.

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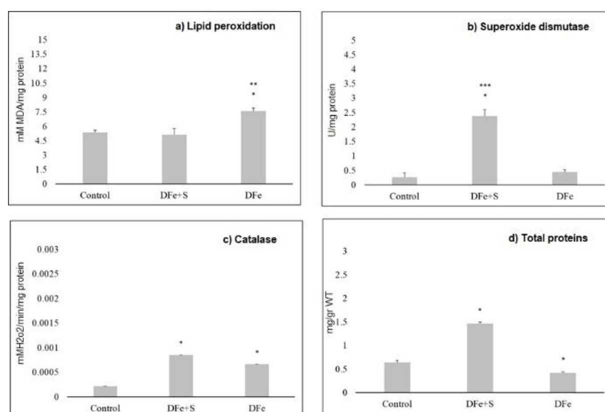
Catalase: CAT levels in the study groups are shown in figure 1c. Catalase levels in DFe+S and DFe females do not show significant differences, however, they are higher than those of control females. 228% higher in DFe+S females and 180% higher in DFe females.

Total protein: As shown in figure 1d, the group with the highest total protein was the ID+S group. 102 % more than the control group and 136 % more than the ID group.

ID females exhibited the lowest total protein levels, 14.6 % lower than control females.



* Female of the control group ($p \leq 0.05$)
**Female from Iron Deficient+Supplemented group ($p \leq 0.05$)
***Female from the Iron Deficient group ($p \leq 0.05$)

Graphic 1 Oxidative stress and antioxidant effect in iron-deficient and supplemented females. Graph "a" shows the levels of lipid peroxidation in the study subjects and is indicative of oxidative stress. Graphs b, c and d; showed the antioxidant effect: b) superoxide dismutase, c) catalase and d) total proteins

Discussion

Iron deficiency (DFe) is a worldwide public health problem, as it causes multiple conditions with major health impacts (DeMaeyer & Adiels-Tegman, 1985; Kassebaum *et al.*, 2014; Stoltzfus, 2001). During neurodevelopment, DFe leads to disturbances such as: disturbances in motor function, anxiety, cognitive and behavioural defects, auditory and visual dysfunction, as well as psychiatric conditions. The pathophysiology of these neurological disorders is complex and includes dysfunctional myelination, abnormal dendritogenesis and synaptogenesis, and even impaired neurotransmitter and hormone synthesis (Algarin *et al.*, 2003; Bakoyiannis *et al.*, 2015).

The neurological alterations of DFe have been attributed to several factors, mainly to a decrease in the incorporation of iron as a molecular structure of proteins. At the antioxidant level, iron is known to be a structural part of catalases (Isler *et al.*, 2002), and it is also known that this trace element participates in oxidative stress processes through the Fenton reaction (Lloyd *et al.*, 1997). Therefore, high levels of iron can cause oxidative stress, and low levels of iron can affect antioxidant defences. It is worth noting that most iron-oxidative stress studies have been conducted at the blood level (Akarsu *et al.*, 2013; Askar *et al.*, 2017b; Diaz-Castro *et al.*, 2008), but it is necessary to know what happens at the central level to learn more about the involvement of iron in neurological effects. Iron has been shown to play an important role in CNS development as it is essential for myelination and axonal development, in fact, it has been established that late supplementation in iron-deficient infants does not restore neurodevelopmental damage (de Ungria *et al.*, 2000). On the other hand, it has been shown at the brain level that iron deficiency tends to increase reactive oxygen species and decrease the in vivo activity of antioxidant enzymes (Thompson *et al.*, 2003) but studies are usually performed in experimental models using males as a sample and little is known in females.

In a previous study, we demonstrated that organs increase iron demand under deficiency conditions, indicating altered organ function in the presence of iron deficiency (Vieyra-Reyes *et al.*, 2017). In the present study, we found that iron-deficient females show the highest levels of lipid peroxidation, indicating that they are the most damaged at the oxidative level. This indicates that oxidative stress cannot be counteracted by antioxidant enzymes. It can be seen that although the levels of superoxide dismutase in iron-deficient females are similar to those of control females, the high brain lipid peroxidation cannot be counteracted by this antioxidant defence. In the case of catalase, the levels are significantly higher than in control females, but they are not sufficient to reduce the damage. This provides further information for the understanding of the neurological, cognitive and behavioural alterations associated with iron deficiency.

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When analysing the effect of supplementation, it was found that the levels of iron bound to haemoglobin are lower than those of control females, however, they are not significantly different. This helps greatly in the regulation of oxidative stress, since thanks to the supplementation, the levels of superoxide dismutase and catalase increase, preventing the levels of lipid peroxidation from being altered. This is also reflected in the increase of total protein levels in supplemented females. This is a transcendent result that supports and sustains the importance of the use of iron supplementation for the prevention of the development of neurological problems and their respective concomitant pathologies.

Conclusions:

- Chronic iron deficiency in females greatly affects the levels of lipid peroxidation in the brain and this effect cannot be counteracted by the antioxidant defence of superoxide dismutase and catalase.

- Iron supplementation in females that suffered from iron deficiency at perinatal level, during gestation and until weaning, equivalent to 21 days postnatal, show normal levels of lipid peroxidation due to the high antioxidant defence activated by increased levels of superoxide dismutase and catalase.

Perspectives:

To develop comparative studies, between females and males suffering from chronic iron deficiency to evaluate the levels of oxidative stress and to establish strategies in order to reach possible solutions to this major public health problem.

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In vitro study of the film thickness of six resin cements

Estudio *In vitro* del espesor de película de seis cementos resinosos

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Abstract

Objectives. Study the differences of the film thickness of six cements to determine that they comply with the standards.

Methodology. Experimental, cross-sectional, *in vitro* study. Film thickness test of resinous cements using the standards of ISO 9917-1.

30 samples were made dividing them into 6 groups of 5 samples each. Group 1: Prime Dent, Group 2, iCem, Group 3: Relyx Ultimate, Group 4: MaxCem, Group 5: TheraCem, Group 6: BisCem. The thickness of two glass slab placed one on top of the other was measured, the resin cement was prepared by placing it (0.05 ± 0.005 ml) at the center of the glass slab with a 3 ml syringe, it was measured again. The weight was removed after 10 minutes, the thickness of both glass slad was measured. Film thickness was calculated by taking the difference between the thickness of the glass slab with and without the sample between them.

Results: An ANOVA test was performed to obtain the statistical value; it was obtained that p=0.000, therefore there are statistically significant differences between the cements. Contribution. The application of dental cements for indirect restorations is used daily, so knowing the film thickness provides security when choosing the ideal material.

Thickness, Cement, Resin

Resumen

Objetivos. Estudiar las diferencias de espesor de película de seis cementos para determinar que cumplan con la norma.

Metodología. Estudio experimental, transversal, *in vitro*. Prueba de espesor de película de cementos resinosos mediante los estándares de la Norma ISO 9917-1.

Se realizaron 30 muestras dividiendolas en 6 grupos de 5 muestras cada uno. Grupo 1: Prime Dent, Grupo 2: iCem, Grupo 3: Relyx Ultimate, Grupo 4: MaxCem, Grupo 5:TheraCem, Grupo 6: BisCem. Se midió el grosor de dos losetas colocadas una sobre otra., se preparó el cemento resinoso colocándolo (0.05 ± 0.005 ml) al centro de la loseta con una jeringa de 3ml, se volvió a medir. Se retiró el peso a los 10 minutos, se midió el grosor de ambas losetas. Se calculó el espesor de película tomando la diferencia entre el grosor de las losetas con y sin la muestra entre ellas.

Resultdos: Se realizó una prueba de ANOVA para obtener el valor estadístico; se obtuvo que p=0.000 por lo tanto existen diferencias estadisticamente significativas entre los cementos.

Contribución. La aplicación de cementos dentales para restauraciones indirectas se usa en el día a día, por lo que el conocer el espesor de película, proporciona seguridad al elegir el material idóneo.

Espesor, Cemento, Resinoso

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Introduction

The evolution of restorative dentistry has brought with it the modification of materials and cementation techniques that seek to improve the working time and duration of the materials in the mouth.

The introduction of new materials has made it possible to reduce working times with the benefit that their properties are not impaired. The new luting materials are created with the aim of achieving a material that has the best characteristics of the natural tooth, that is why we must know the characteristics, limitations and advantages that each one offers us to be able to select the ideal material. Added to that, the process the bonding process is currently widely used helping the cementation of a restoration (Ocejo, 2018).

The properties of resin cements are influenced by the nature of the matrix, type of filler, volume of filler and matrix, filler loading and the polymerization mode that allows the conversion of monomers and that meet the minimum thickness according to ISO 9917-1 (ISO9917-1, 2007).

In the use of dental cements, working with large thicknesses will result in low wear resistance, increased erosion of the luting film exposed to the oral environment, low mechanical strength and low marginal sealing, as well as inadequate seating of the restoration to the tooth surface.

It is important for the operator to know that the film thickness should have a minimum cement thickness that will allow the restoration to support the functional loads of mastication and have an adequate mechanical behavior (Diez, 2022).

When homogeneous cementation films are not handled or there are bubbles, erosions or regions without cementing material, it is easier for microleakage, stress accumulation in uncemented areas or secondary caries to occur, leading to the failure of the restoration.

The present study was carried out to evaluate the film thickness of six self-adhesive resin cements, thus allowing the selection of a luting material that complies with the characteristic of minimum thickness for the correct oral rehabilitation and thus achieve long-term success in the restoration.

The great variety of cements in the market makes it difficult to choose the ideal one, so six of the most used cements in the market are studied to verify that they comply with the qualities they offer.

Over time, new materials have been developed, such as self-adhesive cements, which have been widely accepted due to the reduction of working time.

Currently there are several adhesive cements which ensure optimum adhesion and have better characteristics than those of previous generations, among the factors that are closely related is the film thickness that when handled incorrectly can generate deficiencies in the treatment.

Cementation is determined by the existing space between the restoration and the tooth surface and by the intrinsic capacity of a cementitious material that will occupy that space (bustillos, 2019).

The more complex a preparation is, the greater the possibility of the existence of cement accumulation spaces or air bubble formation, which will affect the fit and the ability to resist mechanical loads (Usechi, 2019).

Currently, resin cements have a fluidity that allows obtaining a sufficiently thin film capable of providing an ideal adaptation of the restoration to the tooth surface, the thickness and homogeneity of the luting layer are related to the mechanical behavior of the cement, directly influencing the durability of the restoration in the mouth (Acosta, 2020 and Manriquez, 2018).

The film thickness in luting materials should not exceed 25 µm above any value stipulated by the manufacturer and the standard (ISO9917-1, 2007).

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For this reason, the need arose to conduct a study on these cementitious materials to determine whether they meet the quality standards set forth in ISO 9917-1 and to compare six resinous cements:

Prime Dent, iCem, Relyx Ultimate, MaxCem, TheraCem, BisCem.

Each of the cements in this research has the ability to adhere to multiple substrates, has a high resistance, insolubility in oral medium, apart from its potential to mimic colors (Camacho, 2020).

The objective is to determine the differences in film thickness of the six cements to determine that they meet the standard and contribute to the ideal choice for each clinical case.

The application of dental cements for indirect restorations is a day-to-day treatment in dental practice (Bustillos, 2019).

In physical properties, it is also advisable to evaluate the cements in addition to film thickness, fluidity, sorption and solubility in order to have a more complete study of all the physical properties of self-adhesive resin cements (Severino, 2022).

Therefore, knowing more precisely one of the most important characteristics, such as film thickness, provides the clinician with certainty when choosing the ideal material.

Methodology

An experimental, cross-sectional, in vitro study was carried out to test the film thickness of self-adhesive resinous cements according to the standards established in ISO 9917-1.

Thirty samples were taken and the study was divided into six groups of five samples each.

- Group 1: Prime Dent

- Group 2: iCem

- Group 3: Relyx Ultimate

- Group 4: MaxCem

- Group 5: TheraCem

- Group 6: BisCem

The present study was carried out in the facilities of the Laboratory of the Faculty of Dentistry of the Universidad Veracruzana, Veracruz Region, during the period August 2020 - August 2022.

As required by the ISO 9917-1 standard, the environmental conditions were standardized, with a temperature of 25° and humidity of 50%.

The procedure was carried out as follows:

The thickness of two tiles placed one on top of the other was measured and the value recorded, then the self-adhesive resinous cement was prepared and (0.05 ± 0.005) ml of the material was placed in the center of the tile with the help of a 3 ml syringe.

After the application of the sample, a second tile was placed and a constant load of 150 N, equivalent to 15.295 kg force x mm^2, was applied for 150 seconds.

The weight was removed after 10 minutes and the thickness of both tiles was measured with the aid of a micrometer (mitituyo) and the film thickness was calculated by taking the difference between the thickness of the tiles with and without the cement sample between them and the data was recorded.

This procedure was performed in the same way and with a calibrated operator performing the same steps for the five samples of the six groups of the study.

Results

The results were as follows:

Group 1: Minimum value of 0.014, maximum value of 0.023 with a mean of 0.0178, variance 0.000022 and standard deviation of 0.00476. See graphic 1.

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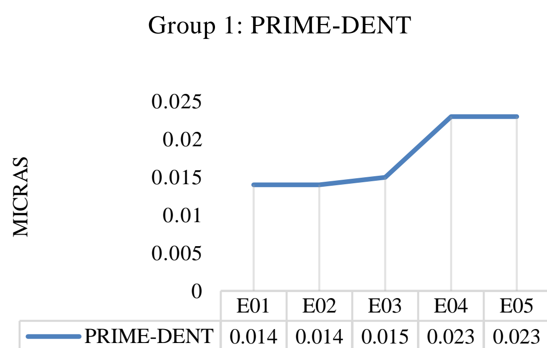
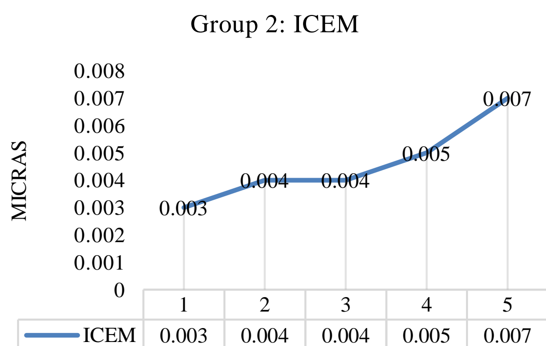


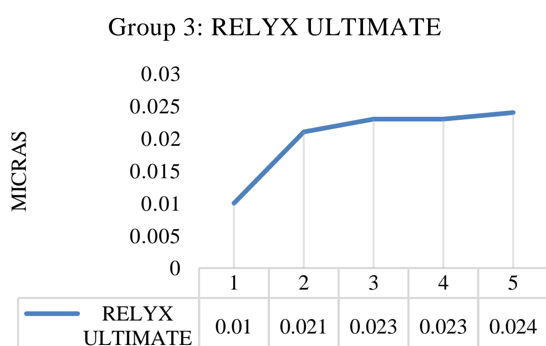
Graphic 1 Results and behavior of Prime Dent Cement

Group 2: Minimum value of 0.003, maximum value of 0.007 with a mean of 0.0046, variance 0.0000023 and standard deviation of 0.001516. See graphic 2.



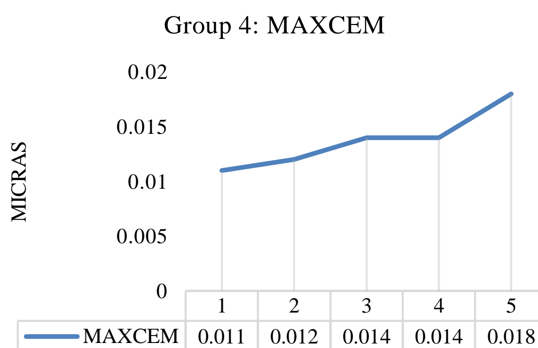
Graphic 2 Results and performance of ICem Cement.

Group 3: Minimum value of 0.01, maximum value of 0.024 with a mean of 0.0202, variance 0.0000337 and standard deviation of 0.005805. See graphic 3.



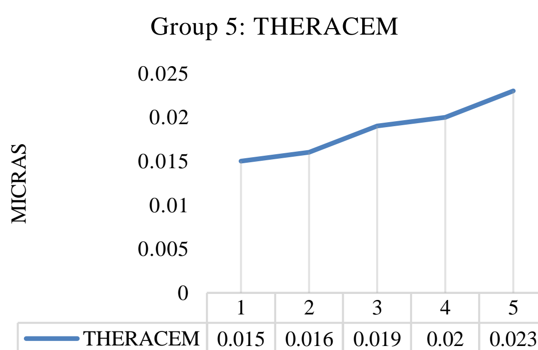
Graphic 3 Results and performance of RelyX Ultimate Cement

Group 4: Minimum value of 0.011, maximum value of 0.018 with a mean of 0.0138, variance 0.0000072 and standard deviation of 0.002683. See graphic 4.



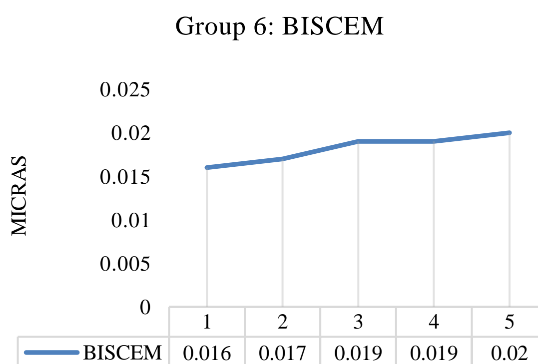
Graphic 4 Results and performance of MaxCemt Cement

Group 5: Minimum value of 0.015, maximum value of 0.023 with a mean of 0.0186, variance 0.0000103 and standard deviation of 0.003209. See graphic 5.



Graphic 5 Results and performance of TheraCemt cement

Group 6: Minimum value of 0.016, maximum value of 0.020 with a mean of 0.0182, variance 0.0000027 and standard deviation of 0.001643. See graphic 6.



Graphic 6 Results and performance of BisCemt cement

All the values obtained by the six groups of cements are detailed in Table 1.

	Prime Dent Group 1	iCem Group 2	Relyx Ultimate Group 3	MaxCem Group 4	TheraCem Group 5	BisCem Group 6
E01	0.014	0.003	0.021	0.011	0.023	0.02
E02	0.023	0.004	0.024	0.014	0.015	0.019
E03	0.014	0.007	0.023	0.018	0.016	0.019
E04	0.023	0.004	0.023	0.012	0.019	0.017
E05	0.015	0.005	0.01	0.014	0.02	0.016

Table 1 Results of the 30 samples that made up the study presenting the values in microns

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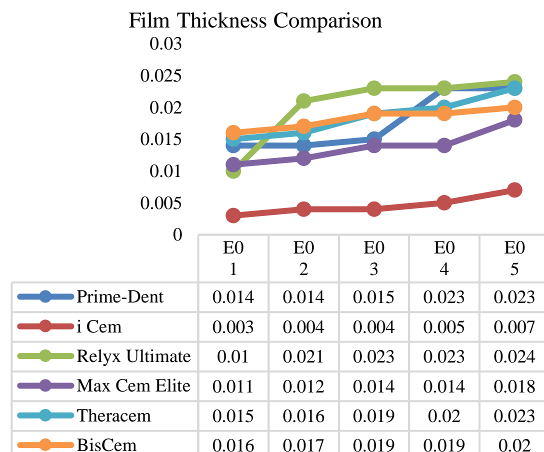
A comparative graph is also made to evaluate the overall performance of the six self-adhesive resinous cements. See graphic 7.



	E0 1	E0 2	E0 3	E0 4	E0 5
Prime-Dent	0.014	0.014	0.015	0.023	0.023
i Cem	0.003	0.004	0.004	0.005	0.007
Relyx Ultimate	0.01	0.021	0.023	0.023	0.024
Max Cem Elite	0.011	0.012	0.014	0.014	0.018
Theracem	0.015	0.016	0.019	0.02	0.023
BisCem	0.016	0.017	0.019	0.019	0.02

Graphic 7 Comparative graph of the values of the 30 samples

An ANOVA test was performed to obtain the statistical value and it was obtained that p=0.000, therefore there are statistically significant differences between the cements.

Once the results were analyzed, it was determined that all the cements comply with the standards, but there is a significant difference between them.

Conclusions

It is important to comment that in spite of the fact that all the cements comply with the norm of having a thickness of .25 microns in the graphs we can see that the most stable cements and with a better clinical behavior are: BisCem in the first place, MaxCem in the second place, MaxCem in the third place and MaxCem in the third place: BisCem in first place, MaxCem in second place and TheraCem in third place.

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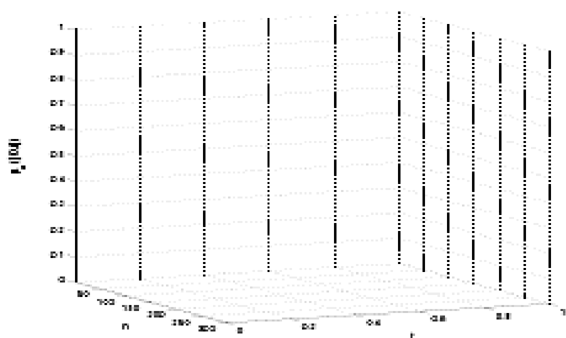

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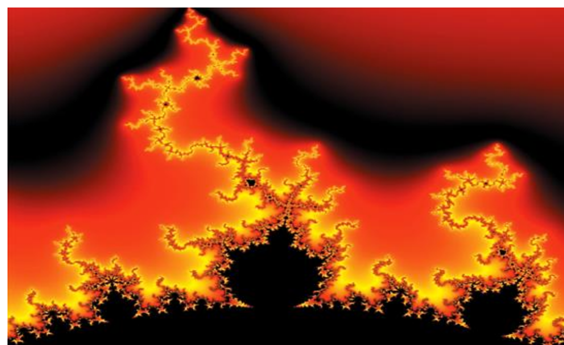

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