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## **Presentation of the Content**

In the first chapter we present, *Betalains from prickly-pear fruit: An alternative natural coloring for food*, by RAMÍREZ-GRANADOS, Juan Carlos, GÓMEZ-LUNA, Blanca Estela, MORALES-VARGAS, Adán Topiltzin and MEJÍA-TENIENTE, Laura, with adscription in the Universidad de Guanajuato, as the following article we present, *Use and application of bioinformatics for the characterization of plant proteomes*, by OSAWA-MARTÍNEZ, Eiko, MINJAREZ, Benito, MORALES-RIVERA, Moisés M. and MENA-MUNGUÍA, Salvador, with adscription in the Universidad de Guadalajara, as the following article we present, *Production of seven varieties of tomato (Lycopersicon esculentum Mill.) pruned to one and two stems under greenhouse*, by VARGAS-ESPINOZA, Everardo, GAYTÁN-RUELAS, Marina, CALDERÓN-RUIZ, Alberto and MORALES FÉLIX, Verónica De Jesús, with adscription in the Universidad Tecnológica del Suroeste de Guanajuato, as the following article we present, *Evaluation of productive and economic parameters of pigs in the final stage*, with three feeding programs, by NOGUEZ-ESTRADA, Juan, AGUILAR-PRICILIANO, Tania, VARGAS-MONTER, Jorge and RODRÍGUEZ-ORTEGA, Leodan Tadeo, with adscription in the Universidad Politécnica de Francisco I Madero.

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## Betalains from prickly-pear fruit: An alternative natural coloring for food

## Betalaínas de tuna: Un colorante natural alternativo para alimentos

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#### **Abstract**

## Food coloring are additives that are usually incorporated into foods to make them more attractive to people. However, some mineral and synthetic dyes used in the food industry are related to harmful effects on human health. Natural dyes, such as betalains extracted from prickly-pear fruit, have fewer restrictions on use and represent a healthier alternative to enhance the appearance of food. In this work, a process to extract betalains from the pulp and peel of Cardona pricklypear fruit was implemented and optimized. The extracted pigment was encapsulated in cornstarch. Then, it was analyzed the effect of the extract/encapsulant ratio on the amount of encapsulated betalains. It was found that the peel of this variety of prickly-pear fruit represents about 48% of its total weight. It was also demonstrated that concentrations of betalains in the peel (12.0 mg/g) and in the pulp (16.5 mg/g) are alike. For these reasons, we consider that the peel of red prickly-pear fruit is an ideal material for the extraction of betalains because it is a waste material with high content of red pigments.

#### Food coloring, Betalains, Prickly-pear fruit

#### Resumen

Los colorantes alimenticios son aditivos que usualmente son incorporados a los alimentos para que sean más atractivos para las personas. Sin embargo, algunos colorantes minerales y sintéticos usados en la industria alimenticia están asociados a efectos nocivos para la salud. Los colorantes naturales, tales como las betalaínas de las tunas, tienen menos restricciones de uso y representan una alternativa más saludable para mejorar la apariencia de los alimentos. En este trabajo se implementó y optimizó un proceso de extracción de betalaínas provenientes de la pulpa y cáscara de tunas Cardona. El colorante extraído fue encapsulado en fécula de maíz. Luego, se analizó el efecto de la relación extracto/encapsulante en la cantidad de betalaínas encapsuladas. Se encontró que la cáscara de esta variedad de tuna representa cerca del 48% de su peso total y que la concentración de betalaínas en la cáscara (12.0 mg/g) es similar a la de la pulpa (16.5 mg/g). Por estas razones, consideramos que la cáscara de tuna roja es un material idóneo para la extracción de betalaínas, ya que es un material de desecho con alto contenido de pigmentos rojos.

Colorante alimenticio, Betalaínas, Tuna

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#### Introduction

Consumers express a strong preference for products with attractive appearance, since color is the first attribute that is judged in food. This is decisive since in innumerable tests it has been proven that when the color of a food changes without altering its shape, aroma or other attributes, a rejection response is obtained from consumers. Food, both in its natural and processed form, has a characteristic and well-defined color by which consumers identify them. Color is often used to determine the pigment content of a food, which in turn is a quality index (González and Vicente, 2007).

It can also provide some information about the edible quality of a food, about its identity or about the intensity of the flavor. Therefore, in many cases the decisive role of color on the person's experience in tasting a food has been proven (Badui, 2006). Color is an organoleptic quality of food and is appreciated through the sense of sight. It is also usually considered psychological a factor appreciation and a criterion for choosing a food product; even in products of plant origin it is related to the possibility of evaluating the degree of maturation.

In this work, color is approached as a quantifiable variable in determining the quality of food quickly, accurately and objectively. Today there are different tools to measure color reliably and are increasingly accurate and easy to interpret. The use of these tools in the food industry is increasing, especially with new digital vision technologies, which are non-invasive techniques for food and allow inspection of an entire production, while ensuring the safety and quality of the food. In addition, the image can be correlated with other properties of food, such as texture, size and distribution of ingredients.

## Justification

The addition of chemicals to food today is a daily occurrence. These substances help in food processing by improving or highlighting a special feature; These substances are called additives. These come from various natural and artificial origins, according to Codex Alimentaius (CODEX, 1995).

The concept of additive refers to any substance that, regardless of its nutritional value, is intentionally added to a food in controlled quantities and for technological purposes.

One of the main additives in foods are dyes that reinforce or vary the color. Processed foods usually have artificial coloration in order to make them more attractive to consumers. Currently these are obtained through three routes that are: mineral, synthetic and natural, currently being the most used synthetic. However, artificial colors are being replaced by natural ones because several studies have shown that these can cause harmful effects in the body. For example, tartrazine causes hives in less than 0.01% of the population exposed to it: while erythrosine is related to thyroid tumors in rats.

For these reasons and the current change in consumer trends, natural dyes are an excellent alternative to enhance the color of food. These are usually obtained from non-food biological materials such as plants or insects or are formed spontaneously by heating a food such as in the case of caramel. Natural dyes are generally considered harmless and the specific limitations in their use are less than those that affect synthetic dyes.

## **Definition of the problem**

People associate the color of food with its quality. Processed foods are usually added with artificial colors to reinforce or modify their color with the intention that consumers accept them for their good looks. However, the correlation between color and quality is not always valid. The abuse of dyes can hide defects or deficiencies of the food, harming the consumer. Moreover, some artificial colors have harmful effects on health, especially when consumed in amounts greater than the recommended daily intake. To avoid these inconveniences, some artificial dyes are being replaced by natural dyes.

## **Hypothesis**

The prickly peel of the Cardona variety is a plant material with a high content of reddish natural pigments that could be used in the food industry to highlight the color of food, giving added value to this material that is commonly discarded by people.

#### Goals

The general objective and specific objectives of this work are presented below.

## Overall objective

Obtain a natural dye for the food industry from the fruit of the cacti genus of Opuntia spp.

## Specific objectives

- Extract the natural pigments from the fruit and determine its concentration through ultraviolet and visible spectrophotometry.
- Propose possible applications of extracted pigments.

#### Theoretical framework

#### **Betalain content**

The quantification of the pigments was carried out by spectrophotometric methods based on the photometric properties of these compounds, according to which they absorb light in certain regions of the visible spectrum (betaxanthines: 470-480 nm and betacyanines: 538-540 nm).

The absorbance that is determined in the extract solutions at the indicated wavelengths must be less than 1. The betalaine content is calculated using the following equation proposed by Castellanos-Santiago and Yahia in 2008, through the absorbance of the extracts of betalains at 538 and 483 nm. For the conversion of absorbance units to concentration units the expression was used:

$$B[mg/g] = (A \times FD \times PM \times V) / (E \times P \times L), \tag{1}$$

where B represents betacyanins or betaxanthines, A is the absorbance at 538 nm for betacyanins and 483 nm for betaxanthines, FD is the dilution factor at the time of reading on the spectrophotometer, PM is the molecular weight (Betanin = 550 g / mol and Indicaxanthin = 308 g / mol), V is the volume of the extract,  $\epsilon$  is the molar extinction coefficient (Betanin = 60000 L / mol-cm, and Indicaxanthin = 48000 L / mol-cm) and L is the width of the cell (1 cm).

## **Extraction of betalains by chemical maceration**

It is a widely used method for the extraction of natural pigments. This being a basic operation whose purpose is the separation of one or more components contained in a solid phase through the use of a liquid phase or solvent. The component is transferred to the liquid phase that receives the name of solute and is the one that is recovered, while the rest of insoluble material or plant material is discarded.

This type of operation can be carried out in one or multiple stages, being one stage in which the two phases are contacted for a while until equilibrium is reached, once reached the mechanical separation of the phases is achieved. The separation can be single or there can be multiple stages, and in turn it can be continuous or discontinuous.

For the extraction of these pigments, the fruit is macerated in water or the material is ground. In most cases, 20 to 50% v/v aqueous or methanol solutions in different percentages are used as solvents to achieve complete extraction (Piatelli, 1981).

## Pigment encapsulation

Encapsulation can be defined as a technique whereby liquid drops, solid or gaseous particles, are covered with a porous polymeric film containing an active substance. This membrane, barrier or film is generally made of chain create a network components to hydrophobic and / or hydrophilic properties (Fuchs et al., 2006). The term microencapsulation is similarly used in the food industry when low molecular weight substances are encapsulated or in small quantities, although the two terms, encapsulation and microencapsulation, are used differently (Yañez et al., 2002).

Among the first practical applications of microencapsulation, the pharmaceutical, medical, textile, food industry (Dutta et al., 2009), pesticide, cosmetics, chemistry (Fuchs et al., 2006), antimicrobial agents (Zong et al., 2009) biomedical and plastics (Dutta et al., 2009).

Regarding the food area, the applications of this technique have been increasing due to the protection of encapsulated materials from factors such as heat and humidity, allowing to maintain its stability and viability. The microcapsules help the food materials used to resist the conditions of processing and packaging improving taste, aroma, stability, nutritional value and appearance of the products (Yañez et al., 2002; Montes et al. 2007).

The microencapsulation technique has allowed to solve some problems that limit the applications of ingredients and food additives, since it can control the elimination of flavorings; as well as reducing volatility, hygroscopicity and reactivity by increasing the stability of products under adverse environmental conditions.

## **Encapsulation methods**

Some techniques of the for used microencapsulation spray are: drying, lyophilization, extrusion, fluidized bed coating, liposome entrapment, coacervation (Gibbs et al., 1999; Santinho et al., 2002), inclusion, centrifugal extrusion, rotational suspension, interfacial separation and polymerization (Wang et al., 2004), among others. The selection of the encapsulation method is based on costs, on the properties of the material to be encapsulated, on the desired size of the microparticles, on the application and on the release mechanism (Pedroza, 2002).

Spray drying is the most widely used encapsulation method in the food industry to encapsulate active compounds and protect materials economically, simply and continuously. Through this technique the solution or dispersion is atomized (with a nozzle or rotating disk) in the form of fine drops in a flow of hot air. When the small drops of liquid come into contact with the hot air a powder is instantly obtained due to the rapid evaporation of the water.

A wide variety of encapsulating materials for food applications have been studied; However, it is important to consider characteristics such as water solubility, permeability, ease of application, low viscosity in concentrated solutions and their hydrophobic or hydrophilic nature. All these factors will influence the characteristics of the final product (Gibbs et al., 1999).

## **Encapsulating materials**

The main encapsulants used for this method are: carbohydrates (starch and derivatives, maltodextrins, corn syrups, cyclodextrins, carboxymethyl cellulose and derivatives), gums (arabic, mesquite, sodium alginate); lipids (waxes, paraffins, fats) and proteins (gelatin, soy protein, caseinates, whey, zein).

These encapsulants must have the ability to provide a stable emulsion during the spray drying process and have very good film-forming properties to provide a layer that protects the active ingredient from oxidation. The physical properties of the microcapsules depend on the temperature of the hot air, the degree of uniformity of the spray and the solids content in the emulsion.

#### Methods and materials

#### Raw material

The prickly pears of the Cardona variety or red prickly pears were obtained from the fruit vendor market in Celaya, Gto., Figure 1.



Figure 1 Tunas of the Cardona variety

## Selection and cleaning of the fruit

The prickly pears were selected having the following considerations: intense color, ripe fruits, healthy and free of damage. This stage is of importance since fruits that are in a state of decomposition can affect the process of obtaining the dye (betalains). For disinfection it was washed with soap and water to remove spines and dirt.

## Conditioning of the raw material

The peel and pulp of the prickly pears were separated manually using good-edged stainless steel knives (Figure 2). Care was taken not to leave pulp attached to the shell. Considering that the dye that has the prickly pear peel degrades easily in the presence of high temperatures and in contact with sunlight, it is stored under refrigeration for later use.



Figure 2 Prickly pears

## 3.4. Obtaining the dye

The extraction of betalaine-type pigments was carried out by means of chemical maceration, placing 270 g of fruit peel and separately another 270 g of pulp that were submerged in distilled water, (Figure 3). 3 extractions of 2 hours were performed until the fruit was discolored. After the procedure, a liter of extract was collected, then a filtration was carried out to eliminate the residue of particles that could interfere with the subsequent procedures.



Figure 3 Red prickly pear coloring extract

The betalaine content of the crude extract was determined by a spectrophotometric method (Castellanos et al. 2008). The aqueous extract was dissolved to ensure that the absorbance (A) was in the optimal range (0.8 < A < 1.0), Figure 4.



**Figure 4** Typical absorbance of dilutions with red prickly pear pigment extract

#### Results

## Description of the fruit

According to the analysis developed in the study, a description of the plant material used was made, Table 1.

Characteristics	Description
Color	Ripe fruit that has an intense reddish peel, like its pulp. Free from damage
	or stains.
Form	Ellipsoid 4.0 cm long and 2.5 cm wide approx.
Texture	Firm crust and soft pulp
pН	5.34 (shell).

**Table 1** Physical and chemical description of red prickly pears

## Weight of prickly pear Cardona

With an analytical balance, the weight of whole fruits and their peels was measured (Table 2). An average of 47.61% of the shell weight was determined in relation to the total weight of the prickly pears. Huaman-Congora studied in 2014 the prickly pears from the Department of Ayacucho in Peru and reported an average of 48.7%.

Sample	Total Weight (g)	Shell Weight (g)	Shell weight percentage (%)
1	121	56.0	46.28
2	120	61.0	50.83
3	118	52.8	44.75
4	130	70.0	53.85
5	127	62.0	48.82
6	132	54.3	41.14
Average	125	59.4	47.61

**Table 2** Percentage of shell weight in relation to the total weight of Cardona prickly pears

Almost 50% of the prickly pear is shell and is commonly discarded, which is why it constitutes an excellent organic material that can be used as a raw material to obtain natural dyes.

## Obtaining the crude extract

In the Methods section the manner in which the extract was obtained was described in detail. The extract was obtained from fresh prickly pears that were washed, cut and separated in shell and pulp until 270 g of each of the latter were obtained. The solvent (distilled water) was used in several washes to obtain a liter of solute that was filtered to avoid interference with the plant material, Figure 5.



Figure 5 Raw extract of red prickly pear coloring

The content of betacyanins and betaxanthines was quantified by the methodology described above by recording the absorbance of the betacyanin extracts at 538 nm and that of the betaxanthines at 480 nm. The absorbances obtained are shown in Table 3.

	Betacyanins	Betaxanthines	Total betalains
Α	7.26 mg/g	4.74 mg/g	12 mg/g
В	11.15 mg/g	5.35 mg/g	16.5 mg/g

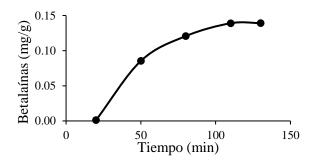
**Table 3** Total betalains content in shell (A) and pulp (B) of red prickly pears

## **Extraction process optimization**

## **Immersion time**

The immersion time of the pulp in the solvent was evaluated during the extraction process to establish the optimum time for maceration. In a beaker, 5 g of prickly pear with 100 ml of distilled water were placed. It was subsequently placed in a magnetic stirring machine at 150 rpm.

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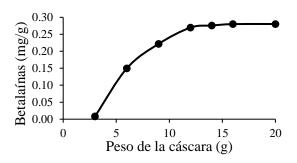
**Graphic 1** Concentration of betalains as a function of the immersion time of prickly pear pulp in the solvent

It can be seen that the concentration of the dye (betalains) in the extract increases between 50 and 110 min. Subsequently, the concentration remains almost constant, reaching a concentration of 0.140 mg of betalains at 110 min as shown in Graphic 1.

Prakash Maran evaluated in 2012 the extraction of pigments from prickly pear pulp using water as a solvent and determined that the optimal time is 115 minutes, which coincides reasonably with the results obtained in this evaluation. On the other hand, Sánchez & Gonzales (2006) determined an optimal immersion time of 10 minutes; however, they used as solvent a water / ethanol solution in relation to 80/20.

## Amount of raw material processed

For the evaluation of the amount of raw material, increasing amounts of prickly peel were macerated from 3 to 20 g in 100 ml of distilled water for 120 min with magnetic stirring at 150 rpm.



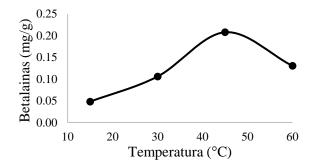
**Graphic 2** Betalaine concentration in the dye extract as a function of the amount of prickly peel used in the maceration process

The concentration of the dye is increased by mashing increasing amounts from 6 to 16 grams of prickly peel. After these quantities, the concentration remains almost constant as shown in Graphic 2. The maximum concentration of betalains was 0.280 mg/g and was obtained from the maceration of 16 g of prickly peel in 100 ml of water.

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## **Maceration temperature**

The maceration temperature was evaluated by placing 5 g of prickly peel in beakers with 100 ml of water, which were heated to a certain temperature and kept for 40 minutes. Subsequently they were allowed to cool before measuring the concentration of betalains.



**Graphic 3** Betalaine concentration as a function of solution temperature

When observing the results, it can be observed that at a temperature of 45  $^{\circ}$  C the greatest amount of betalains with a value of 0.208 mg / g is obtained without affecting its stability (Graphic 3) as predicted by Prakash & Maran (2012), who found that when using an extraction temperature of 40  $^{\circ}$  C and 115 min of heating a maximum concentration of 35 mg / 100g of betalains was obtained.

## **Pigment encapsulation**

One of the most used methods for obtaining natural dyes in powder form is the spray drying technique because it is the most used, economical and effective for the encapsulation of color, flavor and texture. This was used here for being the most convenient technique for its short production times and the use of low temperatures, which is crucial when the products are sensitive to heat because it promotes a high retention of taste, color and nutrients, in addition to have more economic feasibility compared to other techniques.

For the procedure, the extract of 100 g of plant material was obtained with the procedure described above. Subsequently, the pigment was encapsulated with corn starch (Figure 6) as described in the thesis "Obtaining an organic dye for the food industry from pitahaya," by Córdoba Torrez in 2014.



Figure 6 Encapsulation of betalains in cornstarch

This encapsulation was performed for different extract-encapsulant relationships to study differences in color and determine the best relationship for the process of encapsulation of prickly pear pigments, Table 4.

Sample	Corn	Extract	Relationship
	starch (g)	Volume (ml)	
1	25	30	1/1
2	20	30	5/4
3	15	30	5/3
4	10	30	5/2
5	5	30	5/1

**Table 4** Extract-encapsulant ratios used to determine the best tuna dye encapsulation conditions.

Once the ratios were obtained, the beakers were stirred for 6 minutes until the mixtures were homogenized. Once the mixture was homogeneous and free of lumps, an oven was used to dry the sample at a temperature of 45  $^{\circ}$  C for one day to obtain a dry powder (Figure 7).



Figure 7 Drying the encapsulated dye

Once the powder was obtained, it was transferred to cellophane bags to more easily handle the encapsulated dye and weighed to compare the difference of the initial (wet) and final (dry) weight, the results are shown in Table 5.

Shows	Initial sample weight and starch (g)	Final Weight (g)	Weight difference (g)
1/1	50	38.72	11.28
5/4	45	31.49	13.51
5/3	40	23.53	16.47
5/2	35	15.83	19.17
5/1	30	7.49	22.51

**Table 5** Weights obtained before and after encapsulation of the dye

Table 5 shows the loss of weight during encapsulation due to the evaporation of excess moisture in the mixture. With this it can be observed that the larger the corn extract-starch ratio, the greater the weight loss. This could imply that the material can only encapsulate a certain percentage of the pigment so that the smaller ratios would not help encapsulate the entire pigment. However, the use of larger amounts of encapsulants and longer immersion times should be explored to assess whether it is possible to avoid loss of extract and see if there is a higher concentration of the encapsulated pigment.

## **Evaluation of encapsulated betalains**

The color of the encapsulated dye samples was measured by a colorimeter. For measurements, the average color of 10 areas located in random parts of the sample was used. The results are presented below in Table 6.

	Extract / encapsulant ratio (g / g)							
Coord. chromatic	1/1	1/1 5/4 5/3 5/2 5/1						
L	89.69	87.57	88.13	84.68	86.93			
A	10.22	10.88	11.7	12.66	11.41			
В	-6.31	-5.39	-5.72	-4.22	-5.17			

**Table 6.** Average color of the encapsulated extract for different extract / encapsulant ratios.

To determine the total color difference in the three chromatic coordinates the following definition was used:

$$\Delta E = \sqrt{\Delta L^2 + \Delta A^2 + \Delta B^2} \tag{2}$$

It is important to note that  $\Delta E$  indicates the magnitude of the total color difference. For the measurement of the color change, the sample with a 1/1 ratio was considered as the reference sample to determine the difference with respect to the other relationships where the corn starch content is lower.

	Extract / encapsulant ratio (g / g)					
Color variation	1/1	5/4	5/3	5/2	5/1	
ΔL	-2.12	-1.56	-5.01	-2.76	-2.12	
ΔΑ	0.66	1.48	2.44	1.19	0.66	
ΔΒ	0.92	0.59	2.19	1.14	0.92	
ΔΕ	2.403	2.230	5.987	3.215	2.403	

 Table 7 Color difference for various extract / encapsulant ratios

Table 7 shows that the different encapsulations have a color difference. The chromatic coordinate L indicates the brightness of the sample, the value A refers to the region of the color space where the shades range from green (negative) to red (positive), value B refers to the region of the color space where the shades range from yellow (negative) to blue (positive).

When observing the results of the different samples, it was found that the relationship with the most difference in luminosity, red-green and yellow-blue hue, compared to the sample with 1/1 ratio, is the sample with a 5/3 ratio which has a total color difference of 5,987. This is interesting since with this differences in color are observed by modifications in the encapsulation. This may suggest that when using the 5/3 ratio the compound of interest, in this case betalains, is more concentrated and therefore the total color difference is greater than for samples with other relationships.

The time that the extract and the encapsulating agent were in contact was 6 minutes, but from what was observed in the colorimetric analysis it would be interesting to use longer immersion times to know if the analyte can be concentrated more effectively, in addition to quantifying the encapsulated betalains.

## Acknowledgments

This work was carried out with the support of the Rectory of the Celaya-Salvatierra Campus of the University of Guanajuato. The support of Mario Alberto Lozano Servín for his valuable collaboration is also appreciated.

#### **Conclusions**

Prickly pears are an important source of natural dyes. It was found that the weight of the prickly pear peel Cardona represents 47.61% of the total weight of this fruit, which makes it a viable material for the extraction of natural dyes since added value would be given to a waste material. Through the quantitative analysis of the extract, a maximum concentration of 12 mg / g of betalains was obtained in the peel of the prickly pear Cardona; while a maximum concentration of 16.5 mg / g was obtained in the pulp, this being an indication that both the pulp and the skin of the red prickly pears have a high betalaine content.

Considering the cost of the raw material, the most suitable plant material for the extraction of reddish dyes is the shell because it has no commercial value, unlike the pulp that is used as food. On the other hand, when evaluating different variables to optimize the extraction process, it was found that the optimal immersion time for maceration is 110 minutes, since it allows obtaining a higher concentration of betalains. As for the amount of raw material, it was found that, the more matter, the higher the concentration of betalains. In this aspect, a saturated solution with 0.280 mg/g of betalains was obtained by macerating 16 g of raw material in 100 ml of water.

With respect to the effect of temperature on the extraction process, betalains are known to degrade when subjected to temperatures greater than 60 ° C for periods greater than one hour. By subjecting the aqueous extract to different temperatures it was found that the highest concentration of betalains was obtained at 45 ° C; at higher temperatures there is a decrease in betalains in the extract.

The exposure time of the extract at these temperatures is also an important factor since, the longer the exposure time, the greater the degradation of the pigment. In addition, when performing the encapsulation of the dye in the corn starch, the effect of the different extract / encapsulant ratios on the color of the sample was studied. It was observed that at a higher extract / encapsulant ratio, the loss of extract is greater during drying. When performing a colorimetric analysis, it was found that the 5/3 ratio has the largest total color difference with a value of 5,987, which indicates that the sample with this ratio would have a higher betalaine content.

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## Use and application of bioinformatics for the characterization of plant proteomes

## Uso y aplicación de la bioinformática para la caracterización de proteomas vegetales

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#### **Abstract**

#### **Proteomics** and some other cutting-edge technologies have generated information clusters in sequencing and protein studies for plants, which can be used in other areas, such as food in quality control, pharmacological in allergens, characterizations of organisms in studies biological and agronomic for vegetables. The following is a description of the information that can be found in the databases (DB) and their interrelations with other specialized DB, of all the references to describe a protein. For this investigation we used a storage protein, Glutelin-2 in (Zea mays), we show some of the interrelated DB that can offer information for multiple studies of proteins in plants like UniProt KB and STRING-DB.

## Protein, Glutelin-2, Proteomic, Data base, Description

#### Resumen

El uso de la proteómica y algunas otras tecnologías de punta como las plataformas bioinformáticas han generado cúmulos de información en secuenciación y estudios proteicos para diversos organismos vegetales. Cuyos resultados, pueden ser utilizados en otras áreas de investigación, como la alimenticia en el control de calidad, farmacológicas en los alergénicos, las caracterizaciones de organismos en estudios biológicos y agronómicos para vegetales. En el presente estudio describimos la información que se puede encontrar en las bases de datos (BD) públicas y sus interrelaciones a otras bases especializadas, de todas las referencias para describir una proteína y su relación con el proteoma general. Para lo cual, se utilizó una proteína de almacenamiento, llamada Glutelin-2 en granos de maíz (Zea mays), se muestran algunas de las BD interrelacionadas que pueden ofrecer información para múltiples estudios proteómico moleculares como UniProt KB y STRING-DB.

Proteína, Glutelin-2, Proteómica, Base de datos, Descripción

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#### Introduction

Food and its supply in many countries is complemented through imports, especially in the particular case of cereals; taking as an example the corn in Mexico, for the year 2016 it presented a deficit of 12.5 million tons (SAGARPA, 2016) which were imported, having as main origin the USA (González, 2018).

Another important aspect to consider in relation to food is that they regularly share the same geographical origin as the population that consumes them, which creates a greater root in the uses and customs of the food of the different agricultural products. Thus, the increase in the population and the industrialization of these products, increasingly generates an increase in cost and a decrease in their availability. Therefore, it is imperative to implement new techniques that promote greater performance in production rates, in addition to the need for an improvement in the quality of these, as well as ensuring that these foods are free of toxic substances such as metals heavy (Cd, Pb), which represent a high risk factor in the health of the population that consumes them (Chakrabarty et al., 2009, Cao et al., 2017).

In addition, it is worth highlighting the importance in the content of amino acids or the presence of traces of other foods such as flours (Colgrave et al., 2015) Thus, quality controls, health requirements, adequate control of Herbicide or heavy metal contamination indexes, or the identification of genetically modified organisms, are aspects that must be detected in a short time, with precision and efficiency, using a reduced sample of the product to be analyzed, in addition to using techniques that do not increase the final cost of the product (Arvanitoyannis and Vlachos, 2009).

For this purpose, it is that recently the latest technologies and highly precise tools have been implemented in the characterization of the such different agricultural products bioinformatics. proteomics and Thus. proteomics is the technique that is responsible for the description of proteins expressed by a genome at a given time and conditions (Wilkins et al., 1996). Whose technique can be applied in the characterization of cereals and legumes, in different parts of the plant or under different planting and nutritional conditions.

In addition, that its use can be applied in the study of other foods such as honey, milk, flours or fruits, among other materials; Also, it is important to note that these techniques can be coupled to other tools such as liquid chromatography and mass spectrometry by increasing the identification of the protein content of small sample quantities (micrograms) with great precision (Wilkins et al., 1996).

Thus, proteomics based on bioinformatics and mass spectrometry (MS) are methodologies most used the identification, quantification and characterization of proteins and can be a useful tool when it comes to the study of different cereal varieties as for example, corn (Zea mays) (PPDB; http://ppdb.tc.cornell.edu (Sun et al. 2009; Chen et al. 2017), rice (Oryza sativa) http: //gene64.dna. affrc.go.jp/RPD/ (Komatsu et al. 2004) and wheat http://www.wheatgenome.org/ (Vu et al., 2017) In addition, these devices are supported and powered by different bases of international (BD) data; where, free of charge, the sequences of the peptides and the name of the protein detected in the analysis characterization can be located according to their function and / or class even when they come from complex samples such as total or simpler homogenized from a band extracted by conve electrophoresis national, where the results obtained are characterized by being highly accurate, reliable and in a very short time.

As described above, we propose the use of these techniques for the generation of new methodologies in the generation of data with potential in the identification of important properties for quality controls and the selection of a material for food, industrialization or other potential uses such as medical and / or pharmacological. Therefore, the present work has the purpose of showing part of the information contained in the BDs for proteins within plant organisms and their application in the In silico characterization of said crops.

#### **Justification**

The molecular proteomic characterization of organisms or materials in a precise way, with high levels of confidence, quickly and with a low amount of sample, is an attractive methodology and an important source of information in the identification of the quality of a food such as cereals, oils or flours, as well as cultivars that are imported for food or industry.

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Also, it is important to indicate that the proteomic characteristics in the case of study materials allow the generation of new sources of important information for a more detailed and detailed description, in addition to conducting interdisciplinary studies that focus on analyzing different aspects of the organism of interest as well as the characterization and genomic and proteomic behavior of the plant under very specific conditions.

Therefore, the use of internationally available BD can contribute to the generation of new knowledge and information necessary for the different objectives in an investigation, in relation to protein and nutritional content.

#### **Problem**

The use of BD and bioinformatics platforms in the description of the quality and protein content of a crop quickly, accurately and effectively are key properties for the characterization and selection of the main products for human, agricultural or industrial consumption. Therefore, the consultation of information available in the BD, can contribute to complement important properties for decision making in the characterization or destination of the product or object analyzed.

## **Hypothesis**

The use of the information obtained from the BD and its analysis through bioinformatics platforms contributes to the description of a protein or the complete proteome of a culture.

## **Objectives**

Identify the potential of bioinformatics tools and BD in the in silico description of a protein and its relationship with the rest of the proteome in corn grains.

## General objective

Contribute to the description of the corn proteome.

## Specific objectives

Identify and describe the information necessary for a protein through the BD linked to bioinformatics platforms in proteomic analyzes.

#### Theoretical framework

In almost all the world the lack or low availability of food is complemented through imports which increases the final cost of them. For example, in Mexico for the year 2016, 12.5 million tons of corn were imported for national consumption only (Agri-food Outlook for corn 2016). This is even more complex when we highlight that cereals such as wheat, corn, sorghum or rice; They are the largest source of protein consumed primarily by the population of developing and emerging countries. These crops are also deficient in some essential amino acids, such as lysines and tryptophan, which negatively impacts the development and nutrition of the population, especially people with low incomes and vulnerable populations such as children, older adults and pregnant women (FAOSTAT, 2014).

In other contexts, the need for strict quality controls for the various foods offered in the market and the identification of those products with high potential in the diversification of their uses, is another aspect of interest such as the capacity of pharmacological uses, industrial, alternative energy sources or the possibility of being classified and labeling them as possible risk factors and / or allergens, is attractive and necessary for a better offer to the final consumer.

Thus, proteomic analyzes can show the characteristics of these products and raise the processes of quality control and selection of the different agricultural products for human, animal and industrial consumption (Carrera et al., 2017). In addition, the characterization of food through proteomics based on mass spectrometry represents the possibility of an alternative tool in the typing of food accurately and reliably. What would have a favorable impact on food biosecurity and compliance with international safety standards. (Korte and Brockmeyer, 2017; Ortea et al., 2016).

On the other hand, the biological BDs are the repositories of all the information of the genomes and proteins investigated by a large number of researchers and institutions both public and private around the world. In addition, the BDs are integrated by a set of data belonging to the same context, whose objective is to organize the information, structuring it in specific registers and where many of them are available for free to the general public.

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It should also be noted that, each record is composed of specific fields for each peptide, lipid, chemical nucleotide sequence, etc. Where, you have to specify a piece of information called value (in the case of proteins the registration key, or compound name). In order to access the data, programs called BD management systems are developed that make it easier to consult the information contained, which allows a report to be generated through graphics maintaining the integrity of the data. (Rodríguez, 2013). Thus, the biological BD can be focused on flat, animals, viruses, fungi or any other organism (presented as a list of text-type information), but have relational fields, (Leagues) that connect with other databases thus concentrating, more information about the search (Uniprot KB Consortium, 2014).

For the particular case of proteins, which are the central biomolecules responsible for all cellular functions in the living organism, they represent the central focus of proteomic studies, further expanding the panorama of the metabolic, physiological and / or pathological processes of an organism. at a given time or circumstances (Chandramouli and Qian 2009). Thus, proteomics is becoming an indispensable tool for global phenotypic characterization at the molecular level that provides information on the identification of genes and the role of dynamic post-translational modifications (PTM) and protein interactions to other biomolecules, linking the genotype and its metabolisms with the phenotype and functionality (Hu et al ,. 2015).

In addition, different tools have emerged exponentially increasing peptide analyzes thus strengthening the information available in the BDs; One such technique is tandem mass spectrometry (MS / MS). Whose information generated, can be analyzed by the use of various BDs both for complex protein sequences and their post-translational modifications, or be subjected to homology and function analysis through different software. Although, it is necessary to indicate that sometimes the results obtained may present some ambiguity and this may be related to different factors, such as the methodology used in the realization of the project or the incompatibility of the reagents for its analysis, which generates false positives or the loss of the analyzed sample.

## **Research Methodology**

The mapping for the identification of proteomes of specific organisms by mass spectrometry is based on several BD characterizing the different spectrograms obtained in relation to the mass and load of peptide sequences contained in the sample (Abián et al., 2008). In addition, the development of proteomic meta-analyzes represent an invaluable generation of knowledge of the proteome of an organism at specific times and circumstances, which in turn provides a broader picture of the physiological and / or pathological processes of said experimental analyzes and their impact on the metabolism of the organism and finally the impact on the observed phenotype. These studies represent the enrichment of the biological BD and strengthen, in turn, the information contained in the different bioinformatics platforms, thus allowing to administer and take advantage of all this information more efficiently.

It should be noted that, this work is part of the research project: Typing of MR 2008 corn and its progenitors of the BEMARENA Postgraduate Program in Agricultural Sciences of the University of Guadalajara of CUCBA.

For which, the Glutelin-2 corn endosperm storage protein was randomly selected. For this selection, the use of the UniProt KB protein BD was necessary. Identifying proteins characterized in previous works carried out by various researchers and contained in said database, to verify their presence in the plant. A list of proteins was displayed, selecting it based on the coincidences with the name of the protein and the plant species. So this Glutelin-2 protein was chosen with the access code P04706.

### **Materials and methods:**

For the present study, two databases were used mainly; which are online and are freely accessible. In the case of direct reference generation analysis and general corn information analysis, we used the NCBI BD (National Center for Biotechnology Information). For the specific case of the information related to protein level we work with the UniProt KB BD using the access code P04706.

## Kind of investigation

Descriptive applied research, in order to identify the sources to obtain information in the description of a protein belonging to a plant.

- The need to characterize organism and its protein content, to allocate them to different uses.
- The identification of food quality or its origin.
- Obtain sufficient and efficient information on the characterization of a protein.
- Identify the scope of the information obtained in the BD interconnected to MS.

#### **Results**

For this work, the registry for Zea mays Gluterlin-2 was selected, with the UniProt access code KB P04706. From the access code it is possible to access the information contained in the databases and thus know the characteristics of the protein, but it is also possible to do so by means of the exact name of the protein or the name of the gene.

Once on the page and with any of the protein data of interest mentioned above, the search is done in the UniProt KB database, which allows us to access the most updated and reliable information worldwide, as we It provides not only information of the protein of interest but also offers us information contained in other platforms with possible relevance for our investigations;

which we highlight Among interaction with other proteins, biochemical data such as its isoelectric point, (pI) its molecular structure, weight, its secondary dimensional modeling (3D) homology with other proteins, post-translational modifications; In addition to the most relevant works for physiological and metabolic issues within the organisms to study. For this investigation we find the following information:

The protein name for the access code P04706 for corn is Gluterlin-2, where until now, the gene has not been described (N / A), and its subcellular location is the vacuole, (Figure 1A).

Its function is also described which classifies it as a seed storage protein and with a molecular function of nutrient reservoir. It also gives us other alternative names used to identify it as 27 kDa zein or alcohol soluble reduced glutelin. On the other hand, it is possible to know them different post-translational modifications characterized until the moment of the consultation and their implication in the final corn phenotype. In addition, it shows specific information on protein domains highlighting the peptide sequence, location within the protein and function of each domain.

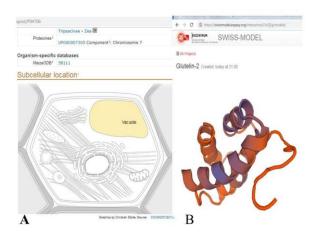
On the other hand, through the STRING platform we can know the functional protein association networks. Where the different labels (Figure 1A, colored lines) of the diagram, redirect us to the characteristics of each of them in this platform https://string-db.org/. For our case study, we see in Figure 1A a diagram interconnecting between the proteins most strongly associated by previous work on our proteins of interest by means of lines of different colors. Each line has a specific meaning of interaction, for example, the union of two proteins by a yellow line indicates that both proteins have been mentioned in an article already published.

Thus, in our case, Gluterlin-2 has been mentioned with Zeina beta in at least two scientific articles such as the one carried out by Yao et al., In 2016, where they indicate that both proteins are essential for morphology in the corn endosperm. It also allows us to identify which are the biological and molecular processes where our protein of interest has been reported, so we can see that in the case of the interaction network shown in Figure 2B the main molecular function is that of nutrient reservoir where five Of the 18 proteins present in the diagram participate in this function and gives us a statistical value of 7.18e-13. In addition, it is possible to know the secondary structure of the proteins contained in the interactome, which can show us the specific domains for each protein in order to identify their functions. Returning to the UniProt KB platform, in the sequence record we find, cross references, in addition to links to other related bases, where we can find the pI: 8.40, average mass 23 689 Da, (https://web.expasy.org).

For this protein that belongs to a plant a specific database for the organism in this case for the corn grain (Zea mays) (https://www.maizegdb.org), 3D protein modeling (Figure 2 A), (https://www.proteinmodelportal.org), allergenic studies (http://www.allergome.org).

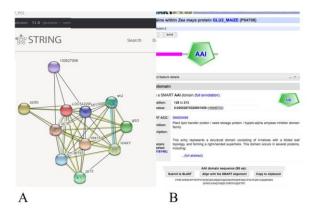
Protein-related articles, in the field of Display Publications in the UniProt KB registry, a text mining system with additional bibliography for more proteins, eGenPub and protein alignment to indicate homology with other proteins, https://npsa-prabi.ibcp.fr/. (Figure 3).

This amount of information shows us how we can identify important data that help us clarify or expand the relationship of the protein under study with the biological, physiological or molecular functions within the plant, thus giving an idea of its manifestation at the phenotypic level within the species under study.



**Figure 1** UniProtKB, protein information under study. Registration of the Glutelin-2 protein in UniProtKB P04706, showing the subcellular location (A) and the 3D structure (B)

The information or fields of each record may vary according to the protein investigated, for this case the Glutelin-2 (Zea mays) record appears with experimental evidence, which provides more information in the fields of this protein's record..



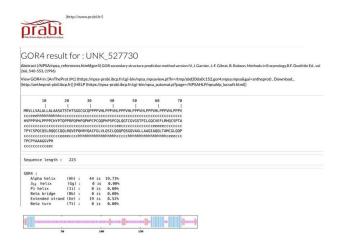
**Figure 2** (A) Databases interrelated with the *Zea mays* Glutelin-2 protein registry (STRING) Domains (B) SMART

#### **Conclusions**

The databases provide relevant and in many cases updated and specific information for vegetable proteins, it is important to interact with other databases, to provide data that can help identify important characteristics that contribute to the study in different areas, as well as specific of a vegetable protein.

identification The of important characteristics such as its pI, its molecular weight, the interaction with other proteins, its secondary structure, shows us a vision of the properties of the vegetable protein under study, an overview of the physiological, pathological and molecular processes that can contribute in the development of interaction studies as in the proteomic meta-analysis of plant species; In addition, the use of tools such as mass spectrometry that allows to identify with good precision the presence of proteins from other species present in the sample, can contribute to food quality control, under circumstances or at a given time or place in the development of crop, also contributing to clarify part of the phenotype observed in a vegetable.

We must also consider that it is only an approach to accurate information, but it does not stop being valuable for the studies that are going to be carried out with vegetable proteins in any of the types of analysis. Therefore, it is important to keep the information on protein recognition up to date and homogenize criteria when naming proteins for all bases and keep the vectors among them updated. In this way complement the studies and research not only in the biological and agronomic area but also in the quality control of food and pharmacological or medical areas.



**Figure 3** Protein alignment to indicate some functional relationship

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Annex: URL of the bases

STRING http://string-db.org.

UNIPROT http://www.uniprot.org

SWISSPROT http://web.expasy.org/docs/swiss-prot\_guideline.html,

#### **SWISS-MODEL**

http://swissmodel.expasy.org/interactive,

MAÍZ MAIZEDB

https://www.maizegdb.org/data\_center/gene\_product?id=58111

Identificación de dominios.

http://smart.embl-

heidelberg.de/smart/set\_mode.cgi?NORMAL= 1

Alineamiento proteínas

https://npsa-prabi.ibcp.fr/cgi-

 $bin/npsa\_automat.pl?page=npsa\_multalin.html$ 

Production of seven varieties of tomato (*Lycopersicon esculentum* Mill.) pruned to one and two stems under greenhouse

Producción de siete variedades de jitomate (*Lycopersicon esculentum* Mill.) conducido a uno y dos tallos bajo invernadero

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#### **Abstract**

The experiment was conducted from May to August 2018 at the Technological University of the Southwest of Guanajuato (UTSOE) in a randomized complete block design with three repetitions, in greenhouse 1 of 700 m2 of the Sustainable and Protected Agriculture Career; with the purpose of evaluating the effect of pruning at one and two stems per plant, on yield, fruit weight and plant variables in seven varieties of saladette tomato produced under greenhouse and in hydroponics. The varieties evaluated were: Natalie, USATX 12227, USATX 9934, Juan Pablo, USATX 24019, USATX 16117, USATX 15538, all established at a density of 31,250 plants or stems per hectare. Pruning at two stems per plant produced the highest yield in the USATX 16117, USATX 9934 and Natalie varieties, producing more than 2.3 kg per plant after six weeks of harvest. For the fruit weight, the USATX 9934 variety with two stems, was the one that produced fruits of greater caliber, being one of the three varieties with the highest yield. For the plant height, the USATX 24019 variety with one stem was the one with the highest height three months after the transplant.

Lycopersicon esculentum, Stem pruning, Yield

#### Resumen

El experimento se realizó de mayo a agosto del 2018 en la Universidad Tecnológica del Suroeste de Guanajuato (UTSOE) en un diseño en bloques completos al azar con tres repeticiones, en el invernadero 1 de 700 m2 de la Carrera de Agricultura Sustentable y Protegida; con la finalidad de evaluar el efecto de la poda a uno y dos tallos por planta, en el rendimiento, peso de fruto y variables de planta en siete variedades de jitomate saladette producidas bajo invernadero y en hidroponia. Las variedades evaluadas fueron: Natalie, USATX 12227, USATX 9934, Juan Pablo, USATX 24019, USATX 16117, USATX 15538, todas establecidas a una densidad de 31,250 plantas o tallos por hectárea. La poda a dos tallos por planta produjo el mayor rendimiento en las variedades USATX 16117, USATX 9934 y Natalie, al más de 2.3 kg por planta al cabo de seis semanas de cosecha. Para el peso de fruto, la variedad USATX 9934 a dos tallos, fue la que produjo frutos de mayor calibre, siendo una de las tres variedades con el mayor rendimiento. Para la altura de planta, la variedad USATX 24019 a un tallo fue la que presentó una mayor altura a los tres meses después del trasplante.

Lycopersicon esculentum, Poda de tallos, Rendimiento

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#### Introduction

Recently, tomato production has increased by about 50 percent, driven by a larger agricultural area, with protected farming systems. Tomato availability is available in all months of the year (SRE, 2015). Mexico is the main exporter of fresh tomatoes worldwide, with about 20% of the volume and 25% of the value traded, which are mainly destined for the US. The country exports around 1.5 million tons per year, which represents between 50 and 70% of the production volume. In Mexico, about 52,374.91 ha of tomato are sown, with an average yield of 56.42 t.ha-1, making it the second most important vegetable in terms of planted area, the most transcendent in terms of volume in the national market and first for its production value (SIAP-SAGARPA, 2018).

In order to seek the transfer of technology to small producers; It was decided to implement this project to evaluate the effect of driving and handling one and two stems per plant on yield and yield components in seven commercial varieties of saladette tomatoes, under greenhouse conditions and hydroponic substrate, to compare and determine the best treatment after six weeks of harvest from two months after the transplant.

## Literature review

The expression of crop yield potential depends on both its genetic constitution and environmental factors (climate, soil), biological factors and the production technique (Sánchez and Escalante, 1988); and that in turn this potential depends on the growth of the plant itself (Alcántar and Trejo, 2012), when considering all its plant organs as roots, branches, leaves, flowers, and of course the stem.

The functions of the stem of a plant are vital for its development. In general, stem functions are related to the structural support of the plant and the transport of nutrients. The structural support provided by the stem allows the leaves, fruits and flowers of the plant to be maintained so that they do not fall to the ground prematurely (Raven et al., 1992). The transport of compounds in the stem is of great importance as well.

This is due to a system of vascular tissues that connect with different parts of the plant and allow the transport of substances to and from the leaves, roots, flowers, fruits and the stem itself (Salisbury and Ross, 1994)

The stem of the tomato plant consists of an axis of 2 to 4 cm thick at its base, on which the leaves, secondary stems (simpoidal branching) and inflorescences develop. Its structure, from the outside in, consists of an epidermis, from which the glandular hairs leave towards the outside; a cortex or cortex, whose outermost cells are photosynthetic and the innermost are colenchymal; a vascular cylinder and spinal tissue. In the distal part is the apical meristem, where the new leaf and floral primordia begin (León, 2006).

Pruning is an indispensable practice for varieties of indeterminate growth produced in protected agriculture, which is carried out 15-20 days after transplantation with the appearance of the first lateral stems, which will be removed, as well as older leaves, thus improving aeration different facilitating cultural Likewise, it helps determine in some commercial types of tomatoes, the number of arms or stems to leave per plant (Vera 2015, cited by Arébalo et al., 2018). Mendoza et al., (2018), in a study carried out to evaluate the effect of pruning one, two and three stems on tomato production, found that single-stemmed plants showed a higher yield, in addition to producing Large fruits in greater percentage.

In another study carried out by Arébalo et al., (2018), when evaluating the effect of pruning and management of tomato plants of the Ramsés variety with one and two stems, they found that despite not having statistically significant differences, the plants at a single stem they produced fruits with better quality indicators such as weight and size of the fruit, while the plants that were pruned in seedlings and after transplantation to form two stems, produced a greater amount of fruits, but of lower quality and therefore lower performance. Corella et al., (2013), when evaluating pruning of one and two stems in five indeterminate varieties of tomato, also found that, the yields in the plants pruned to two stems were much lower than those pruned to a stem. Outstanding in the performance in this case, the Moctezuma, Spartaco and Malinche varieties.

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According to Vera et al., (2015), pruning of two stems per plant allows to harvest a greater amount of medium-sized fruits and lower quality compared to plants that are not pruned. This effect is due to the high demand for nutrients that the plant requires in order to sustain two stems and produce fruits.

On the other hand and being equally important in this process, the nutritional balance plays a fundamental role to take into account from transplantation to the productive stage of the crop (León, 2006). Within the nutrition of the tomato crop, balanced growth, flowering and fruiting or fruit filling solutions can be made and applied, based on an optimum pH of 5.5 to 5.8 and a maximum electrical conductivity of 3.5 dS.m-<sup>1</sup>.

#### Materials and methods

## **Project localization**

The present work was carried out from May to August 2018 at the Technological University of the Southwest of Guanajuato (UTSOE) in the 700 m2 greenhouse 1 of the Sustainable and Protected Agriculture Race, located on the Valle-Huanímaro Highway Km. 1.2 in Valle from Santiago, Gto., Mexico.

## Plant material and planting density

Seven varieties of saladette tomatoes from the USAgriseeds commercial house were established in the greenhouse. The varieties evaluated were: Natalie, USATX 12227, USATX 9934, Juan Pablo, USATX 24019, USATX 16117, USATX 15538, all established at a density of 31,250 plants or stems per hectare, depending on pruning treatments.

## Experimental design and data analysis

The experiment was established based on a randomized complete block design with three repetitions. A total of 14 treatments were evaluated, consisting of the combination of the seven indeterminate varieties of tomato with two pruning levels: one and two stems per plant; performing this last pruning a week after the transplant. The experimental unit for each treatment, consisted of three pots with river sand, each with a plant, either with one or two stems, depending on the treatments.

The data were analyzed using the SAS® statistical package version 9.3 to carry out the analysis of variance (ANOVA), a correlation between the evaluated variables and the Tukey means comparison test ( $p \le 0.05$ ).

#### Variables evaluated

Yield (kg / plant): It was obtained through the harvest of clusters in commercial maturity and weighed with the help of a digital analytical balance, obtaining a cumulative at the end of six weeks of harvest, from two months after transplant.

Fruit weight (g): It was obtained through the weekly harvest of all fruits in commercial maturity per plant, weighed with the help of an analytical balance and the average was obtained after six weeks of harvest.

Apex diameter (mm): It was obtained with the help of a digital vernier, measuring above the last well formed leaf for six weeks to finally obtain an average.

Plant height (m): It was obtained by measuring from the base of the stem to the apex with the help of a flexometer, the accumulated growth after three months after the transplant.

Number of leaves: The total of well-formed leaves was counted after three months after transplantation.

## Agronomic management

Tutoreo of the plant was carried out starting at two weeks after the transplant, in addition to pruning leaves and axillary shoots weekly. Pollinations were done every day manually and with air. The lowering of the plant began once the plants reached two meters high.

As for the thinning of fruits, this work began when the first clusters of fruits appeared in the "marble" stage and as they appeared and developed consecutively on the plant, leaving a maximum of seven fruits per cluster.

Irrigation and crop nutrition were managed based on Steiner's solution taking an electrical conductivity that was  $2.5~\mathrm{dS}$  / m from the transplant stage, up to  $3.0~\mathrm{dS}$  / m at the production stage, with a pH of 5 to 6 , with fertilizer sources of macro and chemical micronutrients.

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Biweekly applications of amino acids, growth hormones and chelated micronutrients were also made.

The management of pests and diseases was carried out weekly or biweekly, beginning with periodic monitoring, implementation of cultural work and through the application of biological products based on fungi and bacteria, organic products based on botanical extracts, sulfur and copper; and low toxicological chemical pesticides.

#### **Results**

In the analysis of variance for the variables of yield and components of the same (Table 1), highly significant differences (p≤ 0.01) were detected in the different treatments for the variables of yield, fruit weight and plant height, which supposes that the management of one and two stems, as well as the genetic potential of the varieties (Sánchez and Escalante, 1988), had an effect on these variables evaluated.

FV	AP	DAP	NHO	PF	RTO
Trat	0.050**	0.1	7.32	433.2**	0.941**
Error	0.009	0.074	5.133	52.51	0.077
CV (%)	5.49	5.72	8.92	11.1	16.55
Mean	1.754	4.75	25.3	65.27	1.67

FV: Source of variation; Trat: Treatment; CV: Coefficient of variation; AP: Plant height; DAP: Apex diameter; NHO: Number of sheets; PF: Fruit weight; RTO: Performance. \*\* Highly significant with  $p \le 0.01$ .

**Table 1** Mean squares and level of statistical significance for the yield and plant variables in the stem pruning experiment in seven tomato varieties under greenhouse. Santiago Valley, Gto. 2018

The coefficients of variation were less than 20% for all the variables evaluated, which shows that there is reliability in the data.

The Tukey mean comparison test (p≤ 0.01) in Table 2, in which it was ordered based on the variable yield from highest to lowest; shows that for this variable, pruning and driving at two stems per plant produced the highest yield, with the USATX 16117, USATX 9934 and Natalie varieties being the best responders to this management, producing more than 2.3 kg per plant after six harvest weeks

These results are contrary to what was found by Mendoza et al., (2018), Arébalo et al., 2018 and by Corella et al., 2013), finding in previous works that varieties pruned to a stem were more yielding.

ISSN 2524-2091 RINOE® All rights reserved Regarding the weight of fruit (Table 2), the same USATX 9934 variety with two stems, produced fruits of greater caliber (86.65 g), thus affecting the yield (2.36 kg / plant); which demonstrates that it is an improved variety to adapt to these management conditions and to express its genetic potential; Although Villamán (2011) mentions that by leaving a second axis, it competes for nutrients, solar radiation and water, affecting the development of the first, which causes a delay in production, so it should be used when conditions environmental allow a longer period of growth.

As for the plant height (Table 2), it was found that the USATX 24019 variety with one stem was the one that grew the most with 2 m height and in counterpart, the variety that grew the least was USATX 9934 with 1.57 m led to two stems; This suggests that a plant with more than one stem must guarantee its optimum growth and development, reducing its vigor in height, as there is more competition for nutrients, solar radiation and water. However, this pattern of behavior was not proportional for all treatments, since not all varieties driven to two stems were those of lower height; then, rather, their behavior responds to the genetic potential as in some varieties of short internodes and to the adaptation of each of the varieties to these management conditions after three months after the transplant.

Treatment (variety)	Yield (kg / plant)	Fruit Weight (g)	Plant height (m)	Apex diameter (mm)	Number of sheets
USATX 16117 2T	2.52 a	73.88 ab	1.84 abc	4.52 a	26.3 a
USATX 9934 2T	2.36 a	86.65 a	1.57 c	4.66 a	22.0 a
Natalie 2T	2.32 a	73.45 ab	1.88 ab	4.54 a	24.3 a
USATX 12227 2T	2.26 ab	75.35 ab	1.70 bc	4.71 a	26.6 a
USATX 15538 2T	2.14 abc	65.61 abc	1.81 abc	4.69 a	25.6 a
Juan Pablo 2T	2.0 abcd	55.64 bcd	1.82 abc	4.69 a	25.3 a
USATX 15538 1T	1.43 bcde	68.66 ab	1.61 bc	4.72 a	25.3 a
USATX 12227 1T	1.40 cde	71.15 ab	1.63 bc	4.94 a	25.3 a
USATX 24019 2T	1.35 cde	46.06 cd	1.69 bc	5.18 a	24.6 a
USATX 16117 1T	1.25 de	60.82 bcd	1.79 abc	4.73 a	26.3 a
Juan Pablo 1T	1.23 de	60.17 bcd	1.61 bc	5.03 a	26.0 a
USATX 9934 1T	1.18 de	69.55 ab	1.68 bc	4-64 a	23.0 a
Natalie 1T	1.14 e	66.18 abc	1.89 ab	4.69 a	26.0 a
USATX 24019 1T	0.86 e	40.68 d	2.0 a	4.82 a	28.3 a
Media	1.67	65.27	1.754	4.75	25.3
DSM	0.836	21.8	0.29	0.819	6.81

T: Stems; DSM: Minimum significant difference; Are means with equal letters within the same column statistically equal (Tukey  $\alpha \le 0.05$ ).

**Table 2** Comparison of means for the yield and plant variables, in the stem pruning experiment in seven tomato varieties under greenhouse. Santiago Valley, Gto. 2018

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In the correlation test (Table 3), the experiment showed highly positive or direct correlation ( $p \le 0.01$ ) between the variables number of leaves with plant height, which demonstrates that for some varieties the more they grew, they managed to develop more leaves, as found for the highest-rise variety USATX 24019 (Table 2), responding to the genetics of the variety.

A highly positive correlation was also found between fruit weight and yield, which shows that yield is favored when larger fruits are developed and harvested, as presented with the USATX 9934 variety with two stems (Table 2).

	AP	DAP	NHO	PF
DAP	-0.251			
NHO	0.540**	0.056		
PF	-0.36*	-0.263	-0.168	
RTO	-0.059	-0.31	-0.07	0.571**

DAP: Apex diameter; AP: Plant height; NHO: Number of sheets; PF: Fruit weight; RTO: Performance. \*: Significant with  $\alpha \le 0.05$ ; \*\*: Significant with  $\alpha \le 0.01$ .

**Table 3** Correlation coefficients between the variables evaluated in the stem pruning experiment in seven tomato varieties under greenhouse. Santiago Valley, Gto. 2018

The indirect or negative correlation (p  $\leq$  0.05) between plant height and fruit weight (Table 3), responds to the fact that some more growing varieties produced smaller fruits, as found for the USATX 24019 variety on a stem; which probably needs more time to develop height and leaf vigor, being a very vegetative variety, until some time when it is adapted to environmental conditions (Villamán, 2011), to start expressing its productive potential.

## **Conclusions**

Pruning and driving at two stems per plant produced the highest yield in the USATX 16117, USATX 9934 and Natalie varieties, producing more than 2.3 kg per plant after six weeks of harvest.

Regarding the weight of the fruit, it was the USATX 9934 variety, which led to two stems, which produced fruits of greater caliber, being also one of the three varieties with the highest performance statistically.

For the plant height, the USATX 24019 variety led to a stem was the one with the highest height three months after the transplant; however, not all varieties driven to a stem were those of greater height.

Pruning at one and two stems had no effect on apex diameter and number of leaves, which simply allowed us to observe the expression of the natural genetic potential of the seven varieties under these production conditions..

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## Evaluation of productive and economic parameters of pigs in the final stage, with three feeding programs

Evaluación de parámetros productivos y económicos de cerdos en la etapa de finalización, con tres programas de alimentación

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#### **Abstract**

The objective was to evaluate the productive and economic parameters of pigs in the final stage with three feeding programs. 240 pigs commercial homogeneous characteristics were used, assigning 40 females and 40 males completely random to the treatments. The animals were fed with 3 diets in flour containing different level of protein: T0 (16 %), T1 (16.42 %) and T2 (17.51). For the analysis of the information, a completely randomized design was used, the means were contrasted with the Tukey test. There were no significant differences (P> 0.05) for the evaluated variables, with the highest consumption of dry matter (CDM) recorded in the T1 (2,065), followed by T2 (2,063) and T0 (1,931 kg.). The daily weight gain (DWG) was for T2 of 0.863 grs., T1 with (0.858) and for T0 of 0.826 grs. With a food conversion (FC) for the proposal T0 of 2,562, T1 (2,671) and T2 3,027 kg. The pigs fed with T1 recorded a cost of 16,869 / Kg. of live weight, followed by T2 with \$14,556 and obtaining more profitability with T0 when obtaining a cost of \$ 13,248. The productive behavior between treatments was similar, but the best profitability is obtained with the T0.

Pigs, Fattening, Behavior, Profitability

#### Resumen

Objetivos El objetivo fue evaluar los parámetros productivos y económicos de cerdos en etapa de finalización bajo tres programas de alimentación comercial. Se utilizaron 240 cerdos con características homogéneas, asignando 40 hembras y 40 machos completamente al azar a los tratamientos. Los animales fueron alimentados con 3 dietas en harina conteniendo diferente nivele de proteína: T0 (16 %), T1 (16.42 %) y T2 (17.51). Para el análisis de la información, se utilizó un diseño completamente al azar, las medias se compararon con la prueba de Tukey. No existieron diferencias significativas (P>0.05) En las variables evaluadas, registrándose mayor consumo de materia seca (CMS) en el T1 (2.065), seguido de T2 (2.063) y T0 (1.931 kg.). La mayor Ganancia diaria de peso (GDP) fue para T2 de 0.863 grs., T1 con (0.858) y para T0 de 0.826 grs. Con una conversión alimenticia (CA) para la propuesta T0 de 2.562, T1 (2.671) y T2 3.027 kg. Los cerdos alimentados con T1 registraron un costo de 16.869/kg. de PV, seguido de T2 con \$ 14.556 y siendo más rentable con T0 al obtenerse un costo de \$13.248. En conclusión, el comportamiento productivo entre tratamientos fue similar, pero la mejor rentabilidad se obtuvo con el T0.

Cerdos, Engorda, Comportamiento, Rentabilidad

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 $<sup>\</sup>ensuremath{^{\dagger}}$  Researcher contributing as first author.

#### Introduction

In volume, pork represents a fifth of the total meat production in Mexico. In recent years, the industry has registered an average annual growth of 2.1%. However, demand has shown a faster growth, causing a significant increase in imports, representing around 45% of apparent consumption. The United States is the main exporter to Mexico; Most of the imported products are fresh, chilled or frozen meats (OECD, 2019).

The evaluation of the cost of production and the indicators of the different production parameters are very important in pig farming. The parameters of pig production have generally improved in the last five years. This improvement did not directly imply a reduction in production costs due to high food prices (Rocadembosch, Amado, Bernaus, Font, & Fraile, 2016).

For the pig industry, food accounts for about 70% of production costs. In recent years, food costs have increased by more than 226.6% since 2008. However, the demand for pork products is increasing, despite rising prices (Banson, Nketsia-Tabiri, Anno, & Kofi Dagbui, 2014). The increase in feeding costs in recent years has generated challenging conditions for pig producers (Saddoris Clemons, Schneider, Feoli, Cook, & Newton, 2011).

Conventional diets contain pig substantial amounts of cereal grains example. corn and wheat) and supplements such as soy flour to provide pigs with the energy and nutrients they need. However, trends in the demand and supply of these conventional foods require pig producers worldwide to seek low-cost alternatives (TWoyengo, Beltranena, & Zijlstra, 2014).

The protein / energy ratio is important for the production yield and utilization of the food resources available to animals. In pig production, a low protein / energy ratio in the diet can be useful to reduce feeding costs and minimize the adverse effects of ammonia release in the environment (Yingying, et al., 2015).

To maximize profit opportunities, producers must develop feeding strategies that result in better yields on food and / or margin on food costs (Saddoris Clemons, Schneider, Feoli, Cook, & Newton, 2011).

ISSN 2524-2091 RINOE® All rights reserved Food conversion represents the highest economic value in scenarios with high food prices (Rocadembosch, Amado, Bernaus, Font, & Fraile, 2016). In the rations of traditional foods they present by stages deficits or excesses of nutrients according to the requirements of the animals; this causes more expensive productive activity and nutritional imbalances and shows that these portions are more expensive than food with the inclusion of unconventional raw materials (Estévez Alfayate, 2016).

In the feeding management of pigs in the final stage, food must be provided that meets the nutritional requirements depending on the level of production that is desired to be achieved, and the genetic potential of the animals, as well as reducing their cost when used in a manner efficient. Based on these elements, the present work aimed to evaluate the productive variables of pigs in the final stage, fed with three different diets in protein to determine the biological and economic optimum.

#### Methodology to be developed

**Location.** The project was carried out with pig producers in La Piedad Michoacán. It is located between the coordinates 20 ° 21 'north latitude and between 102 ° 02' west longitude. Its territory extends to about 284.11 square kilometers and is at an average height of 1,680 meters above sea level.

#### **Installations**

6 pens of 40 m2 each were used with two feeders per pen and three drinking fountains with a firm floor and 1.5 m ponds, roofs of sheets and ventilation on both sides. The flow and availability of water in the pens was monitored every day, under the gram flow technique (in a bottle to determine how much water flows in a minute) expected parameter 2.5 L / min. With lighting-saving bulbs for the entire house.

#### **Treatments**

The farm was considered as an independent experiment due to its particular conditions, three treatments were evaluated:

T0: Food in presentation of flour with 16% protein for pigs in the final stage.

T1: Food in presentation of flour with 16.42% protein for pigs in the final stage.

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T2: Food in flour presentation with 17.51 protein for pigs in the final stage.

#### **Animals**

240 animals were used being 120 females and 120 castrated males, with homogeneous characteristics [age, live weight (PV), genetics, sanitary condition, etc.], of which they were randomly assigned and with the same number of males and females to the treatments.

## Weighing and stamping of animals

The animals were identified at the beginning of the experiment, listing from 1 to 80 for each treatment. Weighing was carried out at the beginning and at the end of the experiment by introducing the animals into a mobile electronic scale type drawer, where the corresponding reading was carried out.

#### **Feeding**

Ad libitum food was provided twice a day, (9:00 am and 3:00 pm), always at the same time. The food was offered with the support of a table based on food consumption by stage (see Table 1).



**Table 1** Feed proposal for pigs in completion stage

Table 2 shows the nutritional composition of each food used in each of the treatments.

NUTRIMENT	Т0	<b>T1</b>	<b>T2</b>
PC%	16	16.42	17.51
METABOLIZABLE	3320	3300	NE
ENERGY (kcal)			
GREASE (%)	3.5	3.09	4.13
FIBER%)	4	3.96	1.97
ASHES (%)	7	NE	3.8
P TOTAL (%)	0.45	0.66	0.409
Total Ca (%)	0.53	NE	0.477

**Table 2** Nutritional composition of the diets of each of the treatments

## **Duration of fattening**

The experimental period was 35 days in order to reach 120 kg of PV per animal.

#### Variables to measure

## Daily food consumption (CDA)

The amount of food offered for each of the treatments was weighed before filling the feeders, and the rejection was weighed the next day.

CDA = Food offered-food rejected

## **Daily Weight Gain (GDP)**

The daily weight gain was calculated considering the final weight (Pvf) minus the initial weight (Pvi) divided between the days of fattening.

GDP = Pvf-Pvi) / fattening days

#### **Food conversion**

It was estimated by dividing the daily food consumption by the daily weight gain.

CA = Daily food consumption / GDP

## Cost per kg of PV produced (\$ / Kg of PV)

The cost per kilogram of live weight produced was calculated by multiplying the food conversion by the price of each food.

## Statistic analysis

A completely randomized design was carried out, being the experimental unit of 80 animals.

$$Y_{ij} = \mu + T \boldsymbol{i} + \boldsymbol{\epsilon} \boldsymbol{i} \boldsymbol{j}$$

The data were analyzed by means of an analysis of variance and comparison of means with Tukey test ( $\alpha \le 0.05$ ) using the statistical software SAS version 9.0.

#### **Results**

The analysis of the evaluated variables, in the three protein levels proposed, showed that there were no significant differences (P> 0.05), in any of the variables evaluated in pigs in the period of completion.

## Cost per kg of PV produced (\$ / kg of PV)

The productive and economic results expressed by the pigs in the final stage using three levels of protein in the diet (T0, T1, and T2), are shown in Table 3.

Treatment	Initial Weight (kg)	Final Weight (kg)	Consumption kg/pig/stage	CDA (kg)	GDP (kg)	CA	kg cattle / pig / stage	Food cost \$ / pig / stage	Cost \$ / kg PV earned
T	84.53	113.46	5339.50	1.93	0.82	2.56	28.92	383.22	13.24
0	7	3	0	1	6	2	7	7	8
T	78.81	108.83	5782.80	2.06	0.85	2.67	30.01	506.32	16.86
1	5	0	0		8	1	5	5	9
T 2	82.09 1	112.30 5	5777.20 0	2.06	0.86	3.02 7	30.21 4	439.78 9	14.55 6

**Table 3.** productive and economic indicators of pig behavior in the final stage with three feeding proposals

When performing the analysis of the productive behavior (Table 3), it was observed that the pigs that consumed T2 feed (30,214kg), recorded more kilograms gained. No differences were observed in the animals of T1 and T0 (30,015 vs 28,927 kg), however, the lowest recorded gain was obtained from the animals of T0 (Table 3). When analyzing the cost generated by pig in completion, the animals of the T0 recorded the lowest cost: 383,227 pesos. While, the animals of T2 generated an investment cost of \$439,789, on the other hand, pigs of T1 had the highest cost generated by investment with \$506,325 pesos per animal.

When analyzing the cost per kilogram of live weight gained, pigs fed T1 treatment recorded a cost of 16,869 / kg of PV, followed by T2 with \$ 14,556 and pigs fed with T0 treatment being more profitable when obtaining a cost of \$ 13,248 / kg of PV.

## Acknowledgment

The authors express their gratitude to the pork producers of La Piedad Michoacán and to the Polytechnic University of Francisco I. Madero for the facilities provided for carrying out the work.

#### **Conclusions**

The completion stage is the most expensive period for food conversion, therefore, economic and efficient diets that impact profitability should be formulated. There were no differences in the productive response of pigs fed the three levels of protein. However, the best conversion and profitability are obtained by feeding pigs in completion with 16% crude protein.

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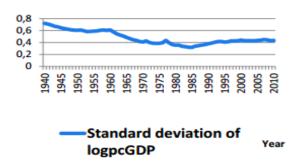
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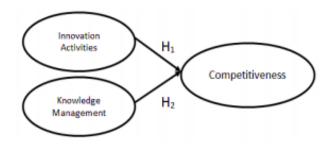


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