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Knowledge Area

The works must be unpublished and refer to topics of Experimental design, Commerce, Options, Rural-Flora and Fauna, Agronomy, Natural and other topics related to Biology and Chemistry.

Presentation of the Content

As a first chapter we present, *Proximal chemical characterization and antioxidant activity of Persea americana leaves cv Hass, Fuerte y Criollo*, by FRAUSTO-MOLINA, Janette, TOVAR-JIMÉNEZ, Xochitl, ÁLVAREZ-GARCÍA, Rocío and TÉLLEZ-JURADO, Alejandro, with adscription in Universidad Politécnica de Pachuca, as a second article we present, *Effect of the use of different types of fishmeal on the physicochemical properties of a fishfeed for Oreochromis niloticus (Nile tilapia)*, by SOTO-RODRÍGUEZ, Diana Laura, GÓMEZ-ALDAPA, Carlos Alberto, CABRERA-CANALES, Zaira Esmeralda and CADENA-RAMÍREZ, Arturo, with affiliation in the Universidad Politécnica de Pachuca, as the third chapter we present, *Effect on the fatty acid profiles of a microalgae strain (Dunaliella tertiolecta) using different lipid extraction techniques*, by GÓMEZ-CORDOVA, Fidel, PALOMAREZ-RUIZ, Irma, SANTOS-BALLARDO, David and MEJIAS-BRIZUELA, Nildia, with affiliation at Universidad Politécnica de Sinaloa, as fourth article we present, *Chemical composition of Tithonia diversifolia (Hemsl.) A. Gray (Asteraceae) and diversity of uses in rural areas*, by ROMÁN-MIRANDA, María Leonor, MORA-SANTACRUZ, Antonio, AVENDAÑO-LÓPEZ, Adriana Natividad and SÁNCHEZ-MARTÍNEZ, José, with ascription in the Universidad de Guadalajara.

Content

Article **ECORFAN Journal-Ecuador**
June 2020 Vol.7 No.12 1-7

Proximal chemical characterization and antioxidant activity of *Persea americana* leaves *cv* Hass, Fuerte y Criollo

Caracterización químico proximal y actividad antioxidante de las hojas de *Persea americana cv* Hass, Fuerte y Criollo

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Abstract

The aim of this work was to determine the proximal chemical composition of leaves and infusion *Persea americana cv* Hass, Fuerte and Criollo, as well as, their antioxidant activity by standardized analytical methods. To carry this out, *P. americana* leaves were analyzed according to moisture content (fresh and dehydrated), only in the sampling area from Criollo variety was found that produced green and purple fruits, therefore, the leaves of these trees were also evaluated, in order to determine if the proximal composition and antioxidant activity depends on these factors. The results indicated that there is a statistically significant difference in the proximal composition between *P. americana* leaves of the three varieties evaluated, as well as, their antioxidant activity, with dry leaves infusion of the Criollo variety with green fruit showing the highest activity antioxidant (70 % Inhibition $ABTS^{+\bullet}$).

Hojas de *Persea americana*, Análisis proximal, Actividad antioxidante

Resumen

El objetivo del presente trabajo fue determinar la composición químico proximal de las hojas e infusión de *Persea americana* de las variedades Hass, Fuerte y Criollo, así como, su actividad antioxidante por medio de métodos analíticos estandarizados. Para llevar acabo esto, las hojas de *P. americana* se analizaron de acuerdo al contenido de humedad (frescas y deshidratadas) y sólo en la zona de muestreo de la variedad Criollo se encontró que esta variedad producia frutos de color verde y morado, por lo cual, las hojas de estos árboles tambien fueron evaluadas, con la finalidad de determinar si la composición proximal y actividad antioxidante depende de estos factores. Los resultados indicaron que existe diferencia estadísticamente significativa en la composición proximal entre las hojas de *P. americana* de las tres variedades evaluadas, al igual que, su actividad antioxidante, siendo la infusión de hojas deshidratadas de la variedad Criollo de fruto verde la que presentó mayor actividad antioxidante (70 % Inhibición $ABTS^{+\bullet}$).

***Persea americana* leaves, Proximal analysis, Antioxidant activity**

Citation: FRAUSTO-MOLINA, Janette, TOVAR-JIMÉNEZ, Xochitl, ÁLVAREZ-GARCÍA, Rocío and TÉLLEZ-JURADO, Alejandro. Proximal chemical characterization and antioxidant activity of *Persea americana* leaves *cv* Hass, Fuerte y Criollo. ECORFAN Journal-Ecuador. 2020. 7-12:1-7.

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Introduction

Persea americana (avocado) is an arboreal species native to Mexico and Central America, it belongs to the Lauraceae family and is classified into four subspecies or horticultural races according to its place of origin: Guatemalan *(P. americana var. Guatemalensis), Antillean (P americana var. drymifolia), Mexican (P. americana var. americana) and Costa Rican (P. americana var. costaricensis),* which have different morphological characteristics and environmental conditions (SIAP-SAGARPA, 2017; Campos-Rojas, 2011).

When the interbreeding between these subspecies takes place it is known as hybridization and the descendant product acquires characteristics that allow a better adaptation to climatic conditions, as well as fruits with different sensory characteristics (SAGARPA, 2011), which influence its proximal chemical composition.

In this sense, the avocado has around 100 varieties and mainly in Mexico it is considered a medicinal plant (Pérez-Álvarez *et al*., 2015). Specifically, the leaves of this species are used as flavoring and flavoring in Mexican gastronomy, while the infusion of avocado leaf has several therapeutic properties such as: antioxidant, digestive, analgesic, antitussive, among others.

Despite this, some studies have been carried out on its proximal composition and biological properties, however, in most cases the horticultural race or hybridization studied is not indicated, so it is relevant to carry out this study in the varieties of commercial importance (Hass and Fuerte cv), as well as, in the variety used in the traditional indigenous medicine of Mexico (Criollo cv) (Campos-Rojas, 2011; Yasir *et al*., 2010; Ezejiofor *et al*., 2013; Adaramola *et al*., 2016; Oboh *et al*., 2016).

In addition, the proximal chemical analysis of a food is the starting point in the evaluation of the macronutrient content so that it can be related to the best combination of some raw material and reach the desired level of the different components of a diet, also considering that the nutritional quality of plant products depends on the micro and macronutrients and on the presence of certain bioactive antioxidant compounds that may have a complementary or superimposed mechanism of action on health and that varies considerably due to environmental, genetic and plant-related factors.

Due to the aforementioned, the objective of this work was to determine the proximal chemical composition of the leaves and infusion of *Persea americana* from the Hass, Fuerte and Criollo varieties, as well as its antioxidant activity by means of standardized analytical methods.

Methodology

Preparation of plant material

The leaves of *P. americana* were collected according to the NOM-109-SSA1-1994 standard.

The sampling area of the Criollo variety belongs to the municipality of Papantla, Veracruz because in this place the leaves are used for therapeutic purposes, for this, samples of endemic trees were taken that produced fruits of different colors (green and purple) with characteristics that correspond to the Criollo variety. While the leaves of the commercial varieties Hass and Fuerte were collected in the municipality of Meztitlan, Hidalgo, in orchards established for commercialization.

In all cases, the leaves were cleaned and divided into two groups, one group was used to perform the fresh analysis and the other group for the analysis with dehydrated leaves.

Infusion of *P. americana* leaves

It was obtained from the fresh and dehydrated leaves of *P. americana* of the Hass, Fuerte and Criollo varieties by decoction of 30 g of sample in 1 L of water for 10 min.

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Proximal chemical analysis of the leaves and infusion of *P. americana*

It was carried out according to the methodology described by the Association Official of Analytical Chemists (AOAC, 1990).

Humidity: It was carried out according to that described in method No. 925.09, the calculation was carried out according to equation (1):

$$\text{Humidity } (\%) = \frac{W_{sample} - W_{final}}{W_{sample}} \times 100 \quad (1)$$

Where:

W_{sample}: Initial sample weight (g).

W_{final}: Weight of dry sample (g).

Ash: It was carried out by means of the gravimetric method No. 923.03. The ash content was obtained by weight difference, for which equation (2) was used:

$$\text{Ashes } (\%) = \frac{W_{sample} - W_{final}}{W_{sample}} \times 100 \quad (2)$$

Where:

W_{sample}: Initial sample weight (g)

W_{final}: Weight of ashes (g)

Fats: The determination was carried out in a Soxhlet equipment following the methodology described in method No. 920.39. The weight difference expressed the percentage of fat and was calculated by means of the following equation (3):

$$Fats\ (\%) = \frac{W_{fats}}{W_{sample}} \times 100 \quad (3)$$

Where:

W_{sample}: Initial sample weight (g)

W_{final}: Weight of fats (g)

Raw fiber: It was carried out according to method No. 962.09, which consists of taking 1 g of vegetable sample (W_0) and adding 200 mL of H_2SO_4 (0.255 N), bringing it to a boil for 1 minute. At the end of the acid digestion 4.54 g of NaOH were added and boiled for 1 h.

Once the time had elapsed, the mixture was filtered with the help of a filter paper at constant weight (W_1), with known ash content (W_2), the filter paper was washed with hot water, to finally fold it and place it in a crucible at weight constant (W_3) to subject it to drying at 100 °C for 24 h (constant weight W_4), subsequently it was brought to ash at 550 °C for 5 h, to finally weigh the sample (W_5). Crude fiber is expressed as a percentage and was calculated according to the following equations (4-6):

$$PF = W_4 - W_3 - W_1 \quad (4)$$

$$PC = W_5 - W_3 - W_2 \quad (5)$$

$$Raw\ fiber\ (\%) = \frac{(PF - PC)}{W_0} \times 100 \quad (6)$$

Where:

W_1: Filter paper weight.

W_2: Weight of ash on filter paper.

W_3: Weight of porcelain capsule.

W_4: Weight of the porcelain capsule with filter paper and the crude fiber residue.

W_5: Weight of the porcelain capsule with the ashes from the crude fiber and the filter paper.

PF: Weight of crude fiber and ash.

PC: Weight of ashes from crude fiber.

W_0: Weight of the plant sample.

Total protein: It was carried out according to method No. 960.52. The result is reported as a percentage of total protein and the correction factor 6.25 was used (equation 7, 8).

$$N_{total}(\%) = \frac{(HCl_m - HCl_B)(0.14)(HCl)}{W_{sample}} \times 100 \quad (7)$$

$$Total\ protein\ (\%) = N_{total} \times 6.25 \quad (8)$$

Where:

HClm: Volume of hydrochloric acid spent in the sample.

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HClB: Volume of hydrochloric acid spent on the blank.

HCl: Normality of hydrochloric acid.

W_{sample}: Initial sample weight.

Total carbohydrates: They were quantified by means of the phenol-sulfuric method described by Dubois *et al*., (1956).

Antioxidant activity of the infusion of *P. americana* leaves

At 20 µL of the infusion, 980 µL of the ABTS + • radical were added, the mixture was incubated at 25 ° C in the dark for 7 min, after the time the absorbance was measured at 754 nm. The results were expressed as a percentage of inhibition of the radical ABTS + • (Miller *et al*., 1996).

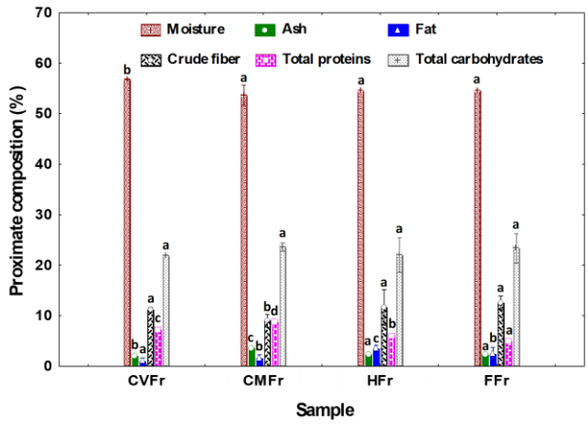
Statistic analysis

The analysis of multiple comparison of means was carried out by the honest significant difference (HSD) method proposed by Tukey using a significance level of 0.05 and the Statistica Statsoft software vs. 7.0.

Results

Proximal chemical analysis of *P. americana* leaves

Graphic 1 shows the results of the proximal chemical analysis carried out on the fresh leaves of *P. americana cv* Hass, Fuerte and Criollo, where it is observed that there is no statistically significant difference (p> 0.05; adjusted R2: 0.89) in the content of total carbohydrates in the evaluated samples, as well as in the crude fiber content only for the CVFr, HFr and FFr samples (p> 0.05; R2 adjusted: 0.72), on the other hand, the total protein content is statistically different among all the analyzed samples (p <0.05; R2adjusted: 0.99), however, the fresh leaves of the Criollo variety that produces purple fruits (CMFr) present higher total protein content (8.72 ± 0.01), while the CVFr sample is the one that It has higher moisture content (53.62 ± 0.5) and lower fat content (1.00 ± 0.01).

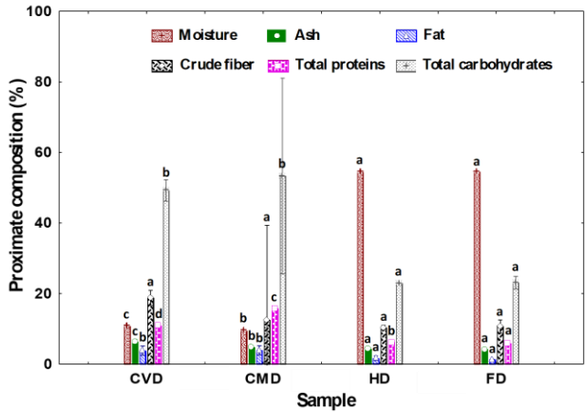


Graphic 1 Proximal analysis of fresh leaves of *Persea americana cv* Hass, Fuerte and Criollo

CVFr: Fresh leaves of P. americana cv Criollo with green fruit; CMFr: Fresh leaves of *P. americana* cv Criollo with purple fruit; HFr: Fresh leaves of *P. americana* cv Hass; FFr: Fresh leaves of *P. americana* cv Fuerte.

Equal letters indicate that there is no statistically significant difference (p> 0.05).

Regarding the proximal chemical composition of the dehydrated samples, in Graphic 2 it is observed that the leaves of *P. americana* cv Criollo are the ones with the highest content of total carbohydrates, total protein and fat, while the content of crude fiber does not present statistically significant difference between the samples evaluated (p> 0.05; adjusted R^2: 0.88). On theñp other hand, the dehydrated leaves of the commercial varieties (Hass and Fuerte) are those with the highest moisture content (54.55 ± 0.01).



Graphic 2 Proximal analysis of the dehydrated leaves of *Persea americana* cv Hass, Fuerte and Criollo

CVD: Dehydrated leaves of *P. americana* cv Criollo with green fruit; CMD: Dehydrated leaves of *P. americana cv* Criollo with purple fruit; HD: Dehydrated leaves of *P. americana cv* Hass; FD: Dehydrated leaves of *P. americana* cv Fuerte.

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Equal letters indicate that there is no statistically significant difference (p> 0.05).

In general, the differences found between the results of the studied varieties may be due to the agroclimatic conditions of the place where the plants grow and their age as indicated by Hussain *et al*., (2013). Likewise, the results obtained in this work are different from those obtained in the literature (Asodu *et al*., 2010; Olaywola, 2013; Gbadamosi and Kalejaye, 2017), it is worth mentioning that in these publications neither the variety studied nor the conditions sample processing (Table 1). The results showed that the main components found in the analyzed samples were total carbohydrates and crude fiber, in this sense, the three varieties analyzed indicate that the crude fiber content (~ 20%) makes the leaves of *P. americana* can be used for animal feed, in the paper industry and also at the level of the human digestive system, where it would help eliminate waste and toxins, in addition to reducing cholesterol, preventing diseases in the small intestine (Yasir *et al*., 2010; Ezejiofor *et al*., 2013; Adaramola *et al*., 2016; Oboh *et al*., 2016).

Component (%)	Asodu *et al*., (2010)	Olaywola (2013)	Gbadamosi & Kalejaye (2017)
Humidity	5.0±2	9.1±0.1	9.3±0.2
Ashes	20.3±3.4	2.9	3.4±0.2
Fats	13.9±6.4	1.3±0.1	6.5±0.2
Crude fiber	40.1±6	2.9±0.1	8.5±0.2
Total protein	26.4±5.2	9.8±0.1	21.6±0.2
Total carbohydrates	13.9±6.4	74.2±0.4	50.7±0.2

Table 1 Proximal analysis of *P. americana* leaves

Table 2 shows the proximal analysis carried out on the infusion of fresh leaves of *P. americana* cv Hass, Fuerte and Criollo, where it is observed that in most of the components there is a statistically significant difference between the infusions of the varieties studied ($p > 0.05$), however, there is no statistical difference in the content of crude fiber and total carbohydrates ($p < 0.05$; R^2 adjusted: 0.98), regarding the higher total protein content is presented in the I-CMFr and I-FFr samples (0.6 ± 0.1 and 0.7 ± 0.1, respectively).

Component (%)	Sample I-CVFr	I-CMFr	I-HFr	I-FFr
	96.9±0.1^a	96.9±0.1^a	97.1±0.1^b	96.8±0.1^a
Humidity	0.3±0.0^a	0.3±0.0^a	0.3±0.0^b	0.3±0.0^a
Ashes	0.1±0.0^a	0.1±0.0^a	0.2±0.0^c	0.1±0.0^b
Fats	0.5±0.4^a	0.5±0.4^a	0.5±0.2^a	0.5±0.3^a
Raw fiber	0.4±0.1^a	0.6±0.1^b	0.4±0.1^a	0.7±0.1^b
Total protein	1.9±0.5^a	1.6±0.3^a	1.5±0.1^a	1.5±0.4^a
Equal letters indicate that there is no statistically significant difference (p> 0.05).				

Table 2 Proximal analysis of the infusion of fresh leaves of *P. americana* cv Hass, Fuerte and Criollo

I-CVFr: Infusion of fresh leaves of *P. americana* cv Criollo with green fruit; I-CMFr: Infusion of fresh leaves of *P. americana* cv Criollo with purple fruit; I-HFr: Infusion of fresh leaves of *P. americana* cv Hass; I-FFr: Infusion of fresh leaves of *P. americana* cv Fuerte.

In Table 3 it is observed that the dehydration process of the leaves of *P. americana* cv Hass, Fuerte and Criollo negatively affects the content of total carbohydrates, total protein and fats, this in comparison with the infusions of the fresh leaves evaluated (Table 2). On the other hand, the crude fiber content in the I-HD and I-FD samples is higher than in the Criollo variety leaves both dehydrated and fresh.

The differences in the proximal analysis of the infusion of *P. americana* leaves may be due to the processing carried out on the sample, as indicated by Valdez-Solana *et al*., (2015), since the dehydration process can modify the content of carbohydrates, crude fiber and fat, mainly because fatty acids can undergo slow degradation processes and proteins and carbohydrates can react with each other causing browning reactions (Maillard reaction) (Venkatesan *et al*., 2006).

Component (%)	Sample I-CVD	I-CMD	I-HD	I-FD
Humidity	97.0±0^a	96.9±0.1^a	97.1±0.1^b	96.9±0.1^a
Ashes	0.3±0^a	0.3±0^a	0.3±0^a	0.3±0^a
Fats	0.03±0^b	0.05±0^a	0.04±0.0^a	0.03±0^b
Raw fiber	0.4±0^a	0.1±0.1^a	0.8±0.3^b	0.6±0.4^b
Total protein	0.3±0.1^b	0.2±0^a	0.3±0.1^b	0.2±0^a
Total carbohydrates	2.0±0.1^b	2.4±0.3^b	1.5±0.0^a	2.0±0.4^b
Equal letters indicate that there is no statistically significant difference (p> 0.05).				

Table 3 Proximal analysis of the infusion of dehydrated leaves of *P. americana* cv Hass, Fuerte and Criollo

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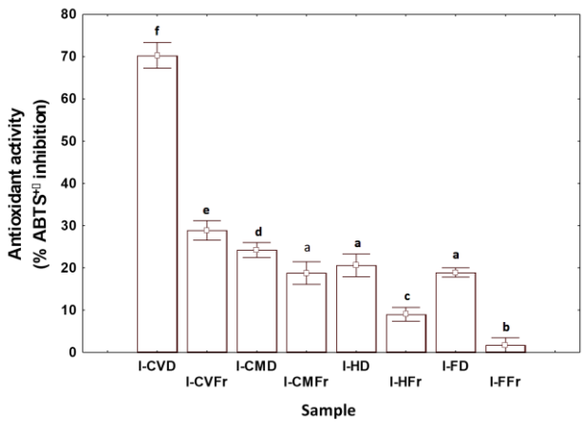
FRAUSTO-MOLINA, Janette, TOVAR-JIMÉNEZ, Xochitl, ÁLVAREZ-GARCÍA, Rocío and TÉLLEZ-JURADO, Alejandro. Proximal chemical characterization and antioxidant activity of *Persea americana* leaves *cv* Hass, Fuerte y Criollo. ECORFAN Journal-Ecuador. 2020

I-CVD: Infusion of dehydrated leaves of *P. americana* cv Criollo with green fruit; I-CMD: Infusion of dehydrated leaves of *P. americana* cv Criollo with purple fruit; I-HD: Infusion of dehydrated leaves of *P. americana* cv Hass; I-FD: Infusion of dehydrated leaves of *P. americana* cv Fuerte.

Antioxidant activity of the infusion of *P. americana* leaves

Regarding the antioxidant activity, it is observed in Figure 3 that the infusion of the dehydrated leaves of *P. americana* cv Hass, Fuerte and Criollo present greater antioxidant activity with respect to fresh leaves, likewise, the green fruit variety Criollo (I-CVD) is the one with the highest activity (70.28±1.22), however, in the infusion of fresh leaves (I-CVFr) its activity decreases (28.90±0.92).



Graphic 3 Antioxidant activity of the infusion of fresh and dehydrated leaves of *P. americana* cv Hass, Fuerte and Criollo

I-CVD: Infusion of dehydrated leaves of *P. americana* cv Criollo with green fruit; I-CVFr: Infusion of fresh leaves of P. americana cv Criollo with green fruit; I-CMD: Infusion of dehydrated leaves of *P. americana* cv Criollo with purple fruit; I-CMFr: Infusion of fresh leaves of *P. americana* cv Criollo with purple fruit; I-HD: Infusion of dehydrated leaves of *P. americana* cv Hass; I-HFr: Infusion of fresh leaves of *P. americana* cv Hass; I-FD: Infusion of dehydrated leaves of P. americana cv Fuerte; I-FFr: Infusion of fresh leaves of *P. americana* cv Fuerte.

Equal letters indicate that there is no statistically significant difference (p> 0.05).

Conclusions

The results indicated that there is a statistically significant difference in the proximal composition between the leaves of *P. americana* of the three varieties evaluated, as well as between the Criollo variety with green and purple fruit. Likewise, the infusion of dehydrated leaves of the green fruit Criollo variety (I-CVD) was the sample that presented the highest antioxidant activity (70% ABTS + • Inhibition).

Therefore, the leaves of *P. americana* could help the development of new products with a high content of crude fiber, having a favorable impact on human health, preventing or delaying gastrointestinal diseases such as intestinal obstruction and cancer. While the I-CVD infusion for its antioxidant activity could be used in the pharmaceutical industry to produce products with medicinal properties.

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Effect of the use of different types of fishmeal on the physicochemical properties of a fishfeed for *Oreochromis niloticus* (Nile tilapia)

Efecto del uso de diferentes tipos de harina de pescado en las propiedades fisicoquímicas de un alimento para *Oreochromis niloticus* (tilapia del Nilo)

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Abstract

The objective of this research was to study the effect of two types of fishmeal on the physicochemical properties of extruded food for *Oreochromis niloticus* (Nile tilapia). Using Pearson's squares, a diet was formulated (moringa, fish meal and corn flours, gelatin and vitamin and mineral premixes) with a protein content of 30 to 32%. The fish meal (sardine or salmon) was added in a 23.7% with respect to total composition of diet. The treatments were moistened with 13 and 17% water and processed in a single screw laboratory extruder. Extruded foods and a commercial (control), were analyzed according to their moisture, apparent density (DA), expansion index (IE), water absorption index (IAA), water solubility index (ISA), hardness and bulk density. The foods formulated with salmon flour at both moistures and with sardine flour at 17% humidity floated at 100%. The IE and DA values of food with salmon meal were like those of the commercial feed. Concluding that, the type of fishmeal used in the formulation of extruded foods influences the physicochemical properties of the product.

Extruded food, Fishmeal, *Oreochromis niloticus*

Resumen

El objetivo de esta investigación fue estudiar el efecto de dos harinas de pescado sobre las propiedades fisicoquímicas de alimentos extrudidos para *Oreochromis niloticus* (tilapia del Nilo). Mediante cuadrados de Pearson, se formuló una dieta a base de harinas de moringa, pescado y maíz, grenetina, premezclas vitamínica y mineral, con un contenido de proteína del 30-32%. La harina de pescado (sardina o salmón) se adicionó en un 23.7% con respecto a la composición total de la dieta. Los tratamientos se humectaron con agua al 13 y 17% y se procesaron en un extrusor de laboratorio de tornillo simple. Los alimentos extrudidos y un alimento comercial (control), fueron analizados de acuerdo con su humedad, índice de expansión (IE), densidad aparente (DA), índice de absorción de agua (IAA), índice de solubilidad en agua (ISA), dureza y flotabilidad. Los alimentos con harina de salmón a ambas humedades y con harina de sardina al 17% de humedad flotaron al 100%. Los valores de IE y de DA de los alimentos con harina de salmón fueron similares a los del alimento comercial. Concluyendo que el tipo de harina de pescado utilizado en la formulación de los alimentos extrudidos influye en las propiedades fisicoquímicas del producto.

Alimentos extrudidos, Harina de pescado, *Oreochromis niloticus*

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Introduction

Feeding in the aquaculture sector represents 50 to 80% of the production cost (Hasan, 2017), therefore, the study of aquaculture feeds is important, as proteins are essential for the stages of growth, maintenance, and reproduction of any living organism. In Nile tilapia (*Oreochromis niloticus*), protein deficiency can induce growth retardation, reduced appetite and, on occasions, deformation of the spine (Llanes *et al*., 2006). The optimal protein content in the ration for tilapia depends on the size or age and ranges from 30 to 50% (Rokey *et al*., 2010).

In addition to the protein content, the Nile tilapia requires floating food, since this species feeds on the surface of pond waters, for this, extrusion technology has been used, which consists of mixing various ingredients, transporting them and thermoforming them in a system of low humidity, high temperatures and high pressures, for a short time, using shear forces originated by a screw (Gil, 2010). During extrusion, proteins are one of the main components affected, because not all of them behave in a similar way (Navarro-Cortez, 2010).

The native proteins are denatured, due to the combined effect of temperature, pressure and shear forces, factors applied inside the extruder barrel, and that promote the increase of the digestibility of the proteins present in the extruded products, mainly due to their denaturation and by the inactivation of antinutritional factors, which decrease its digestibility (Onwulata *et al*., 2003 & Singh *et al*., 2007). The temperature used during the extrusion process, together with the absence of considerable amounts of starch, can reduce the solubility of proteins, consequently losing their functional properties (Pérez-Navarrete *et al*., 2006). In extruded products, with a high starch content, the protein undergoes encapsulation, however, the digestive enzymes of the intestinal tract of the fish dissolve the starch matrix, releasing the protein (Pérez-Robles *et al*., 2008).

In the production of extruded food, another main factor that directly affects the quality of the food is the initial water content. An increase in the processing humidity allows to obtain higher expansion rates, which influence a greater buoyancy of the product and a greater stability in water (Silva and Anderson, 1995).

The initial humidity is also related to the degree of gelatinization of the starch present in the food, however, in processes with low moisture content, gelatinization of the starch can be induced by mechanical cuts to the granules generated by high shear forces. inside the extruder (Vesanthan *et al*., 2001). The degree of gelatinization of starch is associated with the water absorption index (IAA) and the water solubility index (ISA), physicochemical properties that indicate the amount of water absorbed by the starch granules, while a high value of the ISA indicates the content of soluble solids generated by the gelatinization of starch and by the depolymerization and debranching of the polymeric starch chains during the extrusion process (Kannadhason *et al*., 2009; Singh *et al*., 2016 & Lu *et al*., 2019). For this study, it has been decided to work with initial humidity of 13 and 17% because in preliminary tests and under these conditions it has been possible to obtain dry food, with which it is intended to reduce the cost of making the product.

Currently there are no specifications for the formulation of aquaculture feeds, it is known that the main protein source is fish meal, however, the source of this meal is at the discretion of the manufacturer, therefore, in this investigation the behavior of the source of the fish meal (sardine or salmon) at different percentages of initial moisture (13 and 17%) in the mixture (moringa, corn and fish meal, gelatin, vitamin and mineral premix), on the physicochemical characteristics of the food extruded, comparing the responses obtained with those of a commercially available food used as a control, in order to determine its application as food for Nile tilapia.

The development of the work is presented in 4 sections: itroduction, methodology to be developed, results and discussion and conclusions. In the methodology to be developed, the techniques and conditions used to obtain the food are described, as well as the techniques for the physicochemical characterization of the products and commercial food. The results and discussions section presents the results obtained from the physicochemical characterization of extruded food comparing it with a commercial food. The last section presents the conclusions of this research.

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Methodology to be developed

Obtaining extruded feed for Nile tilapia

The diet formulated using Pearson's squares (50.4% moringa flour, 23.7% fish flour, 13% corn flour, 6% gelatin, 3% vitamin premix and 3% mineral premix) was extruded according to the methodology reported by Pérez-Nurseries (2017) with some modifications. At 13 and 17% humidity, two batches (the first with sardine flour and the second with salmon flour) were processed in a single screw laboratory extruder, Brabender 25 L/D brand. Where the heating zones were kept at 80, 100, 125 and 125 °C, from the feed zone to the exit zone, respectively, the screw used had a compression ratio of 2: 1, the feed and screw speeds were 60 and 160 rpm respectively and the exit die used was 4 mm.

Physicochemical characterization of extruded feed

Determination of humidity

AOAC (1990) method 925.09 was used. In trays at constant weight, 3 g of sample were added, and they were kept in the oven at 130 °C for 1 hour, finally the tray with the dry sample was weighed. The moisture percentage was calculated by weight difference according to equation (1).

$$\% \text{ Humidity} = \left(\frac{P_i - P_f}{m}\right) \cdot 100 \quad (1)$$

Where:

Pi = Weight of the tray with sample at the beginning (g). Pf = Weight of the tray with sample at the end (g).m = Weight of the sample (g).

Expansion index (IE) and apparent density (DA)

The EI was calculated according to the methodology reported by Gujska and Khan (1990), dividing the diameter of the extruded feed (pellet) by the diameter of the orifice of the extruder exit die. AD was calculated according to the methodology reported by Wang et al. (1993). With a vernier (Songqi tolos, Stainless Hardened, China) the diameter of 20 samples of 2.5 cm in length was measured.

The diameter was reported as the average of 3 measurements throughout the extrudate and each of the samples was weighed. The DA was determined as the weight of the sample over the volume of the extrudate, which was calculated with equation (2).

$$V = \pi \cdot r^2 \cdot h \quad (2)$$

Where:

V = Volume, r = radius and h = height.

Water absorption index (IAA) and water solubility index (ISA)

The ISA and IAA were determined according to the methodology of Anderson et al. (1970), with some modifications. 1 g of sample (pellets) was weighed on a dry basis, 10 mL of distilled water were added in a 50 mL Falcon tube, the tube with the sample and the water was stirred for 1 min in a vortex (IKA-WERKE, lab dancer, Germany) and centrifuged in a centrifuge (Hermle, 2300, Germany) at 6000 rpm for 30 min. After time, the supernatant was decanted into aluminum trays, previously placed at constant weight, the trays were dried in an oven (Sel lab, USA) for 24 h at 110 °C. The tube with the solid sample was weighed again. The IAA represented the amount of water retained per gram of sample, while the ISA, was expressed as the percentage of dissolved solids in the supernatant.

Hardness

Hardness analysis was carried out on a texturometer (Texturolab, TA.XT-Plus, UK). 60 extruded foods from each treatment (pellets), with a length of 0.5 cm, were analyzed. Hardness was calculated as the force required to cut the product by a compression test. The probe used was a 25 mm cylindrical probe at a distance of 2 mm, a force of 0.049 N, a return distance of 10 mm, a return velocity of 10 mm / s and a contact force of 100 g. The result was reported as the average of the 60 measurements in units of newtons.

ISSN-On line: 1390-9959

Buoyancy

To determine the buoyancy of extruded food in pellet form, the method of Vargas (2003) was used, with some modifications. The number of pellets that floated after 30 min was recorded in a 500 mL cylinder. The result was reported in percentage after analyzing a total of 10 samples.

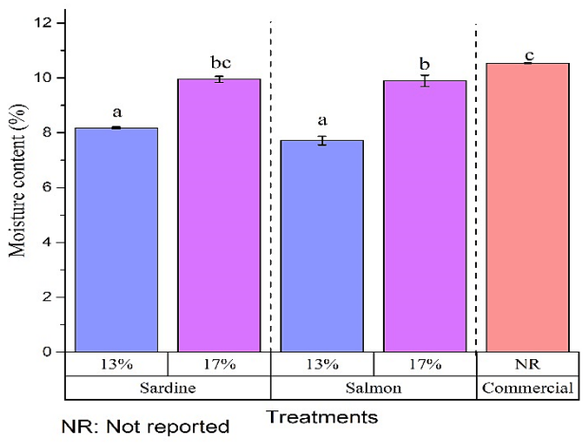
Statistic analysis

A one-way ANOVA was used and the comparison of means ($p \leq 0.05$) was carried out with the Tukey-Kramer test using the NCSS 12 Data Analysis Software Manuals program.

Results and Discussion

Determination of humidity

The results of the moisture content in the treatments with salmon and sardine meals are shown in Graphic 1, where it is observed that after the extrusion process, the humidity of the extruded foods decreased between 5 and 7% with respect to its initial humidity that is, the feeding humidity (13 and 17% respectively), not observing statistically significant differences ($p \leq 0.05$) between the treatments. Food extruded at 17% initial moisture presented moisture percentages like that of commercial food.

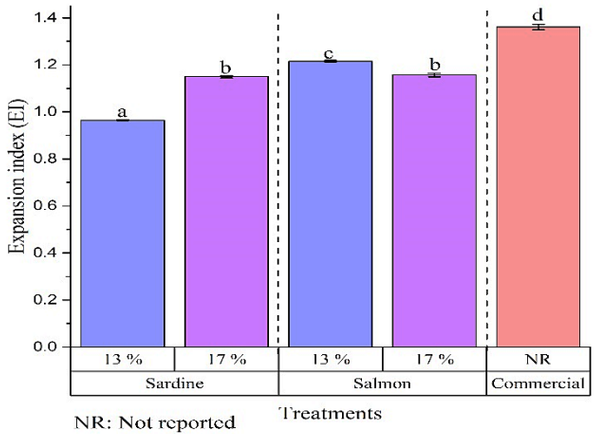


Graphic 1 Moisture content
Source: Our elaboration

Expansion index (IE) and apparent density (DA)

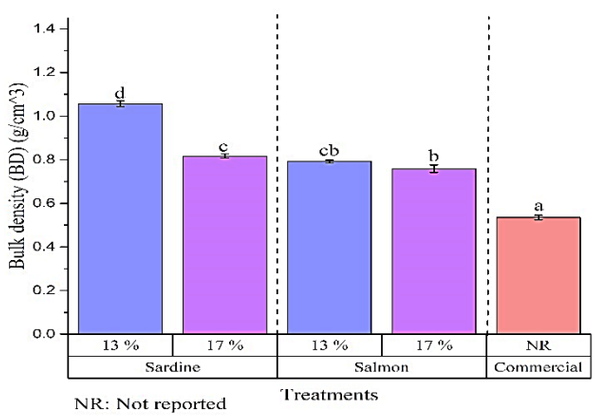
The EI results obtained (Graphic 2) were similar to those reported by Kannadhason et al. (2009), where extruded foods with higher DA values presented lower EI.

The EI decreased significantly when using salmon meal in the formulation, probably due to the type and content of protein present in the meal, since, according to Martin *et al.* (2019), the different types of proteins can undergo structural changes during the extrusion process, and that interfere with the degree of expansion of the pellet.



Graphic 2 Expansion index (IE)
Source: Our elaboration

The DA results (Graphic 3) agree with those reported by Xuelian et al. (2018) who report lower DA in extruded foods processed at higher humidities. Lower DA was observed in extruded foods made with salmon flour, attributed to the water solubility of its components, including proteins (Samuelsen *et al.*, 2013), however, in order to assert this information it is necessary to perform a profile of proteins from each of the flours present in the formulation of the treatments used to obtain extruded foods. Finally, the results indicated that foods similar to commercial food were those extruded at 17% humidity, where despite obtaining higher DAs, buoyancy was not affected (Graphic 7).



Graphic 3 Apparent density (DA)
Source: Our elaboration

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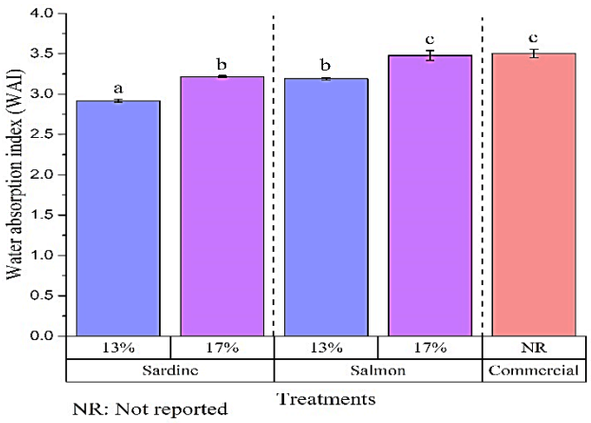
Water absorption index (IAA) and water solubility index (ISA)

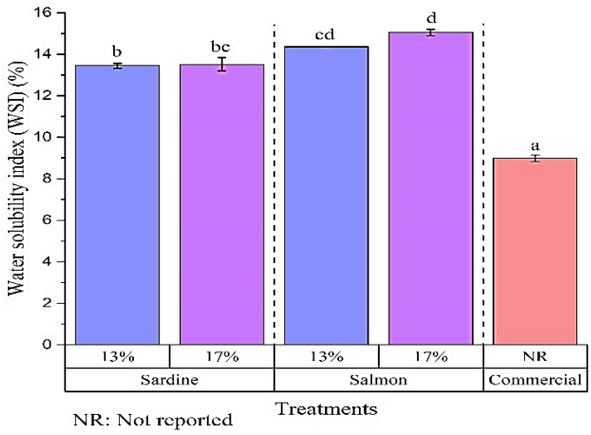
The IAA was slightly higher in extruded foods formulated with salmon flour at 17% humidity (Graphic 4), attributed to the interaction between the hydroxyl groups (OH) of the starch from the corn flour and the OH groups of water present in the food, since according to Lu *et al*., (2019) IAA is related to the proportion of fully gelatinized starch granules that tend to absorb water. In these results, the diet formulated with salmon flour at 17% humidity did not show statistically significant differences ($p \leq 0.05$) with respect to commercial food.



Graphic 4 Water absorption index (IAA)
Source: Our elaboration

The ISA results (Graphic 5), did not present statistically significant differences ($p \leq 0.05$) with respect to the feeding humidity; however, the treatments formulated with salmon meal presented higher ISA, probably due to the content of water-soluble proteins present in each of the fish meals, such as albumin.

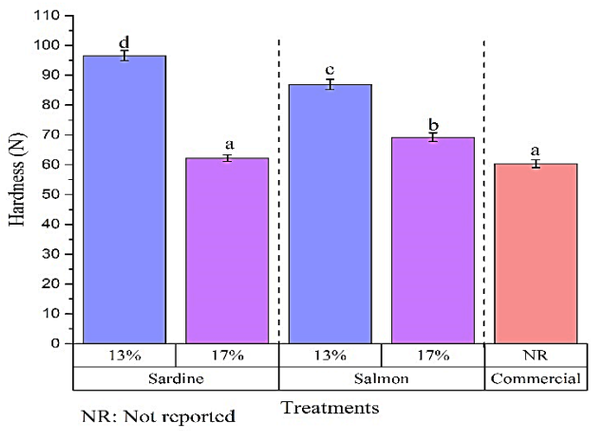
The increase in ISA also indicated a greater degradation of the polymeric chains of starch during extrusion, being beneficial for their assimilation by losing their crystalline structure (Singh *et al*., 2016).



Graphic 5 Water solubility index (ISA)
Source: Our elaboration

Hardness

The hardness of the extruded feeds decreased as the feed moisture content increased (Graphic 6). The lowest result was found in foods extruded with sardine flour at a feeding humidity of 17%, a diet that did not show statistically significant differences ($p < 0.05$) with respect to commercial food, while the results of the rest of the diets they were found above it. The extruded diets at 17% moisture showed values closer to commercial food, however, an increase in the hardness of the pellet could not be detrimental as it contributes to the strength of the pellet for storage (Xuelian *et al*., 2018).



Graphic 6 Hardness
Source: Our elaboration

Buoyancy

The diets formulated with 17% feed moisture showed 100% buoyancy (Graphic 7), while in the extruded feed with 13% feed moisture, only those formulated with salmon meal showed 100% buoyancy while those formulated with sardine flour showed 0% buoyancy.

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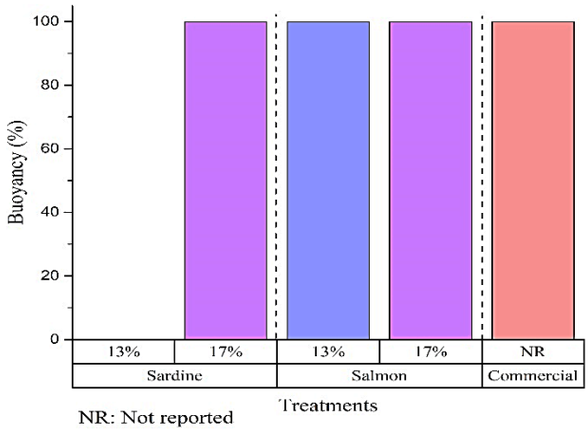

Figure 7 Buoyancy
Source: Our elaboration

Conclusions

The type of fish meal (salmon and sardine) used in the formulation of extruded foods influenced their physicochemical characteristics, being sardine meal the one that had the greatest influence on the characteristics of extruded foods, similar to those of the food. However, the diet with salmon meal at an initial feeding moisture of 17%, substantially improved the physicochemical characteristics of the feed by increasing the expansion of the pellet, decreasing its apparent density and, consequently, contributed to increasing its buoyancy. The formulation and extrusion conditions allowed to obtain slightly harder food compared to commercial food, promoting a decrease in its fracture during storage and feeding of the fish, in addition to its nutritional properties are not affected since the solubility of the same in water is higher than that of commercial food, however, to assert this information, it is necessary to perform in vivo digestibility tests of the extruded food developed.

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Effect on the fatty acid profiles of a microalgae strain (*Dunaliella tertiolecta*) using different lipid extraction techniques

Efecto sobre el perfil de ácidos grasos de una cepa de microalga (*Dunaliella tertiolecta*) empleando técnicas diferentes de extracción de lípidos

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Abstract

Biodiesel is subject of research because it is less polluting when used in pure form or mixed with petroleum diesel. Microalgae are now a material new in research for mass production of biodiesel, so the techniques used for cell growth, biomass extraction and lipid extraction influence the profile of fatty acids susceptible to transesterification and consequently the quality of biofuel. This work shows the effect on the fatty acid profile of a *Dunaliella tertiolecta* strain using two lipid extraction techniques. For this, the culture was carried out in the medium F/2, the recovery of the biomass was carried out by sedimentation-flocculation with NaOH and once dry it was subjected to extraction with solvent by Soxhlet and by ultrasound to obtain lipids under the methodology by Bligh & Dyer. The fatty acids were analyzed by gas chromatography and the profile is made up of saturated-monounsaturated-polyunsaturated fatty acids and shows differences regarding the presence-absence and dry weight content. This represents a contribution to analyze the effect on physicochemical parameters established in Mexican regulations regarding the quality of biodiesel and to determine the potential of the microalgae strain for such production.

Microalgae, Fatty acids, Biodiesel

Resumen

El biodiésel es objeto de investigación debido a que es menos contaminate si se utiliza en forma pura o mezclado con diesel de petróleo. Las microalgas, son actualmente un insumo en investigación para producción masiva de biodiésel, por lo que las técnicas empleadas para crecimiento celular, extracción de biomasa y lípidos influyen en el perfil de ácidos grasos susceptible de transesterificación y por consecuencia en la calidad del biocombustible. Este trabajo muestra el efecto sobre el perfil de ácidos grasos de una cepa de *Dunaliella tertiolecta* empleando dos técnicas de extracción de lípidos. Para ello el cultivo se llevó a cabo en el medio F/2, la recuperación de la biomasa se realizó por sedimentación-floculación con NaOH y ya seca se sometió a la extracción con disolvente por Soxhlet y por ultrasonido para obtención de lípidos bajo la metodología de Bligh y Dyer. Los ácidos grasos se analizaron por cromatografía de gases y el perfil se compone de ácidos grasos saturados-monoinsaturados-poliinsaturados y muestra diferencias respecto a la presencia-ausencia y contenido en peso seco de ellos. Esto representa un aporte para analizar el efecto sobre parámetros fisicoquímicos establecidos en la normativa mexicana respecto a la calidad del biodiésel y para determinar el potencial de la cepa de microalga para tal producción.

Microalga, Ácidos grasos, Biodiésel

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Introduction

Diesel is a petroleum product composed of a mixture of saturated (unbranched) and aromatic hydrocarbons subjected to hydro sulfurization to reduce the sulfur content that it naturally possesses. In Mexico, through NOM-016-CRE-2016, two diesel names are produced and sold, the maximum of 500 ppm of sulfur (equivalent to 500 mg of sulfur/Kg diesel or 0.05% by weight) for use in sector agricultural-marine and industrial diesel for use in direct fire combustion, and the minimum of 15 ppm of sulfur (0.0015% by weight) known as ultra-low sulfur diesel (ULSD) only for use in transport sector in the Metropolitan Zone of the Valley of Mexico, Guadalajara, Monterrey, the Northern Border Zone and imported diesel.

Both denominations generate atmospheric emissions of sulfur oxides and particulate matter as a consequence of combustion processes in each of the engines in which they are used, also causing negative effects on soil, water and human health, a challenging problem to be solved in this century to achieve the sustainable local development worldwide.

This problematic requires research in the area of bioenergy to produce alternative biofuels from varied inputs, easily acquired or extracted, that can be used alone or mixed with petroleum products, technologies for conversion by sustainable processes that give added value to by-products and finally thus reducing energy dependence on imports.

Biodiesel is promising because it has a favorable energy content compared to petroleum diesel, in its pure form it is highly biodegradable, free of aromatic compounds, non-toxic and with sulfur content (< 0.0020%) (Pinzi *et al*., 2009) very similar to ULSD and well below 500 ppm diesel. For mixing, it can be under any proportion, B20 being the most commercialized (B5 the minimum). Its emissions have a lower risk of cancer in the population compared to fossil diesel (Manuale, 2011).

The materials to achieve a quality biodiesel production is a key factor and object of research, ranging from vegetable oils from crops and residuals, animal fats (the traditional ones) that contribute to reduce contamination in bodies of water by direct discharge of these, to more promising inputs (due to their short-term and permanent production) based on a variety of species of some genera of microalgae that produce high lipid content for the transesterification of the fatty acids (FA) that constitute them.

However, some biodiesel parameters such as oxidation stability and low temperature properties are favored or affected by the presence-absence, high-low content of a SFA, MUFA an PUFA, which determines the potential of the input for produce quality biodiesel and ultimately reduce the number of raw materials in practice. This creates the need to also analyze lipid extraction techniques for the study of the FA profile and from there make the decision-making adjusted to energy and economic expenses.

This work shows the FA profile in a strain of the microalgae *Dunaliella tertiolecta* and the variations that exist employed two techniques used for the extraction of lipids, one very fast and efficient such as ultrasound and the other very classic such as Soxhlet, previous recovery of the biomass under an affordable flocculation methodology.

The sections of this article are methodology, results, and analysis regarding the effect of biodiesel properties in a hypothetical production by transesterification of AG and the conclusions that are derived.

Methodology

The culture of the *Dunaliella tertiolecta* strain was carried out for 20 days in the cell growth medium of Guillard & Ryther (1962) known as F/2 prepared in a saline medium (NaCl solution). It began with the transfer of the microalgae to a sterile Erlenmeyer flask with 120 mL of F 2 medium. Once the exponential phase began and in order to achieve a high quantity and concentration of biomass, reactors were inoculated (with an average initial cell concentration of 4 x 10^5 cells mL^{-1}) by volumetric transfer of medium F/2 (Coutteau, 2013) until a final volume of 16 L in 19 L reactors.

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For this, the cell density was determined per day and tripled in aliquots of 1 mL extracted from the culture by counting through a microscope in a double bright line Neubauer Chamber.

The extraction of the dispersed algal biomass in the final volume of work is affected by the dilutions that are made. For effective recovery, a flocculation-sedimentation methodology was adapted, induced by a change in pH of the culture medium from 7.8 to 11.0, adding 1M NaOH and a rest time of 24 h (Beevi, *et al*. 2016; Rojo-Cebreros *et al*. 2016). Subsequently, centrifugation (5 min, 5000 rpm) and lyophilization (-50 ° C, 1.3 Pa, 72 h) were performed to dehydrate it completely (Chen, *et al*., 2011).

The recovery of the fat content present in the dry powder algal biomass was carried out by solvent extraction using two techniques: the conventional Soxhlet solid-liquid extraction method and the solid-liquid extraction method by ultrasonic waves. In both methodologies, the chloroform/methanol mixture was used as a solvent in a 2:1 ratio adapted to the process proposed by Bligh & Dyer (1959) commonly used for the extraction of neutral and polar lipids for energy purposes. In all trials, three replicates were performed.

The Soxhlet extraction was carried out in an average time of 4 h using a 500 mL extractor equipment (composed of a thermomagnetic plate, solvent volumetric balloon, cellulose filter where the biomass was placed, extractor, condenser) coupled to an external cooling system (10 L of water 4 ± 1 °C). Once the process was finished, the sample was placed in a separatory funnel and methanol/distilled water (100/180 mL) was added for the separation of the phases, 24 h later the lower phase (biomass/lipids-chloroform) was extracted and carried to a rotary evaporator (thermal bath 40 °C, 60 rpm, 10 min) to remove solvents. Finally, the lipid extract was stored in amber flasks at 5 °C.

The ultrasound extraction began with the suspension of the biomass in the 2:1 chloroform/methanol mixture and it was centrifuged (200 rpm, 15 min) and then placed in an ultrasonic bath (Branson 1510 power 70 W) at a fixed frequency of 40 KHz for 20 min and achieve the electronic excitation that generates the intramolecular vibrations. It was then centrifuged (150 rpm, 15 min) for the separation of biomass/liquid phase with final adjustment of chloroform/methanol/water at a ratio 2:2:1.8. It was transferred to a decantation balloon and proceeded in the same way as Soxhlet (extraction of the biomass/lipid-chloroform phase, evaporation of the solvent and protection of the fatty extract (González, 2011; King, 2014; Soto-León, *et al*., 2014).

The lipid productivity (mg L^{-1} d^{-1}) was calculated using equation 1, that relates the biomass productivity (biomass produced during the entire exponential growth phase expressed in g L^{-1} d^{-1}) and the total content percentage of lipids quantified by weight difference of biomass before and after extraction (Song, *et al*., 2013).

$$LP = BP \times L \quad (1)$$

The determination of the fatty acids in the lipid extracts was carried out by injecting 1.0 µL of sample into an Agilent® 7820A gas chromatograph coupled to an FID (flame ionization) detector and a DB 5Ht column. The column-oven temperatures were programmed from 50 °C to 180 °C at a rate of 10 °C min^{-1}, then to 320 °C at a rate of 4 °C min^{-1}. The injector temperature was 300 °C and that of the FID detector 320 °C.

The qualitative analysis of the fatty methyl esters that make up the sample (obtained by chemical conversion of the lipid extract of each treatment) was carried out by measuring the retention times and their comparison with reference standards (37 components FAME brand SupelcoTM Mixture C4-C24). The quantitative analysis consisted in the integration of the total area under the peaks shown in the chromatogram assuming that the fatty methyl esters were totally separated. The analyses were carried out in triplicate and the results are presented as a normalized percentage average by weight of each component.

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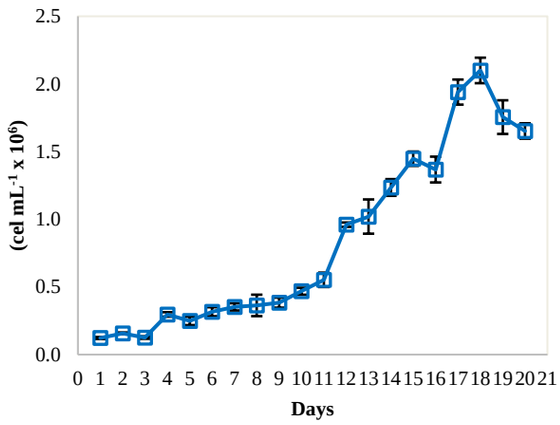
Results

The kinetic behavior of the *Dunaliella tertiolecta* strain in the F/2 culture medium is shown in Graphic 1 as an average of the daily cell count in the working replicas and represents a characteristic sigmoid curve of the growth of microorganisms. The exponential growth phase begins slowly the first 72 h and from there it increases progressively until day 18 where we obtained 2.10 ± 0.12 x 10^6 cells mL^{-1} are reached, considered the maximum cell concentration that declines from day 19 as a result of the competition of microorganisms by nutrients in the middle giving way to the death phase from day 20.



Graphic 1 Cell growth kinetics of *Dunaliella tertiolecta*
Source: self-made

The specific cell growth rate (μ) was 0.21 ± 0.02 d^{-1} for a doubling time (*td*) of the concentration of the microorganisms of 3.34 ± 0.29 *d*, calculated from the averages obtained by the daily counting method. The results obtained are within the values reported by some authors using the same culture medium. For example, for cell density Neto *et al*. (2012) reported a similar behaviour of 22 days, reaching a maximum of 0.81 x 10^6 cel mL^{-1} on day 18, lower than that reported, while, Gárate (2020) under analogous laboratory conditions reached between days 12-14 a maximum of 3.39 x 10^6 cells mL^{-1}, greater than that reported here. Regarding the growth rate and doubling time, El Arrousi *et al* in 2015 reported μ equal to 0.34 d^{-1} and *td* of 2 *d*, Félix (2017) reported μ of 0.27 d^{-1} and a *td* of 2.59 *d*.

It should be noted that all microalgal growth will always present slight or marked differences with respect to another, not only due to modifications to the nutrient medium based mainly on the use of a natural saline medium and an elaborated saline medium and limitation of nutrients, but also by the parameters physical elements that complement it, such as light intensity, photoperiod, temperature, agitation, etc. (Chen, *et al*., 2011; Félix, 2017).

The flocculation efficiency for the recovery of the biomass generated in the crops using 1 M NaOH per pH change was 99 %, which states that it is a simple, affordable and effective methodology, since this chemical agent acts as a powerful destabilizer of the electrostatic charges present in the cell and from the change in acidity due to the increase in the pH of the culture medium, the cells agglomerate and form large flocs, sedimentation at the end of 24 h. The results are similar to those reported by Rojo-Cebreros, *et al.* (2016) that achieved an efficiency of 94.9 % of biomass recovery in a Nannochloropsis sp strain for aquaculture purposes using NaOH 0.5 eq L^{-1} in less than 1 h, Unmalyma, *et al*. (2016) through a study for biomass extraction in *Chlorococcum sp* determined that NaOH was the most effective agent for flocculation with 94 % compared to Al_2SO_4, which was 87 %.

The extraction of accumulated lipids in *Dunaliella tertioelcta* using Soxhlet/Bligh & Dyer ultrasound allowed to determine the percentage content of dry base lipids and the average lipid productivity per day. Thus, at the end of the crops an average biomass productivity of 23.12 mg L^{-1} d^{-1} was obtained, a percentage content of lipids extracted by Soxhlet-Bligh & Dyer of 26.9 ± 0.8 % and an average lipid productivity of 6.22 mg L^{-1} d^{-1}, while, by ultrasound-Bligh & Dyer, a higher lipid content of 33.5 ± 1.1 % and a productivity of 7,813 mg L^{-1} d^{-1} was obtained. These results are within the range reported in the bibliography for this species of microalgae (16.7 to 71 %) (Takagi, *et al*., 2006; Sydney *et al*., 2010 and El Arroussi, *et al*., 2015) and for many marine microalgae (Mata, *et al*., 2010).

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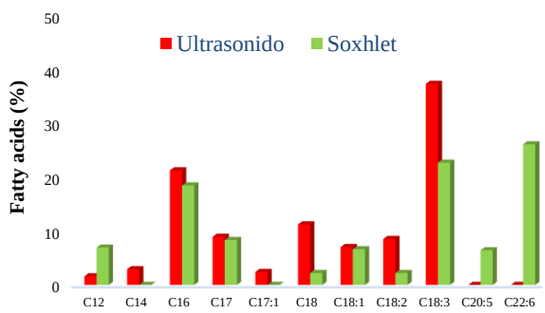
Again, the lipid content will be affected by the same factors that influence microbiological growth already mentioned (different strains of a species of microalgae, nutrients, temperature, light and the variations that may occur in these) as well as the extraction techniques and the solvents they use.

Under this thematic, Araujo, *et al*. (2013) analyzed the lipid extraction efficiency in biomass obtained from *Chlorella vulgaris* by different solvent methodologies (among them Folch, Bligh and Dyer & Soxhlet) applying ultrasound and determined that the Bligh & Dyer methodology presented the highest extraction efficiency (52.5 %) and the Soxhlet extraction was the one with the lowest lipid recovery (1.8 %), attributing the differences to the characteristics of the cell wall and its behavior (greater or lesser affinity) against solvents or a mixture of them and the respective proportion that is used, as well as the power of the equipment, temperature, time, washes.

Suarsini and Subandi (2012) in biomass of *Chlorella vulgaris* and *Spirogya* sp. analyzed the efficiency of three lipid extraction methods (Soxhlet, maceration and ultrasound) as a function of the time used and using n-hexane as a solvent. They established the following: ultrasound (2.33 h, 1.77 %) > maceration (8 h, 1.03 %) > Soxhlet (18 h, 1.58 %) and concluded that ultrasound is an efficient technique for lipid extraction because it uses less extraction time, with very good performance and generates less waste compared to the other two.

The fatty acids (or fatty acid methyl esters or FAME) obtained from the lipids extracted from the *Dunaliella tertiolecta* strain by the proposed techniques and in comparison, with the standard reference standards are shown in Graphic 2.



Graphic 2 Fatty acids in a *Dunaliella tertiolecta* strain using two extraction techniques
Source: Self-made

We identified 9 FA distributed as SFA (C12-C18), MUFA (C17:1-C18:1) and PUFA (C18:2-C22:6) whose percentage content is summarized in Table 1.

FAME	Relative Percentage (%)	
	Ultrasound	Soxhlet
SFA	45.8	35.8
MUFA	9.0	6.8
PUFA	45.2	57.4

Table 1 Composition of fatty acids present in a *Dunaliella tertiolecta* strain
Source: *Self-made*

According to what was obtained, lipid extraction techniques influenced the FAME profile, since Soxhlet yielded 2 new FAME and others did not (for example, C14), in some contents they are slight differences and in others, very marked. However, the profile is in accordance with that reported in the bibliography for each technique and the same nutrient medium (F/2) since generally these coincide in a greater presence of FAME, in compounds from C16 to C18 and C18:3 (Chen, *et al*., 2011; Tang, *et al*. 2011; El Arroussi, *et al*., 2015) and coincides with other marine species of the same or different genus (Tanzi, *et al*., 2013; Tadeo-Sánchez, *et al*., 2014).

The ultrasound extraction efficiency is attributed to mechanical and cavitation processes. Cavitation contributes to the breakdown of the cell wall of the microalgae by the vibrations that are generated, which allows greater and easy penetration of the solvent mixture and by the electrostatic interactions that occur between the two, lipids are quickly released, mechanical processes they help better agitation of the solvent which increases the surface area of contact (Spinella, *et al*. 2016).

Taking into account only the FAME with the highest percentage content, for the SFA, palmitic acid (C16) regardless of the extraction technique, the same happens for oleic acid (C18: 1), in the MUFA, while, for the PUFA, by ultrasound the highest percentage was linolenic acid (C18:3) and by Soxhlet, it was Cis-4,7,13,16,19-docosahexaenoic acid (C22:6).

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Regarding the production of biodiesel adjusted to Mexican or international regulations, the physicochemical parameters such as oxidative stability, flow properties at low temperatures, viscosity, number of cetanes are the most interesting and depend on the FAME present in the raw material of origin.

The ideal is long-chain FAME with low unsaturation number, that is, SFA and MUFA such as C14 (myristic), C16 (palmitic) and C18: 1 (oleic) respectively since they give the biodiesel oxidative stability, high cetane number and lubricity, low viscosity and toxicity, being palmitic acid the one that particularly confers the highest number of cetanes and consequently greater stability to oxidation. However, a high content of SFA compromises the flow properties of biodiesel at low temperatures. Oleic acid is the main methyl ester of biodiesel that allows high lubricity and ignition and low viscosity and toxicity (Pinzi, et al., 2009; Tadeo-Sánchez, *et al*., 2014).

The contribution of PUFA (linoleic and linolenic) is that they favor the operation of biodiesel at low temperatures since they present a lower melting point, which provides greater fluidity of the biodiesel in the engine. The problem is their susceptibility to oxidation, which would affect the storage of biodiesel for a long time, which can be corrected with the addition of oxidizing compounds that can be obtained in the biodiesel production chain or with protection in environments with low light and drafts. air. (Moreira, 2012, Tejeda, *et al.* 2015).

Arias, *et al.*, (2013) proposed that to guarantee quality biodiesel, the convenient thing would be a mixture of FAME produced by different types of microalgae, since the objective is to be able to develop a sustainable, viable and feasible process in the economic and competitive energy terms. with fossil fuels.

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Conclusions

Sodium hydroxide turned out to be an excellent flocculant for the extraction of microalgal biomass due to the time it was used and the percentage of recovery that was obtained.

Cell disruption methods employing solvent extraction such as Bligh and Dyer allow pure lipid extracts even when they are of low dry base content.

The ultrasound extraction was efficient due to the time used (20 min) and the FAME profile obtained. Soxhlet extraction is one of the most used methods for the extraction of fat content in microalgae despite a negative balance in terms of working time and energy consumption, however, it is a reference method with which other methods of extraction. In this investigation, it was carried out in 4 h and yielded a different FAME profile.

The FAME profile together with the lipid productivity and biomass productivity are key factors for decision-making regarding a pilot or mass production of biodiesel.

The FAME profile was compensated by a lower concentration of SFA and a higher proportion of MUFA and PUFA, which coincides with investigations regarding the same species in this work, other species, other microalgae genera and even plant crops and animal fats. The profile obtained contains acceptable percentages of the FAME suitable for transesterification.

We recommend to carry out the production and characterize the biofuel based on the existing Mexican regulations and determine the influence of the FAME obtained, mainly linolenic acid on oxidation stability as it is the characteristic with the greatest impact on the use

Finally, it is also recommended from an economic viability point of view to implement crops with photoperiods since electricity consumption is one of the parameters that negatively impact the process and therefore the long-awaited sustainability, as well as determining the viability for a scale. pilot in environmental conditions with use of solar energy in the region.

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Chemical composition of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) and diversity of uses in rural areas

Composición química de *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae) y diversidad de usos en el medio rural

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Abstract

The objective of this study was to evaluate the nutritional quality of *Tithonia diversifolia*, a shrub species in the Asteraceae family and the diversity of uses in rural areas. A bibligraphic review was conducted in Mexico and other countries in Central America, South America, Africa and Asia. Bromatological analyses of edible material (leaves and petiole) were performed to determine dry matter (DM), fat, ash, crude fiber, crude protein (CP), and nitrogen-free-extract (NFE). Fiber fractions were determined: neutral deterget fiber (FDN), acid detergent fiber (ADF), hemicellulose, cellulose and lignin, from two locations in Jalisco state and one from Colima. Herbarium specimens were reviewed for knowing geographical distribution and rural uses. Results indicate that even without being legume, CP percentages are high with values of 18.42 to 31.54% with high dry matter content up to 98.92%, fiber values (30.81 to 34.08%) for NDF and (22.48 to 31.69%) for ADF. The bibliographic review highlights its forgae use, for ruminants and monogastrics, in beekeeping for its content of néctar and pollen, sucha as medicinal, ornamental and green manure for its contribution of nutritious, mainly phosphorus. *T. diversifolia* is a good option for its nutritional value and diversity of uses are demonstrated as an alternative in semi-intensive animal production systems in both tropical and temperate áreas.

Beekeeping, Crude protein, Dry matter, Fiber fractions, Medicinal use

Resumen

El objetivo de este estudio fue evaluar la calidad nutritiva de *Tithonia diversifolia*, especie arbustiva de la familia Asteraceae y la diversidad de usos en áreas rurales. Se realizó una revisión bibliográfica en México y otros países de Centroamérica, Sudamérica, África y Asia. Se realizaron análisis bromatológicos de material comestible (hojas y pecíolo), para determinar materia seca (MS), grasa, cenizas, proteína cruda (PC), fibra cruda (FC) y extracto libre de nitrógeno (ELN). Se determinó fracciones de fibra: fibra detergente neutro (FDN), fibra detergente acido (FDA), hemicelulosa, celulosa y lignina de plantas, procedentes de dos localidades del estado de Jalisco y una del estado de Colima. Asimismo, se revisaron ejemplares de herbarios para conocer su distribución geográfica y los usos en el medio rural. Los resultados indican que aún sin ser leguminosa los porcentajes de PC son altos con valores de 18.42 a 31.54%, con alto contenido de materia seca hasta de 98.92%, valores de fibra de (30.81 a 34.08%) para (FDN) y de (22.48 a 31.69%) para (FDA). La literatura resalta su uso forrajero, para rumiantes y monogástricos, en la apicultura por su contenido de néctar y polen, como medicinal, ornamental y abono verde por su aporte de nutrimentos, principalmente fósforo. *T. diversifolia* es una buena opción, ya que se demuestra su valor nutritivo y la diversidad de usos, como una alternativa en sistemas semi-intensivos de producción animal, tanto en zonas tropicales como zonas templadas.

Apicultura, Fracciones de fibra, Materia seca, Proteína cruda, Uso medicinal

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Introduction

Tropical livestock based their diet on pastures; these have an extraordinary capacity to produce biomass (Palma, 2005), however, the marked seasonality that exists in most of the country, causes a strong forage deficit in the dry season, especially for extensive livestock farming, which during eight months of drought, consumes the foliage and fruits of forage trees and shrubs, present in the pastures, a valuable resource, as it is the only source of fresh and good quality food during the dry season. Among the species mostly used for animal feeding, legumes stand out, predominating among them, different types of *Acacia* sp., *Leucaena* spp., *Gliricidia sepium*, *Calliandra* spp. and *Caesalpinia* spp., which are characterized by their high protein content, up to 30% in foliage and up to 24% in fruits (Román *et al*., 2004; Palma, 2005; Román *et al*., 2013; Palma *et al*., 2019). On the other hand, in most tropical countries there is a great diversity of plant species, which can contribute to animal feeding (Valenciaga *et al*., 2018; Herrera *et al*., 2020) and reduce production costs in the livestock activity, including *Tithonia diversifolia*, a shrubby plant of the family (Asteraceae), which grows both in tropical and temperate climates.

The types of habitat where it is distributed include: oak forests, tropical evergreen forests, deciduous forests, secondary vegetation, along the roads, as well as in cultivated fields, its altitudinal range goes from sea level to 2500 m. Likewise, in the review of the material of herbarium specimens, collected in the state of Jalisco, they report it in areas near Puerto Vallarta, collected in a tropical deciduous forest and secondary vegetation, associated with *Ficus*, *Enterolobium*, *Hura*, *Gliricidia*, *Hyptis*, *Solanum*, *Tridax* and *Cosmos* among other species.

Studies carried out in Colombia demonstrate the forage potential of this species and its nutritional quality, which without being legume, has high levels of crude protein that range from 14.84 to 28.75%, due to its nutritional value and diversity in its chemical composition (Ríos and Salazar, 1995; Ríos, 1999; Mahecha and Rosales 2005; Mahecha *et al*., 2007; Gallego-Castro *et al*., 2017; Rivera *et al*., 2018), as well as other research carried out in Cuba by (Galindo *et al*., 2018, Valenciaga *et al*., 2018) make this species a viable option to be used in agricultural production systems.

Galindo *et al*. (2018), reported that its use in the animal diet reduces the production of methanogens and protozoa and has beneficial effects on the microbial ecology of the rumen. Lescano-Más *et al*. (2016) stated that in young cattle it contributes to reducing the parasite. Furthermore, this species presents desirable characteristics that make it attractive to be incorporated into silvopastoral systems; including having a large root volume, and a special ability to recover the scarce nutrients present in the soil (Pérez *et al*., 2009), has a wide range of adaptation and distribution in tropical and temperate zones, a characteristic that in some parts of the world, consider it as an invasive species in different ecosystems, mainly in Africa and China (Sun *et al*., 2007; Muoghalu, 2008; Ajao and Moteetee, 2017), it tolerates conditions of acidity and low fertility, it is resistant to poor soils and it can withstand pruning at ground level, it is tolerant to burning and has a rapid growth, it is not demanding of inputs or handling for its cultivation (Mahecha and Rosales, 2005; Pérez *et al*., 2009).

In Colombia they evaluated the production of dry matter with plants obtained through asexual and sexual means; planting 36 plants per plot of (20 m^2), with a density of 1.8 m^2, with weed control and fertilization, obtaining productions of 13, 17 and 19 t / DM / ha, by establishing the cultivation by stakes, plants produced by seed sexual in in vitro conditions and plants obtained by sexual seed, managed in seedlings (Gallego-Castro *et al*., 2015).

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Herrera *et al*. (2020), studied the distribution of this species in relation to climate change and its chemical composition, considering precipitation, temperature and distribution, highlighting that the correlation between climatic factors and chemical composition was variable, with the highest coefficients (r) for phosphorus, with maximum temperature (0.64) and average temperature (0.63), OM and ashes with minimum temperature (0.62 and -0.62), respectively; cellulose with the maximum temperature, total rainfall and the number of days with rain, with values of: (-0.62, -0.69 and -0.73), respectively, and nitrogen (N) with rainfall and its distribution (- 0.81 and -0.82, respectively). Being for the other components, low and not significant correlations; reaching the conclusion that climate factors act individually and / or interrelated, which is relevant to know, for the management of the plant in climate change scenarios.

Tithonia diversifolia is a species native to Mexico and Central America. However, in our country, it has not been used intensively as a forage species in livestock production systems, nor in the diversity of uses it presents, so the objective of this study is:

Objective

Evaluate the nutritional quality of *Tithonia diversifolia* from three different locations, as well as rescue the experiences of other countries on its multiple uses, to enter silvopastoral systems.

Materials and methods

The study began with a bibliographic review, as well as a consultation in the herbarium of the Institute of Botany, of the University of Guadalajara, to know its distribution based on reviewed specimens. The plant was collected in three different locations: one in the municipality of Cuauhtémoc, in the state of Colima, present in weed vegetation, another in El Tuito, municipality of Cabo Corrientes, used as an ornamental and the other collected in the Botanical Garden of the University Center of Biological and Agricultural Sciences. The climates present are:

Warm subhumid with rains in summer Aw1, with rainfall of 1200 mm and mean annual temperature of 24 °C for Cuauhtémoc, and semi-warm subhumid with rains in summer for the site of El Tuito A (C) w1, with 1100 mm of rainfall per year and average annual temperatures between 22 and 26 °C., the same climate as Las Agujas in the municipality of Zapopan, Jalisco, although with lower temperature ranges from 20 to 24 °C. Later, made revisions in the herbaria of the Universidad Nacional Autónoma de México (MEXU) and the Instituto Politécnico Nacional (IPN), to know its geographical distribution, altitude ranges, types of vegetation where it occurs and the uses attributed to it in rural areas.

From the last two sites, a previous evaluation of vegetative propagation was carried out with stakes of lengths between ranges for T_1 (20 to 25 cm), T_2 (26 to 32 cm), and T_3 (33 to 40 cm), With variable diameters between the different lengths. Subsequently, they were subjected to water stress to measure survival.

The edible material was collected in the three aforementioned sites and consisted of leaves and petioles, 500 g of fresh material, which were transported in a cooler, so that the bromatological analyzes could be carried out in the bromatology laboratory to determine dry matter (DM) and crude protein (CP), which are the most relevant parameters in forage species, in addition to forage production and acceptability in consumption by the animal species, using the technique proposed by the Association of Official Analytical Chemists (AOAC, 1990). Likewise, fiber fractions were analyzed: neutral detergent fiber (NDF), Acid detergent fiber (ADF), cellulose, hemicellulose and lignin, by the method of (Van Soest and Wine, 1967).

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Results and Discussion

Vegetative propagation

In the evaluation of vegetative propagation, it was observed that the best values of the variables evaluated were those from El Tuito. Likewise, the highest number of species with regrowths were for treatments 1 and 2 (Table 1), which after submitting them to a 4-month drought period, were the only treatments, which of 10 plants evaluated at least 4 of they managed to survive, not the T_3 ones (lengths of 33 to 40 cm), of which none of them survived, probably due to a greater demand for water. In this regard, in other studies, it is highlighted that this species can be propagated sexually (by seed) or by cuttings; recommending vegetative reproduction (Pérez *et al*., 2009). These authors and (Hartmann and Kester 1995, cited by Pérez *et al*., 2009) indicated that propagation by cuttings produces a more efficient rooting; if the cutting and planting conditions are optimal, thus allowing greater survival and favoring their ability to produce biomass. Ríos and Salazar (1995) achieved productions of 82, 57 and 42 tons per ha, at densities of 2600, 1800 and 760 plants / ha, with 110 days after sowing and with irrigation application. Also, Mauricio *et al*. (2017) highlighted reproduction by cuttings, recommending a length of 20 to 40 cm, buried vertically and at a shallow depth, the minimum and maximum length of the treatments suggested in this study.

Treatment	Diam. (mm)	Sprouts	L (cm)	Survival
AT_1	17.77	3.42	11.62	4
BT_1	19.57	4.83	10.64	9
AT_2	17.85	2.63	12.13	5
BT_2	18.82	4.14	9.76	6
AT_3	20.32	4.00	20.38	0
BT_3	19.28	3.83	8.77	0
T1 (Length: 20-26 cm); T2 (Length: 26 to 32 cm); T3 (Length: 33 to 40 cm)				

Table 1 Behavior of the shoots of the *Tithonia diversifolia* cuttings, from Las Agujas (A) and El Tuito (B), Jalisco.

It is a forage plant, with high levels of protein and high digestibility, contributing in an important way to animal nutrition, both for ruminants and monogastrics, it is also used in the supplementation of poultry feed, to take advantage of its carotene content and give color to egg yolk and chicken meat (Ríos, 1999). Therefore, this study presents its chemical composition that makes it attractive in animal production.

Chemical composition

Regarding its nutritional quality, it can be observed that it presents high levels of protein (18.42 to 31.54%), compared to legume species, which are characterized by their high protein content: *Tithonia diversifolia* has higher values than many tree species of this family and the results obtained in this study, for the materials from Cuauhtémoc, Colima and El Tuito are higher than those reported by other authors among them (Rosales, 1996; Navarro and Rodríguez, 1990 and Olivares, 1999), the latter, who in turn found high levels of calcium (2.3%) in this species, despite growing in acid soils. *Tithonia diversifolia*, presents high dry matter contents of 98.44%, except the material from Las Agujas, Zapopan that presents 21.96%.

The contents of the evaluated parameters are also higher than that reported by Gallego-Castro *et al*., (2017), for dry matter, who report values of 12.45 to 12.90% and of crude protein, values of 12.76 to 14.10%. It must be considered that these differences are probably due to soil conditions, environmental characteristics and part of the analyzed plant, since in this study the edible material consisted of leaves and petioles, in contrast to the aforementioned authors, who analyzed leaves and tender stems. Regarding the ash content, the values were similar in both studies (15.50 to 16.19%), with the exception of the material obtained in the municipality of Cuauhtémoc, Colima with a value of 18%. Ponce, (2019), reported DM contents of 90% and CP of 16.09% after 30 days of regrowth. The nutritional quality of arboreal or shrub species such as *Tithonia*, accumulate as much nitrogen as legumes, in addition to presenting high phosphorus contents.

For their part, Pérez *et al*. (2009), indicated that the nutritional quality depends on the phenological stage of the plant; which generally ranges from 14.8 to 28.5% after flowering and advanced growth; dry matter content 14.1 to 23.2% in advanced growth and after flowering and nitrogen-free extract from 1.91 to 2.4% for advanced growth and after flowering, respectively (Navarro and Rodríguez, 1990), lower values in all parameters, compared with those that occurred in each of the sites of the present study.

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Also the values reported by Téllez and Mendoza (2014), indicated crude protein contents of 19.5%, however, it should be noted that these authors evaluated the entire plant and although they do not indicate its phenological stage, the value was slightly higher in plants from Las Agujas, Zapopan, but inferior to those mentioned in Cuauhtémoc and El Tuito. Medina *et al*. (2009), who carried out a study in Trujillo, Venezuela evaluated morphostructural variables and biomass quality, in plants in the initial growth stage with protein contents of 21.3 to 23.7%; Values similar to this study for the Cuauhtémoc and Zapopan sites, but lower than those reported for plants from El Tuito (Table 2).

Regarding the content of fiber fractions, there were low values (30.81, 33.30 and 34.08%), with respect to (NDF), and (22.48, 25.74 and 31.69%), for (ADF), which indicates that it presents high digestibility of dry matter; that suggests a better animal behavior in its consumption (Table 2); These values are lower than those reported in Colombia in the Upper Tropics for NDF from 50.21 to 53.81 and ADF from 48.18 to 48.87% (Gallego-Castro, *et al*., 2017). On the other hand, Ponce (2019) indicated content of neutral detergent fiber (NDF) of 67.24% and acid detergent fiber (ADF) of 45.84%, high values where the digestibility of the dry matter would be from low to regular, limiting consumption from the animal. Téllez and Mendoza, (2014), reported NDF contents of 58.8% and for ADF of 42.2% with an *in vitro* digestibility of dry matter (DIVMS) of 57.6%. For their part, Medina *et al*. (2009), indicated similar values, to those presented in this study, for fiber fractions: with contents of 33.27% for (NDF) and 27.37% for (ADF) and a high DIVMS of 68.9 to 73.4%.

Determination	Cuauhtémoc, Colima	El Tuito, Jalisco	Zapopan, Jalisco
Dry matter	98.44	98.92	21.96
Crude protein	21.64	31.54	18.42
Ethereal Extract	3.12	2.73	2.60
Ashes	18.00	15.70	17.69
Fiber	27.07	26.30	10.31
NFE	28.61	22.65	50.98
NDF	30.81	34.08	33.30
ADF	22.48	25.74	31.69
Lignin	16.23	19.39	23.67
Cellulose	6.25	6.35	8.02
Hemicellulose	8.33	8.34	1.61
NFE= nitrogen-free extract			

Table 2 Chemical composition of leaves and petioles of *Tithonia diversifolia*, based on dry matter in (%)

Diversity of uses

Tithonia diversifolia has multiple uses in most countries of origin and where it has been introduced, including its use as green manure, due to its rapid growth, high capacity to fix nitrogen and accumulation of phosphorus, with beneficial effects on poor soils (Scrase *et al*., 2019). In Kenya it is used as a source of nitrogen, phosphorus and potassium in maize and rice crops (Jama *et al*., 2000), also for the control of termites (Adoyo *et al*., 1997).

Another alternative for use is as an insecticide since its insecticidal properties have been demonstrated to combat the leaf defoliator ant (Pantoja-Pulido *et al*., 2017). Due to the beauty of its yellow or orange flowers and its prolonged flowering, it is cultivated in several countries in Central America, South America, Asia and Africa, for ornamental purposes. Within the bibliographic review, we can cite very varied uses (Table 3), from ornamental, medicinal, forage and also due to its high content of pollen and nectar for the production of honey, contributing in an important way to the beekeeping industry. It is a soil improver due to its high nutrient content (nitrogen, phosphorus and potassium) and its rapid decomposition, which makes it available for other crops, improving the recycling of nutrients from these elements.

Use	Description	Country	Date
Control of gastrointestinal stronglypids	It was made in young cattle, during the rainy and un rainy season	Cuba	Lescano-Más, *et*al., 2016
Beekeeping profit	Producer of nectar and pollen	Mexico, Colombia Philippines	Roman *et*al., 2006; Rios, 1999; Cairns, 1997, cit. Rivers,1999
Forage	Feeding goats in cutting and hauling systems Ramoneo of sheep, feeding tilapias and incorporates into rations to feed hens.	Philippines Colombia Cuba	Cairns, 1997, cit. Rios, 1999 Galindo *et*al., 2017

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	Consumption of Holstein cows It feeds rabbits and pigs		
Attraction of beneficial insects	Attraction of pollinators and beneficial insects	Colombia	Rios, 1999
He is credited with Insecticide Activity	It is used by farmers for pest control	Africa	Pantoja-Pulido *et*al., 2017
Medicinal	In the treatment of eczema and skin lashing in pets. To decrease abortions and cannibalism in conejas	Guatemala Colombia Venezuela	Nash, 1976, cit. Rivers. 1999 Mahecha and Rosales, 2005
Living and windbreaker fences	Protection and conservation of water sources. Like a windbreaker curtain around the apiaries	Colombia	Rios, 1999
Green fertilizer and soil improver	Incorporation of biomass for its rapid decomposition on in rice and maize crops. In bean crops with a screening system, *T. diversifolia was found to*have high levels of N, P and K Used to recover grass-invaded soils	Kenya Costa Rica Philippines	Jama, 2000, Thor *et*al., 2002 George, *et*al., 2001 Rios, 1999

Table 3 Different uses of *Tithonia diversifolia*, reported in the literature

In many countries its main use is as forage and medicinal, for various ailments, so its two main uses are described in more detail:

Forage use

Tithonia diversifolia has been used as animal feed in several countries such as Cuba and Colombia, mainly in CIPAV and in the University of Sao Joao del Rei-Brazil (UFSJ), highlighting its nutritional quality that varies depending on the phenolic stage of the plant, its Forage production based on dry matter of 5.6 to 8.1 t / ha / year and on fresh basis was obtained from 24.7 to 41.3 t / ha / year (Mauricio *et al*. 2017). It is a species with good biomass production capacity and rapid recovery after cutting, which depends on the sowing density, soil characteristics and the vegetative state. Due to its high protein value, it is used in both ruminants and monogastrics; This species is used for cutting and hauling: for sheep, cattle, pigs, rabbits and buffalo; as well as in grazing together with grasses in the herbaceous stratum, in the food diet, it is generally used pre-dried or ground in the form of flour and feed (Pérez, *et al*., 2009).

In Colombia it is part of the intensive silvopastoral systems (SSPi), many of which have been carried out by CIPAV, together with forage grasses and some other tree species, including *Leucaena leucocephala* and *Guazuna ulmifolia*.

Mahecha *et al*., (2007), pointed out the advantages in the use of *Tithonia diversifolia* foliage as a forage supplement for dairy cows, with no significant difference between the use of concentrates and different inclusions of the foliage of this species of up to 35%. In forage banks with a density of 12,500 plants / ha, productions of 107.6 t / ha / per year of green forage and 24.6 t / ha / year of DM were obtained (Tellez and Mendoza, 2014).

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Medicinal use

Tithonia diversifolia is valued by many cultures, for its medicinal properties. It is a species used in traditional medicine, due to its multiple properties, due to the presence of secondary metabolites such as antimicrobial and anti-inflammatory (Sousa *et al*., 2019) to combat malaria (Afolayan *et al*., 2016), indicating that the extracted extracts with dichloromethane and methanol 1: 1 of *Tithonia diversifolia* and *Lawsonia inermis* were more effective against the *Plasmodium* parasite parasite than the aqueous extracts, used in traditional medicine; *T. diversifolia* has also been highlighted for its use against diabetes (Sari *et al*., 2018) and cancer (DiGiacomo *et al*., 2015).

Antioxidant properties are attributed to it; González-Sierra *et al*. (2019), indicated that the roots have a higher antioxidant capacity with 1.10 mg; followed by the leaves with 1.08 mg and finally the stems with 0.50 mg of ascorbic acid / mg of extract. They also reported phenol, flavonoid, coumarin, quinone and terpenoid content. It is important to point out that the concentration of these metabolites varies according to the phenological stage of the plant, the time of year, the characteristics of the soil, the region where the sample is obtained and environmental conditions of the area; noting that both the nutrient content in the soil, mainly Ca and P, as well as climatic conditions, seems to affect the presence of volatile constituents, mainly the content of sesquiterpenes in the leaf (Sampaio and Da Costa, 2018). *T. diversifolia* has shown variability in the content of secondary metabolites (Rivera *et al*., 2018), hence the difference in the results and the versatility of the plant, to adapt to different environments.

The oxidant activity can be associated with the content of phenols and flavonoids, which is explained by the redox properties of phenolic compounds (Gutiérrez-Sierra *et al*., 2019). The antioxidant activity, in general, is given by its ability to sequester free radicals, iron chelator, as well as the inhibition of oxidase enzymes. These metabolites are capable of avoiding or attenuating oxidative stress, due to reactive oxygen species (ROS), which prevents the oxidation of important biomolecules (proteins, nucleic acids, lipids and sugars).

This is associated with the appearance of diseases such as: cancer, Alzheimer's, aging, cataracts, diabetes, hypertension, cardiovascular diseases, among others (Valco *et al*., 2007; Sies, 2010; Dzialo *et al*., 2016). The extracts of the roots and leaves of *T. diversifolia* presented the highest concentrations of phenols and flavonoids (González-Sierra *et al*., 2019), (Table 4).

Disease Type	Part of the plant used	Countries	Bibliographic reference
Diabetes, malaria, snake bite, gastric ulcer, rubella and wounds	Leaves and roots	Costa Rica, Republic of the Congo, Kenya, Nigeria, Uganda, Mexico and Venezuela	Ajao and Moteetee, 2017 Afolayan et al., 2016
Bruises, abscesses	Stems and leaves	Venezuela	Frei, *et al*., 1998
Viper bite	Leaves as an antidote	Kenya	Owuor *et al*., 2005
Liver problems	The leaves in cooking	Colombia	Ríos, 1999
Malaria remedy		Guatemala	Nash, 1976, cit. Ríos, 1999
Hits	Macerated leaves like árnica	Cuba	Ríos, 1999
Spasms and cold	Cooking leaves	Colombia	Ríos, 1999
Malaria		Mexico and Nigeria	Heinrich, 2000; Ajaiyeoba *et al*., 2006
Dermatological problems, wounds	Toasted leaves	India	Heinrich, 2000; Frei, *et al*., 1998

Table 4 Use in traditional medicine of *Tithonia diversifolia* and parts used

The information collected in the herbarium specimens indicates a wide geographical distribution, as well as different habitats, where it occurs, including home gardens and coffee plantations, the altitudinal ranges include from those close to sea level to altitudes of 2000 m, generally in cloud forest and the main uses are as ornamental and medicinal, only in two states its use as forage is reported (Table 5).

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State	Common name	Habitat	Altitude	Use
Campeche	sunkak margarita	Acahual Smsp	20, 80	Ornamental
Chihuahua		Oak forest	1400	Ornamental
Colima	tacote, arctic	Ruderal	400, 500, 1100	Ornamental
Warrior	Margarita		1900	Ornamental
Jalisco	daisy, tacote, garnic, sunflower	HC, Sbc, Pine Forest, Smsp, Smsc and BMM	50,400, 1500, 1900	Ornamental
Oaxaca	Arnica	Oak Forest, Ruderal	2000	Medicinal
Tabasco	arctic, bitter, lion's hand and carolina	HC and Smsc	25	CV and Medicinal
Veracruz	aggregate, gigantic, bitter, maroon, maroon sunflower and tamchich	Sasp, Sbc, Pine Forest, Acahual, Cafetales, Oak Forest, Ruderal and BMM	110, 120, 152, 530, 1250, 1300, 1750, 2190	Medicinal in wounds, swelling, to cure rashes. The leaf and sap, Ornamental and Forage are used
Yucatan	Amargosa	Smsc and Smsp	10, 20	Ornamental, Medicinal and Fodder

Table 5 Types of habitat, altitude and uses in rural areas of Tihonia diversifolia

Conclusion

The results indicate that due to its high nutritional quality, its wide distribution and its diversity of uses, *T. diversifolia* is a viable option to be used in semi-intensive animal production systems, both in tropical and temperate zones.

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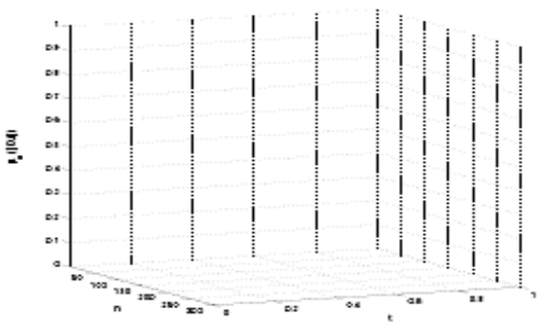

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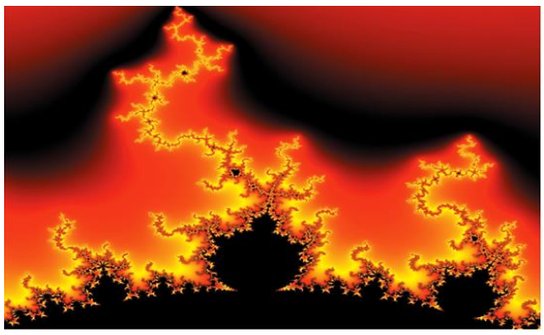

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