Biochemical and Functional Characterization of *Guazuma ulmifolia* Lam Biomass: A Novel Source of Fructose and Fiber for Sustainable Applications

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ABSTRACT

Objective: Quantification of phytochemical content of *Guazuma ulmifolia* Lam biomass from Veracruz, Mexico, with the aim of carrying out future biotechnological applications, such as animal feed formulations.

Design/methodology/approach: This work characterized the peel and seed of *G. ulmifolia* L. Fructose and glucose were identified as the primary components in the peel extract using high-performance liquid chromatography. HPLC also revealed traces of unknown components. In addition, combined glucose and fructose were quantified at different extraction times (up to 90 min) and in triplicate using refractometry as a rapid and complementary technique. The seed extract was analyzed using Fourier transform infrared spectroscopy, and the dry peel underwent bromatological evaluation to determine the analytical constituents.

Results: High-performance liquid chromatography results corroborated the presence of fructose and glucose after 90 min of extraction, with retention times of 2.1 and 2.4 minutes, respectively. HPLC analysis revealed a larger area under the chromatogram at a retention time of 2.1 minutes, indicating that fructose (76.2%) was the predominant sugar in the peel extract. The concentration of combined fructose and glucose in the extract obtained was 12.2 %wt at the beginning of the extraction and 36.727 %wt at the end of the extraction (90 minutes). The total soluble saccharide content (fructose and glucose) in the peel was 15.42 %wt. Bromatological analysis of the remaining biomass showed a crude fiber content of 35.4% and a crude protein content of 7.12%

Limitations on study/implications: The seasonal nature of the fruit *G. ulmifolia* L. posed a limitation for its conservation and storage.

Findings/conclusions: These findings suggest that *G. ulmifolia* L. biomass, particularly from dried fruit, is rich in carbohydrates and crude fiber, presenting a potential alternative for livestock fodder and its sugares may serve as an alternative feed for honey bees. Finally, and applying the adjusted mathematical model, the refractometry technique is proposed as a rapid alternative for the measurement of sugars in extracts of *G. ulmifolia* L., avoiding the use of robust techniques.

Keywords: *Guazuma ulmifolia* L., biomass, fructose, analytical constituents.

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INTRODUCTION

Recently, the veterinary industry has been looking for food alternatives through biomasses that the environment generates naturally in a cycle of circular economy and

reproduction [1-5], as well as obtaining natural products for human consumption that are free of preservatives and synthetic additives that harm animal and human health in the medium term [6-8]. There are different biomasses that have been used for different applications such as microalgae biomass, which are used to extract biofertilizers and biofuels [9-11]. Also, due to the problem of large accumulations of *Sargassum* spp. biomass in different parts of the world [12-13], its use has been studied to generate ecological filters for the treatment of contaminated water [14]. Additionally, it has been shown to be a natural source of alginates, cosmetics, biopolymers and bioplastics [15-18]. In another case, *Agave sisalana* biomass is being used to generate green plasticizers, fructans, and even biofuels [19-21]. Globally, we can find many more sources of biomass for the generation of products of daily use in humans and the veterinary industry that have recently become of commercial interest [22].

In Mexico there are several sources of organic biomass, including some that have been mentioned previously, such as agave, coconut, and the large quantities of sargassum that reach the coasts of the Yucatan peninsula. Another important source is the *G. ulmifolia* L. tree, which has been used as a source of fodder for cattle and goats mainly [24-25]. In addition, this tree has beneficial health properties, for example, it has been reported to reduce oxidative stress, contains anti-diabetes properties, and its fruit is rich in dietary fiber and phenolic compounds [26-30]. The dried fruit of the *G. ulmifolia* L. tree has been used in traditional medicine, treating some diseases such as diarrhea, elephantitis, control and treatment of hemorrhage and uterine pain [31]. Despite the above-mentioned properties, no attention has been given to expanding its use in animal food formulations at present. Although there is information of phytocomponents of *G. ulmifolia* L. from other countries [32], there is limited information on the phytochemical composition of the native species of Veracruz, Mexico. For this reason, a phytochemical characterization of the shell and the seed is shown in the present work.

In addition, the glucose and fructose content of fruits is a determinant key to the sweetness of these fruits. Accurate monitoring of these sugars allows a predictive evaluation of the quality of vegetable products. High-performance liquid chromatography (HPLC) coupled with a high-performance mass spectrometer is commonly employed to measure glucose and fructose in different fruit extracts of *G. ulmifolia* L. [33-34]. However, the availability of HPLC-MS equipment in Veracruz is not very high for performing daily monitoring. For this reason, refractometry measurement of sugars in aqueous extracts of the seed shell is included, as a rapid and cheap methodology for the determination of glucose and fructose.

MATERIALS AND METHODS

Collection, treatment and seed separation of *G. ulmifolia* L. fruit

G. ulmifolia L. dried fruit was collected from two different regions of Mexico, from the coast of the State of Chiapas and the Huasteca region of the State of Veracruz. Disinfection of the dried fruit was carried out by washing using neutral soap and a 10% sodium hypochlorite solution for 10 minutes. Drying was carried out for 36 hours at a temperature of 60 °C in a conventional oven (Memmert UN110). Subsequently, the seed was extracted from each fruit and stored in a Falcon tube at room temperature, as well as the fruit peel in sterile plastic containers for further processing.

Obtaining the peel and seed extracts

To obtain the peel extract, 100 g of the disinfected dried fruit peel was placed in a 1000 ml beaker. 500 ml of distilled water was added, and the heating process was started until it reached 60 °C, maintaining the temperature for 60 minutes. The resulting extract was brought to boiling point by maintaining the temperature for 90 minutes. During this boiling process, seven samples were extracted at different times (0, 20, 30, 40, 50, 70 and 90 minutes) and were stored in Eppendorf tubes at 4 °C. The extraction process was repeated three times for the same fruit peel to ensure complete extraction of soluble solids. The peel of the fruit was removed from the liquid solution and taken to a drying process for 36 hours at 60 °C for the determination of the total soluble solids of the sample by weight difference.

The seed extract was carried out in a 1:10 ratio (weight: volume) of seed and distilled water, whereby 10 g of seed was placed in 100 mL of distilled water in a beaker and heated for one hour at 60 °C until a viscous consistency was reached. The extracted mucilage was separated from the seed and stored in 250 mL glass containers and stored refrigerated at 4 °C.

Lyophilization of seed extract and FTIR characterization

The mucilage was freeze-dried using a 4.5 L benchtop freeze-drying system (LABCONCO FreeZone 4.5 Liter Benchtop Freeze Dry System). Prior to lyophilization, the mucilage was flash frozen (−81 °C) for 24 hours. Subsequently, the sample was subjected to the freeze-drying system at a temperature of −47 °C and a pressure of 0.25 ± 0.1 mbar for 72 hours. Finally, the lyophilized sample was placed in Eppendorf cups. 1 mg of freeze-dried sample was placed on the sample holder for analysis by FTIR. The spectroscopy system (Thermo Scientific Nicolet iS10 SMART iTR) was operated at a resolution of 8 cm^{-1} and acquisition range of 400-4000 cm^{-1}.

Characterization of the peel extract by HPLC (LC/MS spectra of sugars)

Both extracts from *G. ulmifolia* L. fruits and reactive grade standards of glucose and fructose were injected into the UHPLC (Dionex Ultimate 3000) equipped with a ACQUITY UPLC BEH Amide 1,7 micrometer column (2.1×100 mm). The system was operated with acetonitrile and ammonium hydroxide solution (0.1% NH_4OH) with a volume ratio of 75/25. The samples were diluted in an acetonitrile/water solution (1/1) before being injected.

Obtaining the calibration curve and characterization of the peel extract by refractometer

Six dilutions (w/w) were prepared with distilled water of pharmaceutical secondary standard (fructose, Sigma Aldrich, CAS 57-48-7) to obtain the calibration curve. Table 1 shows the dilution ratios for the different concentrations used. Refractometry measurements were carried out in triplicate using a Brix Digital 0-90% digital refractometer (Brixometer, Aosihui brand) using 80 μL of the different dilutions of fructose as shown in Table 1. Dispersion analysis of the raw data was performed using OriginLab v 8.0 software. Final concentration of soluble carbohydrates was calculated by using the Brix measurements and the total mass of the peel extract.

The extracts obtained from the fruit of *G. ulmifolia* L. at 0, 20, 30, 40, 50, 70 and 90 minutes of the process were evaluated by the refractometer and the carbohydrates concentration was calculated by applying the mathematical model adjusted from the calibration curve and the total volume of the peel extract (42 g).

Bromatological analysis of *G. ulmifolia* L. peel flour

For the bromatological analysis, the *G. ulmifolia* L. fruit peel was ground (Insely 1000A mill) to a flour consistency. 500 g of the flour were placed in a completely sealed plastic container and sent to the certified external laboratory (EuroNutec Laboratory). The parameters analyzed were: ash (AOAC method 942.05), nitrogen free extract (FAO manual difference), crude fiber (method 06AI-75-025), fat ethereal extract (method 06AI-75-048, official AOAC 2003.05), moisture (method 06AI-75-049, NMX-F-083-1986), crude protein by Dumas (method ISO_16634-1_2008).

RESULTS AND DISCUSSION

Characteristics of the fruit of *G. ulmifolia* L.

G. ulmifolia L. fruit has five cavities, where the small seeds are located. The fruit has an average length of 2.5 cm and an approximately 1 cm width. A fruit contains an average of 50 seeds with light brown shades and whose average dimensions are 0.5 cm long and 0.3 cm wide (Figure 1).

The peel presents a unique and very characteristic aroma of some type of floral honey or even sugar with some characteristic flavoring. The biochemical properties of the dried fruit (shell and seed) are of great importance for future research that will provide relevant information for the development of alternative biotechnological applications.

Characterization of the seed extract by FTIR spectroscopy

The FTIR spectrum presented in Figure 2 corresponds to the mucilage extracted from the seed of *G. ulmifolia* L. It shows a large and broad peak centered at 3292 cm^{-1} that is due

Table 1. Relationship of concentrations used for calibration curve generation. Abbreviations: F, Fructose; DW, Distilled Water; C, Concentration.

F (g)	DW (g)	C (%wt)
0.250	0.250	50
0.250	0.500	33.3
0.250	0.875	22.3
0.250	1.460	14.7
0.250	2.460	9.2
0.250	4.710	5

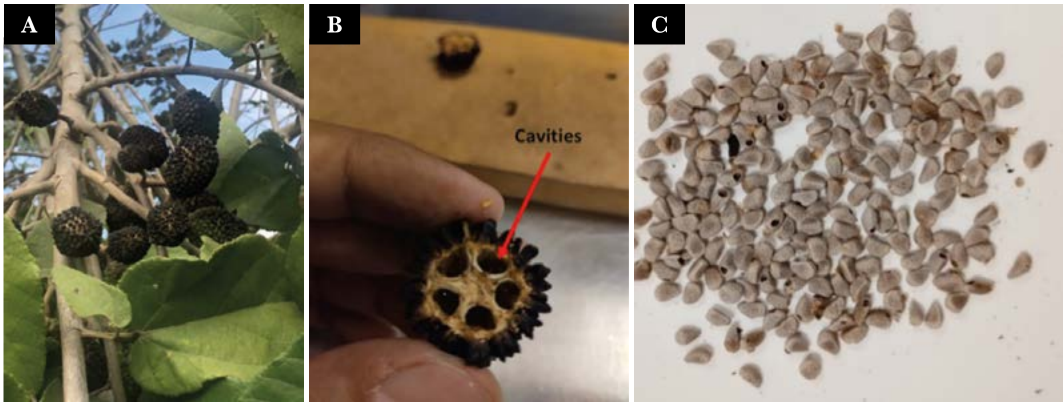

Figure 1. *Guazuma ulmifolia* L. fruit A) Representative image of *G. ulmifolia* L. tree with dried fruit. B) Cross section of the fruit where the five cavities of the fruit are observed. C) Seeds of *G. ulmifolia* L.

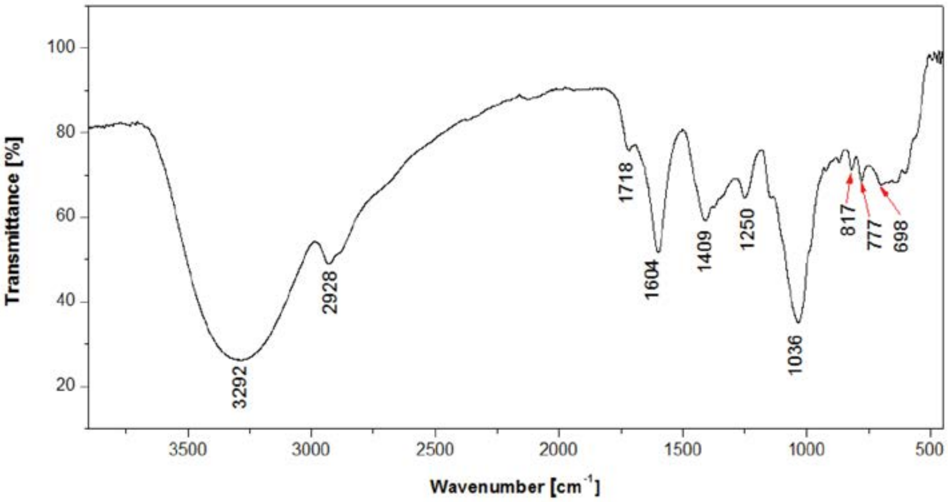

Figure 2. FTIR spectra of freeze-dried mucilage extracted from *G. ulmifolia* L. seed.

to stretching of the O-H bond. The peaks centered at 2928 and 1409 cm^{-1} were assigned to elongation and bending motions of the C-H bond, respectively. The signal located at 1250 cm^{-1} was associated with C-O-C bond elongation from an ester group, while the neighboring peak located at 1036 cm^{-1} was associated with elongation of C-O bonds of alcohols. The vibrations described so far strongly suggest the presence of polysaccharides in high concentration. Additionally, the vibrations located at 1718 and 1604 cm^{-1} were associated with bond stretching. C=O and C=C, respectively. Ramírez and collaborators reported a high concentration of polysaccharides and fiber in the seed extract of *G. ulmifolia* L. [35], considering these results, we could associate the vibrations to aromatic rings and ketone groups present in the lignin.

Characterization of peel extract by HPLC (LC/MS spectra of sugars)

Glucose and fructose standards exhibited molecular ions at m/z 179, indicating the presence of the ions [glucose−H]− and [fructose−H]− (Figure 3). [36]

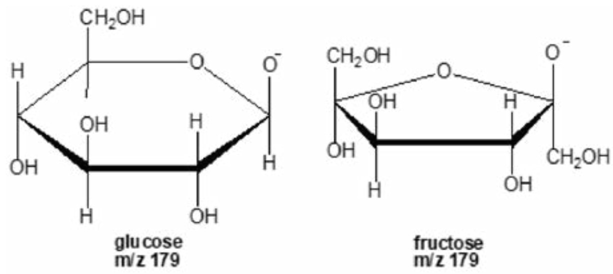

Figure 3. Anions [glucose−H] − and [fructose−H]− ions.

Figure 4A shows chromatogram of the fructose standard and chromatogram of the glucose standard (Figure 4B), with retention times of 2.1 min and 2.4 min, respectively. Chromatogram C, corresponding to the *G. ulmifolia* L. fruit extract, displays two clear peaks with retention times of 2.1 min and 2.4 min, with m/z values of 179, corresponding to fructose and glucose, respectively (see supplementary information). Instinctively, the area under the curve of the chromatogram indicates that the fructose content in the extract of *G. ulmifolia* L. is the highest. Interestingly, the chromatogram of *G. umifolia* L. fruit extract presented two additional peaks centered at 0.8 and 4 min. The small area of these peaks indicates that their concentration was very small: therefore, almost all the soluble carbohydrates consisted of fructose and glucose (76.2 and 23.8 %wt, respectively) in the present study.

Characterization of the extract (glucose plus fructose) of the peel by refractometry

The Brix degrees allowed to measure the total number of soluble solids present in the *G. ulmifolia* L. fruit extract. Considering that HPLC analysis showed that almost all the weight of soluble solids in the extract consisted of fructose and glucose (76.2 and 23.8%), in

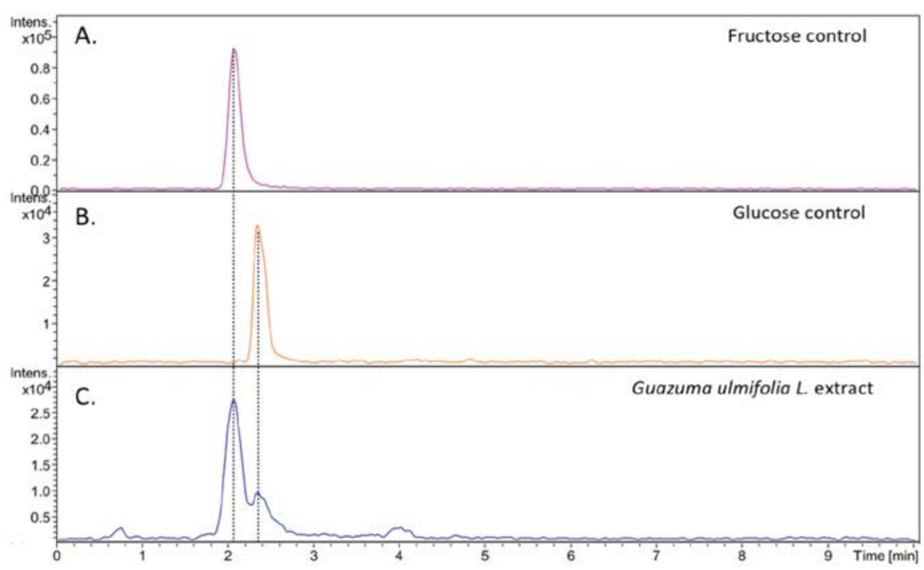

Figure 4. Qualitative analysis of chromatograms. A. Chromatogram of fructose control, B. Chromatogram of glucose control, C. *G. ulmifolia* L. extract chromatogram.

the present section it is evaluated the refractometry as a quick technique to determine the total carbohydrates (glucose and fructose) by using fructose as calibration sugar. This sugar content must be quickly determined for a potential large scale application, for instance, in the area of beekeeping, and food industry. The calibration curve was obtained by using a digital refractometer with the different standard fructose concentrations as defined in Table 1. For each concentration Brix degrees were determined, obtaining a calibration curve with a linear behavior with an $R^2=0.967$ (Figure 5A).

The following equation (equation 1) determines the concentration (C) of fructose, with BG being the Brix degrees for each concentration evaluated.

$$C = 1.550(BG) - 2.023 \tag{1}$$

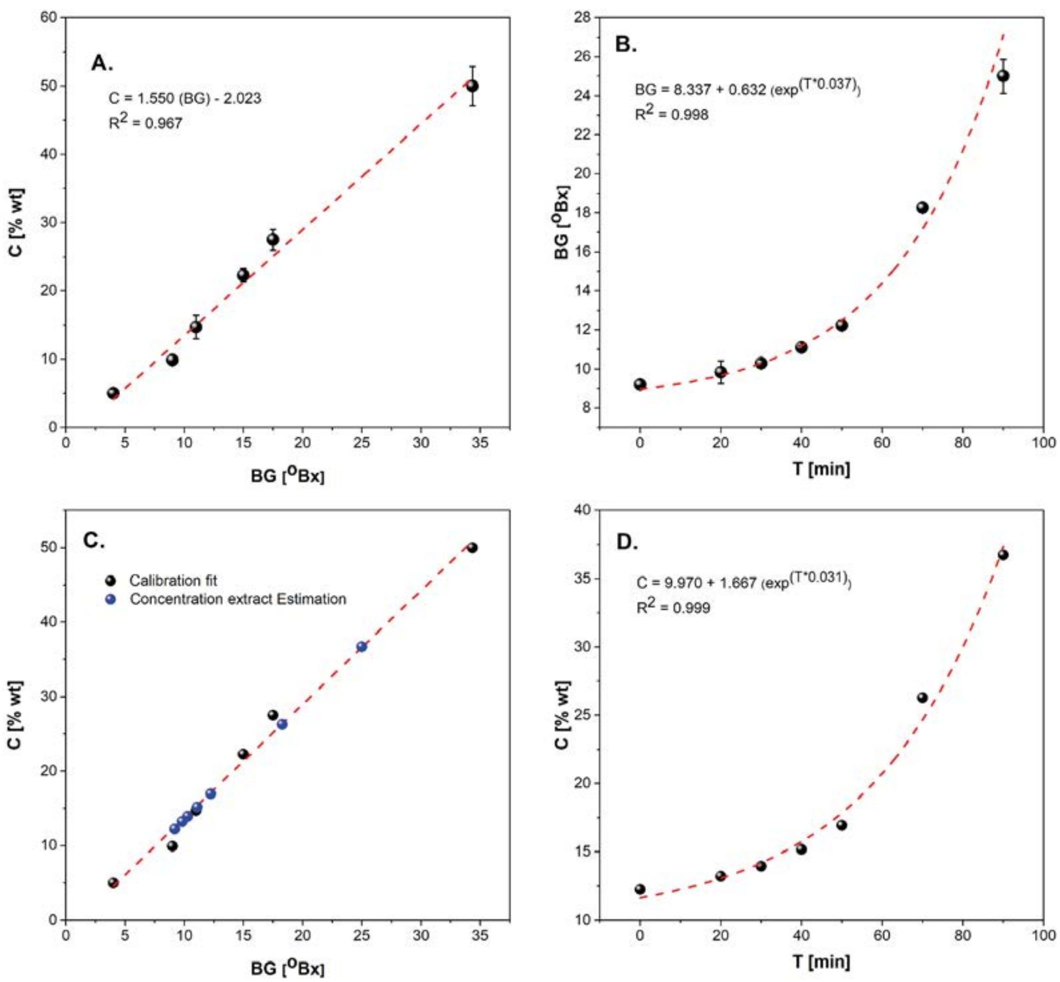

Figure 5. Brix measurement and determination of fructose concentration. A. Calibration curve with an exponential regression obtained from the standard fructose standard, B. Exponential behavior curve during the extraction process. Exposure of *G. ulmifolia* L. extract at different times after its boiling point, C. Concentration obtained from each sample of *G. ulmifolia* L. extract, compared with the calibration curve obtained from the standard, D. Estimation of fructose and glucose concentration during the extraction process as a function of time.

On the other hand, the *G. ulmifolia* L. fruit extract was heated until it reached boiling point. Afterwards, samples were obtained at 0, 20, 30, 40, 50, 70, and 90 minutes to evaluate the degrees °Bx. The behavior of the BG during the extraction process as a function of time (T) reflected an exponential increase with an R^2=0.998 (equation 2, Figure 5B)

$$BG = 8.337 + 0.632\left(\exp^{T \times 0.037}\right) \tag{2}$$

Figure 5C shows the comparison between the concentrations used for the calibration curve and the results of fructose concentration (C) obtained at different times of the extraction process. These results are summarized in Table 2. Using equations (1) and (2) it was possible to estimate the behavior of fructose and glucose concentration as a function of time during the extraction process (Figure 5D) with an R^2=0.999. The concentration increases exponentially due to the amount of water that evaporates during the extraction process. Applying the calibration function obtained in Figure 5A (equation 2), a determination of the fructose plus fructose concentration was made from the Brix degrees obtained in each sample obtained from the extract at different times. According to the time range (T) exposed the extract to boiling point, the glucose plus fructose concentrations for the *G. ulmifolia* L. extract ranged from 12.23 %wt (T=0 min, BG=9.200) to reach a viscous solution of 36.727 %wt (T=90 min, BG=25).

The total concentration of soluble saccharides in the *G. ulmifolia* L. peer calculated from the total volume of extract and the glucose plus fructose concentration is 15.42% wt. If the results from HPLC are considered, a 11.75 %wt of fructose and 3.7%wt of glucose are the total mass concentration in the peel of the fruit. Pereira *et al.,* reported a total concentration of carbohydrates of mono and disaccharides of 25.94 % wt for *G. ulmifolia* L. pulp and seed extract [30]. The source of the extract could explain the difference between these results, as the peel seed was used in the present study.

Although the calibration curve was obtained with fructose, the present results showed that refractometry is not selective for this sugar and can be used for a quick and cheap determination of combined glucose and fructose concentration in *G. ulmifolia* L. aqueous

Table 2. Fructose concentrations used to obtain the calibration curve and determination of fructose plus fructose concentration during different times in the extraction process. Abbreviations: C, Concentration; BG, Degrees Brix; T, Time.

Calibration curve			***G. ulmifolia* L. extract**			
C (%wt)	**BG (°Brix)**	σ	**T**	**C (%wt)**	**BG (°Brix)**	σ
50	35.333	0.012	0	12.23	9.200	0.264
33.3	17.000	0.014	20	13.22	9.833	0.577
22.3	15.000	0.035	30	13.94	10.300	0.173
14.7	11.000	0.020	40	15.182	11.100	0.100
9.2	9.000	0.030	50	16.94	12.233	0.230
5	4.000	0.057	70	26.29	18.267	0.251
			90	36.727	25.000	0.866

extracts. However, validation measurements with other techniques must be performed in future work.

Bromatology analysis of *G. ulmifolia* L. peel flour

The use of the total biomass of the fruit can be an alternative natural source of complementary fiber for cattle, pigs, horses, among others. For this, the bromatological analysis of *G. ulmifolia* L. meal is essential for the determination of analytical constituents (ash, nitrogen free extract, crude fiber, fat, ethereal extract, moisture and crude protein) that will allow to generate a balance in the formulations that integrate the *G. ulmifolia* L. meal. The samples were analyzed by an external laboratory authorized by SENASICA (Servicio Nacional de Sanidad, Inocuidad y Calidad Agroalimentaria), which is a regulatory body that promotes the application and certification of food contamination risk reduction systems and their agri-food quality, in order to facilitate application and commercialization. Table 3 shows the bromatology results and the method applied.

In the resulting analytical constituents shown in Table 3, it can be observed that it has a high content of nitrogen-free extract (47.81%), which are soluble carbohydrates (starches, sugars, organic acids, pectins and mucilages) and can be easily digested. Another parameter with high content was crude fiber (35.4%), which is non-nitrogenous organic substances. Crude protein or crude protein (7.12%) is normally one of the constituents of special importance in the veterinary industry, since it has a significant impact mainly in the growth and production stages. Finally, in a small fraction of the biomass is found the ethereal extract fat (2.72%).

Table 3. Analytical constituents determined from the peel of *G. ulmifolia* L. in the form of flour through a bromatological analysis.

Constituent	Method	Results (%)
Ash	AOAC 942.05	2.76
Nitrogen free extract	FAO manual difference	47.81
Crude fiber	06AI-75-025	35.40
Fat ethereal extract	06AI-75-048, AOAC 2003.05	2.72
Moisture	06AI-75-049, NMX-F-083-1986	4.19
Crude protein	ISO_16634-1_2008	7.12

CONCLUSIONS

The extract from the peel of *G. ulmifolia* L. reveals the presence of both fructose and glucose, with fructose being more abundant (almost 3 times than glucose). This was confirmed through High-Performance Liquid Chromatography (HPLC) coupled with high-resolution mass spectrometry, which provided accurate monitoring and identification of the sugars based on their retention times and molecular ions. The concentration of total soluble saccharides (fructose and glucose) in the peel extract was determined using a digital refractometer. The saccharides concentration is a function of extraction time. The maximum saccharide concentration (36.727) was achieved at longer extraction time

(90 min). The total content of soluble glucose and fructose in the peel was 15.42 %wt. The freeze-dried seed extract was analyzed by FTIR spectroscopy, where the presence of vibrations corresponding to polysaccharide and lignin functional groups were verified. Finally, the biomass of *G. ulmifolia* L. has a high content of nitrogen free extract (47.8%), crude fiber (35.40%) and crude protein (7.12%), which makes it a source of biomass that can be balanced to integrated rations for animals.

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