REVISTA BIO CIENCIAS
http://revistabiociencias.uan.edu.mx
https://doi.org/10.15741/revbio.12.e1772
ISSN 2007-3380

Original article / Artículo original

Aqueous extract of agave pulquero (*Agave salmiana*) leaves as a source of dietetic fiber and compounds with antioxidant capacity

Extracto acuoso de pencas de maguey pulquero (*Agave salmiana*) como fuente de fibra dietética y compuestos con capacidad antioxidante

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Please cite this article as/Como citar este artículo: Ayala Niño, A., Pavón Silva, T.B., Zarazúa Aguilar, Y., Sánchez Franco, J.A. (2025). Aqueous extract of agave pulquero (Agave salmiana) leaves as a source of dietetic fiber and compounds with antioxidant capacity. *Revista Bio Ciencias*, 12, e1772. https://doi.org/10.15741/revbio.12.e1772

Article Info/Información del artículo
Received/Recibido: October 01th 2024.
Accepted/Aceptado: February 24th 2025.
Available on line/Publicado: March 12th 2025.

ABSTRACT

Agave salmiana is a Mexican endemic maguey, primarily used to produce pulque, a fermented beverage derived from its sap (aguamiel). When the sap production ceases, the maguey leaves (pencas) are mainly used as forage. This study aimed to prepare and evaluate aqueous extracts from maguey leaves. The extracts were obtained using water at 70 °C and three leaf conditions: un-scraped (US), partially scraped (PS), and fully scraped (FS). The extracts were analyzed for phenolic compounds, ascorbic acid, antioxidant capacity (DPPH), and chelating activity during preparation and up to eight months of storage. The phenolic compound content (2913.33 ± 277.9 mg GAE/L), ascorbic acid content (758.02 ± 56.57 mg AA/L), and antioxidant capacity (930.7 ± 44.09 µmol TE/L) increased during extract preparation. Dietary fiber content ranged between 12.6 – 16.4 % in the extracts. Additionally, phenolic compounds (kaempferol and quercetin) were identified in all three extracts using HPLC UV-VIS. By the eighth month of storage, phenolic compounds and antioxidant capacity increased in the PS and FS extracts. These findings suggest that *A. salmiana* maguey leaves can be used to produce aqueous extracts rich in bioactive compounds with antioxidant capacity and dietary fiber, which are beneficial in treating and preventing diseases.

KEY WORDS: Bioactive compounds, Shelf life, Chelating activity, Ascorbic acid, Phenolic compounds, Functional food, Antioxidant capacity, Flavonoid.

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ISSN 2007-3380

RESUMEN

El *Agave salmiana* es un maguey endémico mexicano, cuyo principal producto es el pulque, fermento de su aguamiel. Cuando cesa la producción de aguamiel, las pencas del maguey son utilizadas principalmente como forraje, por lo que el objetivo de este estudio fue elaborar y evaluar extractos acuosos de pencas de maguey. Para su obtención se utilizó agua a 70 °C y tres estadios de pencas, sin raspar (US), siendo raspado (PS) y completamente raspado (FS). A los extractos se les cuantificó el contenido de compuestos fenólicos, ácido ascórbico, capacidad antioxidante (DPPH) y actividad quelante, durante su elaboración y hasta ocho meses de almacenamiento. El contenido de compuestos fenólicos (2913.33 ± 277.9 mg GAE/L), ácido ascórbico (758.02 ± 56.57 mg AA/L) y la capacidad antioxidante (930.7 ± 44.09 µmol TE/L) aumentaron durante la elaboración de los extractos. Se cuantificó un 12.6 - 16.4 % de fibra dietética en los extractos. Además, se identificaron compuestos fenólicos por HPLC UV-VIS (kaempferol y quercetina) en los tres extractos elaborados. Al octavo mes de almacenamiento, se observó un aumento de los compuestos fenólicos y su capacidad antioxidante en los extractos PS y FS. Con estos resultados se concluyó que las pencas de maguey *A. salmiana* pueden ser empleadas para la obtención de extractos acuosos ricos en compuestos bioactivos con capacidad antioxidante y fibra dietética, sustancias implicadas en el tratamiento y prevención de enfermedades.

PALABRAS CLAVE: Compuestos bioactivos, Vida de anaquel, Actividad quelante, Ácido ascórbico, Compuestos fenólicos, Alimento funcional, Capacidad antioxidante, Flavonoide.

Introduction

The agave or pulquero maguey plant (*Agave salmiana*) is considered a succulent widely distributed worldwide, the *Agave* genus encompasses approximately 210 species, of which 159 are endemic to Mexico, among these, 40 are used for alcoholic beverage production such as pulque, a pre-Hispanic beverage of great cultural significance (García-Mendoza *et al.,* 2019).

Pulquero maguey is primarily cultivated in the central region of Mexico, mainly in the State of Mexico and Hidalgo, where different parts of the plant are used for several purposes (Lara-Avila *et al.,* 2017). The heart or “piña” of the maguey serves a primary role in the production and concentration of aguamiel, a fermented substance that gives rise to pulque. This whitish, viscous, slightly acidic alcoholic beverage holds nutritional significance due to its high content of substrates and microorganisms identified as prebiotics and probiotics (Escalante *et al.,* 2016), which, when consumed, confer beneficial health effects (Cunningham *et al.,* 2021).

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When aguamiel is concentrated, it produces maguey syrup, which has gained popularity due to its association as a healthy product positioned as a functional food for diabetic people (González-Montemayor *et al.,* 2019). The aguamiel extraction involves a “scraping” technique performed on mature maguey plants, a process that takes over five years for the plant to reach maturity (Valdivieso-Solís *et al.,* 2021). It has been reported that during the scraping process and the maturation of the maguey, there is an impact on the concentration of saponins, soluble fiber, and carbohydrates (Pinos-Rodríguez *et al.,* 2008; Puente-Garza *et al.,* 2017[a]), these substances are of interest due to their positive effects on certain pathologies. Maguey leaves are commonly used in culinary dish preparation; however, they are not always fully utilized, leading to waste. Maguey leaves account for up to 50 % of the plant’s mass, and in many cases, they are discarded at the harvesting site, this practice can pose an environmental hazard, as the leaves can serve as reservoirs for the proliferation of pathogenic microorganisms, as well as insects and rodents (Velázquez-De Lucio *et al.,* 2024).

Conversely, evidence suggests that maguey leaves are a rich source of bioactive compounds with antioxidant capacity, dietary fiber, phenols, and flavonoids, substances beneficial to health (Medina-Mendoza *et al.,* 2022).

Dietary fiber is a crucial component of the diet for human health, however, it is recognized that fiber consumption has recently declined due to the extensive incorporation of ultra-processed foods and diets such as the Western diet. This trend has led to an increase in non-communicable diseases, particularly among individuals with lower intake of soluble and insoluble fiber. This situation can be addressed through dietary modifications and appropriate lifestyle changes (Zhi-Wei *et al.,* 2021).

Therefore, the present study aimed to obtain an aqueous extract from maguey leaves (*A. salmiana*) as a source of dietary fiber and antioxidant compounds for the development of a functional food. Moreover, this research represents the first study utilizing an extract from maguey leaves, a plant native to central Mexico with significant cultural and practical relevance.

Materials and Methods

Raw material

Maguey leaves were collected in the Otumba de Gómez Farias municipality, State of Mexico, Mexico, during February and March 2023. The leaves were harvested from the same cultivation area and classified into three different scraping stages of the maguey with aguamiel production, considering that each maguey plant produces aguamiel for approximately six months: (1) US, (2) PS, and (3) FS. All maguey plants were of similar age (roughly 12 years). Once harvested, the leaves were stored in refrigeration at 4°C until use.

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Maguey leaves processing

The maguey leaves were cleaned, peeled, and cut into small pieces for blending. Subsequently, they were heated in water at 70 °C for 15 minutes to extract compounds. Insoluble fiber was manually removed and pressed to extract the majority of solids from the extract. The obtained liquid was concentrated at a constant temperature of 45 °C using direct heating on an industrial stove (Industrial Stove with Oven, Coriat©, Mexico) until reaching a concentration of 35 ± 1 °Brix (Portable Refractometer FG-113 Brix/ATC 0~32 %, The Scientific©, U.S.A.). Aliquots were collected every 15 minutes (up to 45 minutes) for subsequent analysis, ensuring that all extracts had the same total soluble solids concentration. The final samples were frozen and lyophilized (Labconco© FreeZone6 Lyophilizer, Kansas, USA) for four days at a constant temperature of -55 ± 1°C and a constant vacuum pressure of 0.140 mbar. Finally, they were stored under refrigeration for eight months. Analyses were conducted every two months during storage. For all determinations, the lyophilized extracts were rehydrated to 65 %.

Proximal composition determination

Moisture content was determined using the oven-drying method (Official AOAC Method 920.151, 2006). For this purpose, crucibles were tared, and a five-gram aliquot of the sample was weighed into them, samples were then placed in a drying oven at 115 °C for three hours until a constant weight was achieved. Finally, the crucibles with the samples were weighed, and the difference was calculated to determine the percentage of water content (Electric Drying Oven, Scorpion Scientific©, Mexico).

Protein content was evaluated using the Kjeldahl method (Official AOAC Method 920.152, 2006) with an automatic Kjeldahl system (Kjeldatherm© Digestion System, Germany). This method determined the nitrogen concentration in the sample, which was multiplied by the conversion factor (6.25) to calculate the protein percentage. The method involved digesting one gram of the sample with 15 mL of concentrated sulfuric acid, one Kjeltabs CX catalyst tablet, and 2 mL of deionized water. During digestion, ammonium sulfate was formed, which, in the presence of an excess of 32 % sodium hydroxide, released ammonia. The released ammonia was distilled and captured in 3 % boric acid, forming ammonium borate, which was then titrated with 0.98 N hydrochloric acid.

Gravimetric incineration method (Official AOAC Method 940.26, 2006) was used to determine the ash content, employing a muffle furnace (Muffle Furnace© 1500 Model FDI535M Thermolyne, U.S.A.). Crucibles were tared, and five grams of the samples were weighed. The samples were dehydrated and then subjected to direct flame calcination using a Bunsen burner under an extraction hood. After calcination, the samples were placed in the muffle furnace (550 °C) for five hours for incineration. Subsequently, the samples were cooled in a desiccator for 15 minutes, and the residue was weighed. The ash content was reported as a percentage (AOAC, 2006).

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Total dietary fiber determination

Total dietary fiber was determined using the enzymatic-gravimetric procedure with the SIGMA dietary fiber equipment (Total Dietary Fiber Kit ©, SIGMA TDF100).

Aliquots were prepared in quadruplicate and subjected to an enzymatic digestion process, which involved the degradation of proteins (protease) and starch (α-amylase and amyloglucosidase). Subsequently, a precipitation process was performed using 78 % ethanol, followed by 95 % ethanol, and finally 99.7 % acetone, using Gooch crucibles (F 40-60 microns). Protein and ash content in the residue were determined as previously described.

The calculation of dietary fiber content was performed using Equation 1.

Equation 1: $$\%TDF = \frac{(\text{Residual mass - Protein mass - Ash mass - Blank})}{Sample\ mass} * 100$$

Reducing Sugars

Reducing sugars were determined following the method reported by Bouaziz *et al.* (2014), which employs 3,5-dinitrosalicylic acid (DNS) to oxidize reducing sugars (RS) while undergoing simultaneous reduction.

The method began with the preparation of the DNS solution. For both, the standard curve and the study samples, 30 µL of the sample and 180 µL of DNS were mixed and incubated in boiling water for 15 minutes. After incubation, 1,230 µL of deionized water was added to cool the sample, and absorbance was measured at 540 nm.

A standard curve was prepared using a stock glucose solution (10 mg/mL), from which the following concentrations were obtained: 0, 1, 2, 3, 4, and 5 mg/mL. For each concentration, 30 µL was treated using the same method. Absorbance was measured at 540 nm using a microplate reader (Biotek Power Wave© XS, U.S.A.), and results were reported as milligrams of reducing sugars per liter (mg RS/L) with the equation ($y = 0.1846x - 0.0022$, $R^2 = 0.9996$).

Sample preparation for extraction analysis

One gram of lyophilized maguey leaf extract was combined with 10 mL of methanol-water (80:20; v/v) and mixed using a vortex to ensure homogeneity. The mixture was then centrifuged (Beckman Coulter Centrifuge©, AllegraTM 25R, California) at 3,000 rpm for 5 minutes at 4°C. The supernatant was collected and concentrated using a rotary evaporator (Rotavapor Büchi Labortechnik©, AG CH-9230, Switzerland). The concentrate was subsequently resuspended with 10 mL of methanol-water (50:50; v/v) and stored at −20°C for further analysis (Puente-Garza *et al.,* 2017[b]). All three extracts were prepared in the same manner for each sample.

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Determination of phenolic compounds

The content of phenolic compounds was quantified using the Folin-Ciocalteu method as described by Georgé *et al.* (2005). A standard curve of gallic acid was used to compare the results of the treated samples.

Samples were diluted 1:50 with deionized water. Additionally, a standard curve for phenolic compound concentration was prepared using a solution of 400 mg of gallic acid per liter of deionized water. From this stock solution, the following concentrations were prepared: 0, 100, 200, and 300 mg/L. For the curve, 100 µL of each concentration was used.

The technique for both, the standard curve and the study samples, involved adding 100 µL of Folin-Ciocalteu reagent (diluted 1:10 in deionized water) and 400 µL of 7.5 % sodium carbonate solution. Samples were vortexed and left at room temperature for 30 min. Absorbance was measured at 764 nm using a microplate reader (Biotek Power Wave© XS, U.S.A.). Results were expressed as milligrams of gallic acid per liter (mg GA/L) ($y = 0.005x + 0.032$, $R^2 = 0.998$).

Quantification of ascorbic acid

The colorimetric method for ascorbic acid described by Dürüst *et al.* (1997) was employed. This method uses 2,6-dichlorophenolindophenol (DCPI) as a reagent for both, the samples and the standard reference curve, of ascorbic acid.

Firstly, the sample was diluted 1:100 in deionized water, and the following solutions were prepared: DCPI, 1 M acetate buffer at pH 6, and an ascorbic acid solution. A standard curve was constructed using the ascorbic acid solution at concentrations of 0, 10, 20, 30, 40, and 50 mg/L. For the curve, 100 µL of each concentration was used.

The procedure for both, the standard curve and the study samples, involved mixing 100 µL of the diluted sample, 100 µL of acetate buffer, and 800 µL of DCPI. A 0.4 % oxalic acid solution was used as the blank.

Absorbance was measured at 520 nm using a microplate reader (Biotek Power Wave© XS, U.S.A.), and results were expressed as milligrams of ascorbic acid per liter (mg AA/L) ($y = -0.0027x + 0.1498$, $R^2 = 0.9995$).

Antioxidant capacity

The antioxidant capacity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH, stable free radical) method as described by Morales & Jiménez-Pérez (2001). This was compared to a standard Trolox curve (a substance capable of scavenging free radicals).

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Samples were diluted 1:9 and a 0.07 % DPPH solution in methanol was prepared. A standard curve of Trolox, which is the reference molecule, was obtained using the following concentrations in ethanol: 0, 50,100, 200 and 300 µmol Trolox/L

For the technique applied to both, the curve and the study sample, 100 µL of the diluted sample and 500 µL of the DPPH solution were mixed, vortexed, and left at room temperature for 60 minutes. Afterward, the mixture was centrifuged at 10,000 rpm for five minutes at 4 °C, and the absorbance of the supernatant was measured at 517 nm (Biotek Power Wave© XS microplate reader, USA). Results were expressed as µmol Trolox equivalents per liter (µmol TE/L) ($y = -0.0012x + 0.9615$, $R^2 = 0.9922$).

Chelating activity determination

Chelating activity was determined using the method described by Gulcin *et al.* (2003), which evaluates the chelating activity of ethylenediaminetetraacetic acid (EDTA) as a reference molecule for ferrous ion (Fe^{2+}).

The procedure involved adding 100 µL of the sample to amber Eppendorf vials, followed by 50 µL of ferric chloride solution and 450 µL of methanol. Mixture was vortexed and left to rest for five minutes at room temperature. Subsequently, 400 µL of ferrozine solution was added, and the samples were left to rest at room temperature for another ten minutes. After this period, the absorbance was measured. EDTA at 0.1 M was used as the reference chelating agent, and deionized water under the same conditions served as the control sample.

Absorbance was read at 562 nm (Biotek Power Wave© XS microplate reader, USA), and the results were reported as a percentage of chelating activity calculated using Equation 2.

Equation 2. $$\%CA = \frac{AC - AS}{AC} * 100$$

Where:

% CA= Percentage of chelating activity.

AC = Absorbance of the control at 562 nm.

AS = Absorbance of the sample at 562 nm.

Phenolic compound identification

Phenolic compounds were identified using an HPLC UV/VIS system (Agilent©, 1100 Series, Waldbronn, Germany) for the three maguey leaf extracts and their comparison. The procedure was carried out following Puente-Garza *et al.* (2017[b]). A Zorbax Eclipse© XDB-C18 column (4.6

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x 150 mm, five µm) was used. The mobile phases consisted of: Phase A (water with 0.1% formic acid) and Phase B (acetonitrile with 0.1 % formic acid) at a flow rate of 0.8 mL/min.

The gradient was configured and executed as follows: 82 % of Phase A was maintained during the first 15 minutes and then reduced to 25 % over ten minutes, held for five minutes before being reduced to 0 % over ten minutes, allowing the incorporation of 100 % Phase B for an additional ten minutes. Data were collected at 340 nm to quantify flavonoid content using kaempferol and quercetin reference standards (Sigma-Aldrich, St. Louis, MO).

The results were compared with reference standards treated under the same conditions according to the analysis method. Chromatograms were obtained and analyzed using HP Agilent© ChemStation software (California, USA). All solvents used were HPLC-grade.

Microbiological analysis

Aerobic mesophilic bacteria count was performed on Standard Methods Agar, incubated at 30°C (Precision Innovents© Incubator, Thelco Laboratory, USA) for 48 hours (Richardson, 1985). *Enterobacteriaceae* count was carried out on Violet Red Bile Glucose (VRBG) agar, incubated at 37°C (Felisa© Incubator, Mod. F3133D, Mexico) for 24 hours (Westhoff, 1981). Samples were analyzed at 0, 2, 4, 6, and 8 months of refrigerated storage.

The results were reported in Colony Forming Units per milliliter (CFU/mL).

Experimental design

For the extraction experimentation of bioactive compounds, a 4x4 experimental design with three repetitions was used, which was assigned for the determination of antioxidant capacity: phenolic compounds, ascorbic acid, DPPH, and chelating activity (4) by the extraction time of compounds (4) (at 0, 15, 30, and 45 min).

For the analysis of bioactive compound content and their bioactivity during storage, a 5x3 design with three replicates was used. The evaluation included phenolic compound content, ascorbic acid, DPPH, chelating activity, and reducing sugars (5) across *A. salmiana* maguey leaf extracts (3) (US, PS, and FS).

Statistical analysis

All determinations were performed in triplicate. Phenolic compounds, ascorbic acid, reducing sugars, antioxidant capacity, chelating activity, and microbiological activity were quantified during refrigerated storage over eight months. The data obtained were processed using one-way analysis of variance (ANOVA) and Tukey's pairwise comparisons with a 5 % significance level for the means. Statistical analysis was conducted by comparing results within the same treatment and between treatments. All analyses were performed using SPSS software (IBM SPSS © Statistics, version 25, 2022).

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Results and discussion

Phenolic compounds, ascorbic acid, antioxidant capacity (via the DPPH method), and chelating activity were quantified during the aqueous extraction process of maguey leaf (*A. salmiana*). Throughout the process, the content of phenolic compounds (Figure 1: A) and ascorbic acid (Figure 1: B) demonstrated higher concentrations at the end of the extraction treatment. This increased antioxidant capacity as measured by the DPPH method (Figure 1: C). In contrast, chelating activity did not show significant changes from the beginning to the end of the treatment (Figure 1: D). This behavior aligns with multiple studies where antioxidant capacity has exhibited a concentration-dependent activity profile, reflecting increased values due to the accumulation of compounds with antioxidant potential (Manssouri *et al.,* 2020).

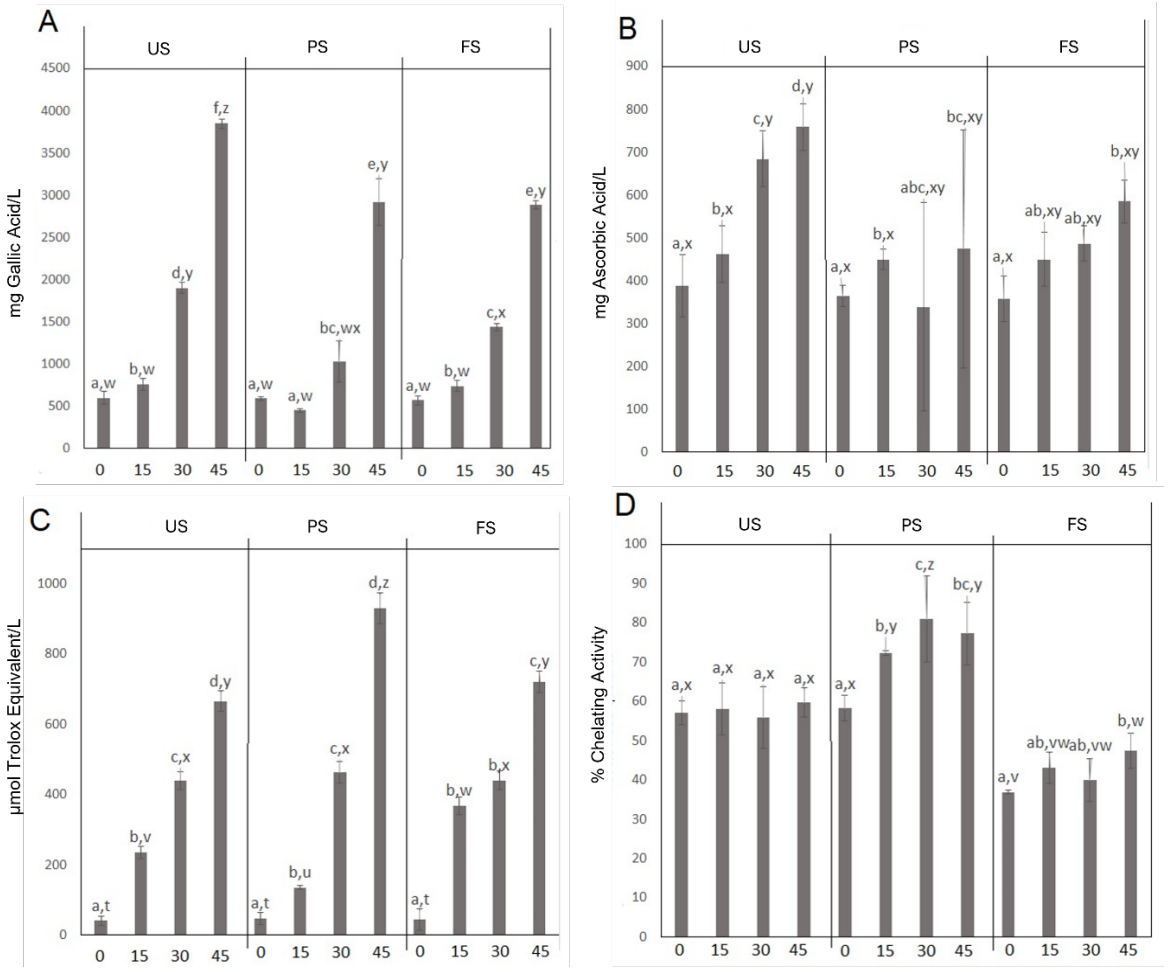

Figure 1. Bioactive compounds content and antioxidant capacity during the preparation of the aqueous extract of *A. salmiana* leaves.

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It is known that the application of thermal treatments to food matrix can break various membranes containing bioactive compounds. The rupture of these membranes allows the release of compounds with antioxidant capacity, such as phenolic compounds (phenols, flavonoids, and anthocyanins). This behavior was observed in the present study, as there was an increase in the concentration of phenolic compounds and, consequently, an increase in their antioxidant capacity at the end of the extraction process (Barba *et al.,* 2017).

During the extraction process, boiling temperatures were not reached; however, a significant amount of moisture was removed, resulting in approximately 35 °Brix. The concentration effect during extraction could be related to the increase in phenolic compounds and ascorbic acid, as well as their antioxidant capacity. Similarly, it was observed that the extract from the US maguey leaf had the highest concentration of phenolic compounds and ascorbic acid at the end of the extraction process compared to PS and FS. Meanwhile, the extract from the PS maguey leaf showed the highest antioxidant capacity and chelating activity at 45 and 30 minutes, respectively, during the extraction process.

As the phenolic compounds and ascorbic acid increased, a rise in the concentration of saponins, molecules with significant biological activity, was also observed (Brindhadevi *et al.,* 2023). These substances are found in high concentrations in maguey leaves (Puente-Garza *et al.,* 2021). Leal-Díaz *et al.* (2015) described a decrease in saponins when the maguey reaches maturity, however, in that study, the results were obtained from aguamiel, not from the maguey leaves themselves. In contrast, a study published by Pinos-Rodríguez *et al.* (2008) reported an increase in saponins in the leaves during the mature stage of maguey. Therefore, the maturity phase appears to be a crucial factor influencing saponin content.

Table 1 presents the physicochemical composition of the aqueous extract from maguey leaves at three maturity stages, which showed no significant differences in moisture content. It was observed that the FS extract contained the highest protein content. Additionally, higher levels of ash and dietary fiber were found in the US and FS extracts, resulting in lower levels of these components in the PS extract. Similar results were reported by Pinos-Rodríguez *et al.* (2008), who noted higher protein and dietary fiber content in maguey leaves with greater maturity. The differences in the physicochemical composition of maguey leaves are attributed to the plant's metabolism (Pinos-Rodríguez *et al.,* 2008).

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Table 1. Physicochemical composition of aqueous extracts of *A salimiana leaves*.

Compounds	US	PS	FS
Moisture (%)	63.4±0.20^{a}	64.4±0.06^{a}	58.6±0.20^{a}
Protein (%)	0.82±0.02^{b}	0.60±0.0^{a}	1.48±0.03^{c}
Ashes (%)	2.77±0.0^{b}	1.85±0.04^{a}	2.61±0.0^{b}
Dietetic fiber (%)	15.1±0.02^{b}	12.6±0.0^{a}	16.4±0.01^{b}
pH	5.12±0.01^{a}	5.01±0.01^{a}	5.05±0.01^{a}
º Brix	36±0.40^{a}	35±0.30^{a}	35±0.50^{a}
Titratable acidity	6.9±0.03^{a}	6.4±0.01^{a}	6.62±0.05^{a}

Different letters significantly differ according to multiple range significance at $p < 0.05$.

The high dietary fiber content in the US and FS extracts was attributed to the concentration of total solids, as confirmed by the measurement of Brix degrees. This suggests that maguey leaf extracts could have positive effects on consumers. When comparing the fiber content to other food matrices such as prickly pear (Hernández-Becerra *et al.,* 2022), cahuiche (a wild cranberry) (Sánchez-Franco *et al.,* 2019), and tortillas (Domínguez-Zárate *et al.,* 2019), the composition and content in maguey leaf extracts are equal or greater than those of minimally processed foods. As mentioned previously, phenolic compounds, ascorbic acid, antioxidant capacity measured by DPPH, and chelating activity were monitored during storage for up to 8 months under refrigeration conditions at 4 °C.

As shown in Table 2, phenolic compounds increased in all three extracts (US, PS, and FS) during storage. The extract with the highest phenolic content was FS. The presence of compounds such as phenylalanine ammonia-lyase, which is activated under stress or low temperatures, has been reported to increase the concentration of phenolic compounds during refrigerated storage (King *et al.,* 2022).

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Table 2. Bioactive compounds and changes in their bioactivity during storage.

		Months				
Bioactive compound	Extract	0	2	4	6	8
Phenolic compounds mg GA/L	US	3847±55.07 ^{b,yz}	3873.3±15.27 ^{b,z}	3813.33±64.2 ^{b,y}	3806.66±61.65 ^{b,y}	3343.33±77.85 ^{a,x}
	PS	2913.33±77.9 ^{a,v}	2873.33±15.27 ^{a,uv}	2966.66±55.07 ^{b,v}	2932.33±15.27 ^{b,v}	3213.33±64.29 ^{c,w}
	FS	2850.0±50 ^{a,v}	3156.66±40.41 ^{b,w}	3461.66±53.71 ^{c,x}	3643.33±17.87 ^{d,x}	3715.66±61.63 ^{e,y}
Ascorbic acid mg AA/L	US	758.02±56.57 ^{d,z}	696.29±65.75 ^{c,y}	683.95±42.76 ^{c,y}	585.18±56.57 ^{b,x}	498.76±21.83 ^{a,y}
	PS	474.07±6.96 ^{a,w}	511.11±37.07 ^{b,wx}	535.80±21.38 ^{b,wx}	548.14±37.08 ^{b,wx}	486.42±37.07 ^{a,w}
	FS	585.18±37.03 ^{ab,x}	597.53±56.57 ^{b,x}	585.18±56.57 ^{ab,x}	561.72±42.7 ^{ab,wx}	523.45±21.38 ^{a,wx}
Antioxidant capacity µmol TE/L	US	665.27±29.26 ^{a,v}	804.16±48.11 ^{bc,x}	910.50±50.23 ^{c,y}	862.50±12.78 ^{c,xy}	763.83±30.04 ^{b,w}
	PS	930.7±44.09 ^{ab,y}	887.83±44.09 ^{a,xy}	849.61±48.11 ^{a,xy}	937.50±25.45 ^{ab,y}	996.61±49.72 ^{a,z}
	FS	730.83±30.04 ^{b,w}	629.16±25.81 ^{a,v}	599.16±30.04 ^{a,v}	754.16±26.78 ^{b,w}	796.04±12.72 ^{c,x}
% Chelating activity	US	59.67±2.30 ^{a,w}	54.15±2.04 ^{c,y}	50.49±1.90 ^{d,z}	52.82±1.16 ^{c,y}	46.24±2.30 ^{b,x}
	PS	77.23±1.43 ^{b,yz}	75.85±1.16 ^{ab,y}	79.52±0.67 ^{b,z}	68.75±2.04 ^{a,x}	69.53±1.70 ^{a,x}
	FS	47.47±3.49 ^{b,v}	54.25±1.29 ^{c,u}	55.25±0.94 ^{c,u}	49.52±1.16 ^{b,v}	39.46±0.49 ^{a,u}
Reducing sugars mg RS/L	US	274.85±3.79 ^{a,y}	279.90±1.74 ^{a,y}	281.24±8.81 ^{a,y}	274.69±1.36 ^{a,y}	293.29±4.81 ^{b,z}
	PS	123.29±7.93 ^{a,r}	139.10±3.80 ^{b,s}	147.47±4.43 ^{b,t}	156.61±5.68 ^{c,t}	175.98±4.42 ^{d,u}
	FS	239.8±4.41 ^{a,w}	242.69±3.61 ^{a,w}	259.87±4.81 ^{b,x}	279.05±2.05 ^{c,y}	294.29±2.36 ^{d,z}

Standard deviation (SD, ±): (n = 3); Values in the same column with different superscript letters are statistically significant ($p < 0.05$), a,c in same treatment; v,z between treatments. GA: gallic acid; AA: ascorbic acid; TE: Trolox equivalent; RS: Reducing sugars.

However, this behavior may vary from sample to sample due to the presence of phenolic compounds, leaf maturity, enzymatic activity, protein content, and carbohydrates (some of which exhibit cryoprotective effects) as well as biosynthesis occurring during and after harvest (Neri *et al.,* 2020). The increase in phenolic compounds during storage is associated with cellular degradation, enabling these compounds to be released from the cellular structure (Sablani, 2015).

On the other hand, a decrease in ascorbic acid was observed in all three extracts, with a more pronounced deterioration in the US extract. Elevated temperatures and water activity

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can accelerate the rate of ascorbic acid degradation (Lee & Labuza, 1975). For this reason, the extracts were dehydrated and stored under freezing conditions (-16 °C).

As a result, there was an approximate 25 % decrease in ascorbic acid in the US extract, which could be related to the presence of Fe^{3+} ions, known to accelerate or enhance the oxidation of ascorbic acid (Giannakourou & Taoukis, 2021). Additionally, the US extract reported a high ash content, indicating the presence of minerals. Furthermore, the stability of ascorbic acid is more frequent within a pH range of two to four. In this context, the extracts had a pH of five, a factor that may have contributed to the oxidation of ascorbic acid (Herbig & Renard, 2017).

In the three extracts, different behaviors were observed for antioxidant capacity and chelating activity, showing both increases and decreases. In the case of chelating activity, its reduction could be related to exposure to metal ions, which have been shown to influence the reactions of foods and extracts during storage (Boukhatem *et al.,* 2020). Conversely, antioxidant capacity exhibited a significant increase up to eight months of storage, particularly in the PS and FS extracts, whereas for chelating activity, the US extract demonstrated the lowest activity compared to its initial levels.

The increase in antioxidant capacity during frozen storage has been documented in various plant materials (Bhattacharya *et al.,* 2018). This enhancement may be attributed to crystal formation during the freezing process, which damages cell membranes and disrupts the physical structures of the food matrix, leading to the release of compounds with antioxidant capacity (Neri *et al.,* 2020). While this effect can be detrimental to fresh products, reducing final quality due to texture alterations, it could be a beneficial strategy when aiming to release phenolic compounds with antioxidant potential (Bhattacharya *et al.,* 2018).

Regarding the identification of phenolic compounds (HPLC UV/VIS), a diglycoside and a triglycoside of kaempferol, as well as a glycoside of quercetin, were identified equally in all three extracts. This aligns with what was reported by Puente-Garza *et al.* (2017[b]), who also conducted on the leaves of *A. salmiana*, suggesting that aqueous extraction could be a viable method for obtaining bioactive compounds. The lack of differences in the phenolic compound content among the three aqueous extracts adds value to the utilization of maguey leaves that have completed their scraping process for aguamiel extraction and have reached the end of the maguey plant's life cycle.

Finally, it is well known that thermal treatment is an effective strategy for controlling certain microorganisms and enzymes that cause food decomposition. However, the type of thermal treatment applied depends on the intended storage conditions. If the food is to be refrigerated for a short period, as in the case of the US, PS, and FS extracts, pasteurization is applied. Conversely, if the goal is to store the product at room temperature for extended periods, high temperatures applied for short durations are used to ensure better preservation of the food (Espinosa *et al.,* 2020).

The obtained extracts showed no growth of aerobic mesophiles or enterobacteria from

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day zero to the eighth month of analysis for US, PS, and FS under refrigeration. This confirms that no cross-contamination occurred and that the procedures for preparing and storing the extracts were appropriate.

Conclusions

The extracts from *A. salmiana* agave leaves exhibit a significant concentration of bioactive compounds such as dietary fiber and flavonoids, substances that provide positive health effects for consumers. Prolonged refrigeration storage led to the degradation of bioactive compounds and, consequently, a decrease in antioxidant capacity in the US extract. However, storage also showed a protective effect and a recovered antioxidant capacity due to phenolic compounds in the PS and FS extracts. Agave leaves at different maturation stages could be utilized in the development of functional foods for human consumption, as they contain substantial amounts of dietary fiber and bioactive compounds with antioxidant capacity. The thermal treatment applied during the extraction of agave leaf extracts ensured no microbial growth, even after eight months of refrigerated storage. This outcome would increase extract shelf life, making it suitable for subsequent application in food matrices.

Author contributions

Work conceptualization: AAN, JASF; Methodology development: AAN, TBPS, YZA, JASF; Software handling: AAN; Experimental validation: AAN, TBPS, YZA, JASF; Data analysis: AAN, JASF; Data management: AAN, TBPS, YZA, JASF; Writing and manuscript preparation: AAN, TBPS, YZA, JASF; Drafting, review, and editing: AAN, TBPS, YZA, JASF; Project administration: JASF.

All authors of this manuscript have read and approved the published version.

Funding

This research did not receive external funding.

Ethical declarations

Not applicable.

Informed consent statement

Not applicable.

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ISSN 2007-3380

Conflict of interest

The authors declare no conflict of interest.

References

Official Methods of Analysis. [AOAC]. (2006). “humedad: 920.151; proteína: 920.152; cenizas: 940.26”. AOAC International, 18th edition, Washington, D.C. USA.

Barba, F.J., Mariutti, L.R.B., Bragagnolo, N., Mercadante, A.Z., Barbosa-Cánovas, G.V., & Orlien, V. (2017). Bioaccesibility of bioactive compounds from fruits and vegetables after termal and nonthermal processing. *Trends in Food Science and Technology,* 67, 195-206. https://doi.org/10.1016/j.tifs.2017.07.006

Bhattacharya, M., Hota, A., Kar, A., Sankar Chini, D., Chandra Malick, R., Chandra Patra, B., & Kumar Das, B. (2018). *In silico* structural and functional modelling of Antifreeze protein (AFP) sequences of Ocean pout (*Zoarces americanus*, Bloch & Schneider 1801). *Journal of Genetic Engineering and Biotechnology*, 16(2), 721-730. https://doi.org/10.1016/j.jgeb.2018.08.004

Brindhadevi, K., Chidambaram, M., Kavitha, R., Govindaraj, R., Chinnathambi, A., Salmen, S. H., Prabakaran, D. S., & Natesan, V. (2023). Extraction, antioxidant, and anticáncer activity of saponins extracted from *Curcuma angustifolia*. *Applied Nanoscience*, 13(1), 2063-2071. https://doi.org/10.1007/s13204-021-02096-9

Bouaziz, A., Masmoudi, M., Kamoun, A., & Besbes, S. (2014). Optimization of Insoluble and Soluble Fibres Extraction from *Agave americana* L. Using Response Surface Methodology. *E-Journal of Chemistry*, 13. https://doi.org/10.1155/2014/627103

Boukhatem, M. N., Boumaiza, A., Nada, H. G., Rajabi, M., & Mousa, S. A. (2020). Eucalyptus globulus Essential Oil as a natural Food Presercative: Antioxidant, Antibacterial and Antifungal Properties *in vitro* and in Real Food Matrix (Orangina Fruis Juice). *Applied Science,* 10(16), 5581. https://doi.org/10.3390/app10165581

Cunningham, M., Azcarate-Peril, M. A., Barnard, A., Benoit, V., Grimaldi, R., Guyonnet, D., Holscher, H. D., Hunter, K., Manurung, S., Obis, D., Petrova, M. I., Steinert, R. E., Swanson, K. S., Sinderen, D., Vulevic, J., & Gibson, G. R. (2021) Shaping the Future of Probiotics and Prebiotics. *Trends in Microbiology,* 29(8), 667-685. https://doi.org/10.1016/j.tim.2021.01.003

Domínguez-Zarate, P. A., García-Martínez, I., Güemes-Vera, N., & Totosaus, A. (2019). Texture, color and sensory acceptance of tortilla and bread elaborated with Maya nut (*Brosimim alicastrum*) flour to increase total dietary fiber. *Ciencia y Tecnología Agropecuaria*, 20(3), 699-719. https://doi.org/10.21930/rcta.vol20_num3_art:1590

Dürüst, N., Dogan, S., & Dürüst, Y. (1997). Ascorbic acid and element contents of food of Trabzon (Turkey). *Journal of Agriculture and Food Chemistry,* 45(6), 2085-2087. https://doi.org/10.1021/jf9606159

Escalante, A., López-Soto, D. R., Velázquez-Gutiérrez, J. E., Giles-Gómez, M., Bolívar, F., & López-Munguía, A. (2016). Pulque, a Traditional Mexican Alcoholic Fermented Beverage: Historical, Microbiological, and Technical Aspects. *Frontiers in Microbiology*, 7, 1026. https://doi.org/10.3389/fmicb.2016.01026

Revista Bio ciencias
ISSN 2007-3380

Espinosa, M. F, Sancho, A. A., Mendoza, L. M., Mota, C. R., & Verbyla, M. E. (2020). Systematic review and meta-analysis of time-temperature pathogen inactivation. *International Journal of Hygiene and Environmental Health,* 230, 113595. https://doi.org/10.1016/j.ijheh.2020.113595

García-Mendoza, A. J., Franco-Martínez, I. S., & Sandoval-Gutiérrez, D. (2019). Cuatro especies nuevas de Agave (*Asparagaceae, Agavoideae*) del sur de México. *Acta Botanica Mexicana,* 126, e1461. https://doi.org/10.21829/abm126.2019.1461

Georgé, S., Brat, P., Alter, P., & Amiot, M. J. (2005). Rapid determinations of poliphenols and vitamin C in plant-derived products. *Journal of Agriculture and Food Chemistry*, 53(5), 1370-1373. https://doi.org/10.1021/jf048396b

Giannakourou, M. C., & Taoukis, P. S. (2021). Effect of Alternative preservation Steps and Storage on Vitamin C Stability in Fruit and Vegetable Products: Critical Review and Kinetic Modelling Approaches. *Foods*, 10(11), 2630. https://doi.org/10.3390/foods10112630

González-Montemayor, A. M., Flores-Gallegos, A. C., Serrato-Villegas, L. E., López-Pérez, M. G., Montañez-Sáenz, J. C., & Rodríguez-Herrera, R. (2019). 6 - Honey and syrups: Healthy and natural sweeteners with functional properties. *Natural beverages,* 13, 143-177. https://doi.org/10.1016/B978-0-12-816689-5.00006-7

Gulcin, I., Buyukokuroglu, M. E., & Kufrevioglu, O. I. (2003). Metal chelating and hydrigen peroxide scavenging effects of melatonin. *Journal of Pineal Research*, 34(4), 278-281. https://doi.org/10.1034/j.1600-079X.2003.00042.x

Herbig, A. L., & Renard, C. M. (2017). Factors that impact the stability of Vitamin C at intermediate temperatures in a food matrix. *Food Chemistry*, 220, 444-451. https://doi.org/10.1016/j.foodchem.2016.10.012

Hernández-Becerra, E., Aguilera-Barreiro, M. A., Contreras-Padilla, M., Pérez-Torrero, E., & Rodriguez-Garcia, M. E. (2022). Nopal cladodes (*Opuntia Ficus Indica*): Nutritional properties and functional potential. *Journal of Functional Foods*, 95, 105183. https://doi.org/10.1016/j.jff.2022.105183

IBM Corp. (2022). IBM SPSS Statistics for Windows (Version 25.0) [Computer software]. IBM Corp.

King, E. S., Noll, A., Glenn, S., & Bolling, B. W. (2022). Refrigerated and frozen storage impact aronia berry quality. *Food Production, Processing and Nutrition*, 4(3). https://doi.org/10.1186/s43014-021-00080-y

Lara-Avila J. P., & Alpuche-Solis, A. G. (2017). Análisis de la diversidad genética de agaves mezcaleros del centro de México. *Revista Fitotecnia Mexicana*, 39(3), 323-330.

Lee, S. H., & Labuza, T. P. (1975). Destruction of ascorbic-acid as a function of water activity. *Journal of Food Science*, 40(2), 370-373. https://doi.org/10.1111/j.1365-2621.1975.tb02204.x

Leal-Díaz, A. M., Santos-Zea, L., Martínez-Escobedo, H. C., Guajardo-Flores, D., Gutiérrez-Uribe, J. A., & Serna-Saldivar, S.O. (2015). Effect of *Agave americana* and *Agave salmiana* ripeness on saponins content from Aguamiel (Agave Sap). *Journal Agriculture and Food Chemistry,* 63(15), 3924-3930. https://doi.org/10.1021/acs.jafc.5b00883

Manssouri, M., Znini M., & Majidi, L. (2020). Studies in the antioxidant activity of essential oil and various extracts of *Ammodaucus leucotrichus* Coss. & Dur. Fruits from Morocco. *Journal of Taibah University for Science*, 14(1), 124-130. https://doi.org/10.1080/16583655.2019.1710394

Medina-Mendoza, C., Mendoza-Tolentino, Y., & Cervantes-Miranda, J. (2022). Determination of

Revista Bio ciencias
ISSN 2007-3380

bioactive compounds in stalks of maguey pulquero (*Agave salmiana*). Revista de Ingeniería y Tecnologías para el Desarrollo Sustentable, 10(1), 34-41.

Morales, F., & Jiménez-Pérez, S. (2001). Radical scavenging capacity of Maillard reaction products as related color and fluorescence. *Journal of Agriculture and Food Chemistry*, 72(1), 119-125. https://doi.org/10.1016/S0308-8146(00)00239-9

Neri, L., Faieta, M., Di Mattia, C., Scchetti, G., Mastrocola, D., & Pittia, P. (2020). Antioxidant activity in frozen plant foods: Effect of cryoprotectants, freezing process and frozen storage. *Foods*, 9(12), 1886. https://doi.org/10.3390/foods9121886

Pinos-Rodríguez, J. M., Zamudio, M., & González, S. S. (2008). The effect of plant age on the chemical composition of fresh and ensiled *Agave salmiana leaves*. *South African Journal of Animal Science*, 38(1), 43-50. http://dx.doi.org/10.4314/sajas.v38i1.4108

Puente-Garza, C. A., Espinosa-Leal, C. A., & García-Lara, S. (2021). Effects of saline elicitors on saponin production in *Agave salmiana* plants grown *in vitro. Plant Physiology and Biochemistry*, 162, 476-482. https://doi.org/10.1016/j.plaphy.2021.03.017

Puente-Garza, C. A., García-Lara, S., & Gutiérrez-Uribe, J. A. (2017[a]). Enhancement of saponins and flavonols by micropropagation of *Agave salmiana*. *Industrial Crops & Products,* 105(15), 225-230. https://doi.org/10.1016/j.indcrop.2017.05.014

Puente-Garza, C. A., Meza-Miranda, C., Ochoa-Martínez, D., & García-Lara, S. (2017[b]). Effect of *in vitro* drought stress on phenolic acids, flavonols, saponins, and antioxidant activity in *Agave salmiana. Plant Physiology and Biochemistry* 115(1), 400-407. https://doi.org/10.1016/j.plaphy.2017.04.012

Richardson, G. H. (1985). Standard methods for the examination of dairy products, 15th ed. APHA. CALSP. Washingtong, D.C. pp: 412.

Sablani, S. S. (2015). Freezing of fruits and impact on anthocyanins. *Processing and Impact on Active Components in Food,* 18, 147-156. https://doi.org/10.1016/B978-0-12-404699-3.00018-4

Sánchez-Franco, J. A., Ayala-Niño, A., Cariño-Cortés, R., Hernéndez-Fuentes, A. D., Castañeda-Ovando, A., Campos-Montiel, R. G., Román-Guerrero, A., & Jiménez-Alvarado, R. (2019). *Vaccinium leucanthum* Schlechtendahl fruit, a new source of dietary fiber and antioxidant compounds. *Revista Mexicana de Ingeniería Química*, 18(3), 901-911. https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Sanchez

Valdivieso-Solís, D. G., Vargas-Escamilla, C. A. Mondragón-Contreras, N., Galván-Valle, G. A., Gilés-Gómez, M., Bolívar, F., & Escalante, A. (2021). Sustainable Production of Pulque and Maguey in Mexico: Current Situation and Perspectives. *Frontier in Sustainable Food Systems*, 5(1). https://doi.org/10.3389/fsufs.2021.678168

Velázquez-De Lucio, B. S., Hernández-Domínguez, E. M., Falcón-León, M. P., Téllez-Jurado, A., & Álvarez-Cervantes, J. (2024). Revalorization of degraded maguey pulquero substrate for Lycopersicon esculentum germination. *Current Research in Microbial Sciences*, 7, 100283. https://doi.org/10.1016/j.crmicr.2024.100283

Westhoff, D. C. (1981). Microbiology of ultrahigh temperature milk. *Journal of Diary Science*, 64(1), 167-173. https://doi.org/10.3168/jds.S0022-0302(81)82545-3

Zhi-Wei, G., En-Ze, G., & Quiang F. (2021). Soluble Dietary Fiber, One of the Most Important Nutrients for the Gut Microbiota. *Molecules,* 26(22), 6802. https://doi.org/10.3390/molecules26226802