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Determinación del índice glucémico en jarabe de agave obtenido por tecnología emergente

Determination of the glycemic index in agave syrup obtained by emerging technology

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RESUMEN

Introducción: El jarabe de agave es de los subproductos más relevantes y cada vez más populares en el mercado, considerado como un edulcorante natural. Su índice es considerado bajo, pero existe controversia al respecto, por lo que el objetivo principal de esta investigación fue evaluar el índice glicémico de jarabe de agave obtenido por calentamiento óhmico en voluntarios aparentemente saludables.

Metodología: Se obtuvo jarabe de agave por calentamiento óhmico. Tras reclutar voluntarios, se seleccionaron los aptos para el estudio siguiendo el protocolo oficial internacional ISO 26642:2012 y protocolos anteriormente establecidos. Por punción capilar se evaluó el área bajo la curva de respuesta del jarabe de agave contra glucosa como estándar, con cargas de 50 g de carbohidratos.

Resultados: Únicamente 15 voluntarios fueron clasificados como aptos para el estudio con un rango de edad de 21-33 años. Tras realizar las ecuaciones correspondientes se obtuvo un índice glicémico *In vivo* de 35.

Conclusiones: El Jarabe de agave obtenido por calentamiento óhmico presenta un índice glicémico *In vivo* sutilmente más elevado que lo reportado ampliamente por autores, aunque mantiene la clasificación de bajo.

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Palabras clave: Calentamiento óhmico, respuesta glicémica, *In vivo, Agave salmiana.*

ABSTRACT

Introduction: Agave syrup is one of the most relevant and increasingly popular byproducts on the market, considered a natural sweetener. Its glycemic index is considered low, but there is some controversy surrounding this. Therefore, the main objective of this research was to evaluate the glycemic index of agave syrup obtained by ohmic heating in apparently healthy volunteers.

Methodology: Agave syrup was obtained by ohmic heating. After recruiting volunteers, those eligible for the study were selected following the official international protocol ISO 26642:2012 and previously established protocols. The area under the response curve of agave syrup against glucose as a standard was evaluated by capillary puncture, with 50 g carbohydrate loads.

Results: Only 15 volunteers were classified as eligible for the study, with an age range of 21-33 years. After performing the corresponding calculations, an in vivo glycemic index of 35 was obtained.

Conclusions: Agave syrup obtained by ohmic heating has a slightly higher in vivo glycemic index than that widely reported by authors, although it maintains its low classification.

Funding: This research was supported by the Secretaría de Ciencia, Humanidades, Tecnología e Innovación (Secihti) scholarship program in collaboration with the UAdeC, with scholarship number 1082548. Additionally, support was provided by the Department of Food Science and Technology, UAAAN.

Keywords: Ohmic heating, Glycemic response, *In vivo, Agave salmiana*.

KEY MESSAGES

- Agave syrup has been under-studied in *In vivo* analyses.
- There is controversy surrounding the use of fructose-based sweeteners.
- The in vivo glycemic index of agave syrup obtained by ohmic heating is 35, which is considered low.
- In an appropriate diet, it can be a natural sweetener with beneficial properties for the consumer.

INTRODUCTION

Plants belonging to the Agave genus have been present for millennia. They are important to many regions in the Americas, drought-resistant, and environmentally resilient. Furthermore, their use can be comprehensive, favoring the region's circular economy: from the stalk, core, leaves, flowers, and sap, to the agro-industrial waste left after processing. Agave syrup is one of the most relevant and increasingly popular byproducts on the market, considered a natural sweetener obtained by concentrating the carbohydrates in the sap or nectar of the agave, also called aguamiel [1,2].

It has been described as a source of bioactive compounds suitable for everyone, with the thermal process for obtaining this syrup being the traditional and most popular method among producers. However, this process, which usually involves cooking with wood as the main fuel, significantly reduces the bioactive compounds that are beneficial to human health. This provides an opportunity to obtain this syrup without losing properties or characteristics, with ohmic heating being a viable option for preserving these compounds [3]. Ohmic heating is claimed to be an efficient emerging technology, with lower cost and processing time than its traditional version; as a pretreatment in agave bagasse, it improves the release of bioactive compounds such as phenolic compounds and fiber; likewise, no significant effects on nutritional and functional properties has been reported [4,5].

The high fructose content, followed by fructans (agavins), bioactive compounds (saponins, polyphenols and flavonoids) and minerals (iron, calcium, potassium and magnesium) contained in this syrup presents an excellent prebiotic potential and low glycemic index for the prevention, treatment and control of chronic non-communicable metabolic diseases [5,6]. The glycemic index is a tool for classifying foods based on the quality and quantity of carbohydrates they contain. Foods high in this index are associated with a higher prevalence of chronic non-communicable metabolic diseases, mainly increasing the risk of mortality from strokes. Therefore, although still controversial, the consumption of products low in this index is widely recommended as part of a healthy lifestyle [7–9].

There is literature on the glycemic index in syrups and honeys; however, most of this data is obtained through *In vitro* processes, while *In vivo* studies on agave syrup are scarce and highly variable. Indices ranging from 10 to 36 are normally estimated and reported, but clear and reliable studies are lacking [10,11]. *In vivo* studies are the gold standard for metabolic responses [12], therefore, the main objective of this research was to evaluate the glycemic index of agave syrup obtained by ohmic heating in apparently healthy volunteers.

METHODOLOGY

Agave syrup

The production of agave syrup by ohmic heating is shown in **Figure 1** following the protocol described by Martínez-Zavala (2023) [5]. Agave sap (Aguamiel) from *Agave salmiana* was used, provided by local producers in Guadalupe Victoria, Saltillo, Coahuila, Mexico (24°54'25.25"N 101°06'15.93"W). It was transported and stored at 4°C to prevent fermentation into Pulque, derived from the microorganisms naturally present in the Aguamiel. Initially, the Aguamiel had 89% moisture and a concentration of 10°Bx, measured by the Universal Portable Refractometer (Model: ACT, Brand: JuanJuan, China) at 20°C. Before taking measurements, the refractometer's accuracy was standardized using distilled water at 20°C.

Subsequently, the emerging technology was used: The stainless-steel electrodes were immersed in a flat-bottomed cylindrical glass jar (Diameter: 15.5 cm and Long: 15 cm), with a distance of 12.6 cm between them and connected to a Variable Voltage Transformer (0-300 VAC, 60 Hz, Brand: NAPEE, Model: 2S/10A-140/GVD, Mexico). These cells and devices were designed, manufactured, and/or modified in the Functional Foods and Nutrition Laboratory of the Universidad Autónoma de Coahuila. The process required a constant voltage of 120 V (9.52 V/cm) until the aguamiel reached 70 °Bx, pH 4.7 and 25% moisture. This process takes approximately 65 minutes. The temperature was monitored throughout the process and recorded every 10 minutes with a thermometer (Taylor, SAMA Mercury, Model: 6337M, China); it did not exceed 90°C.

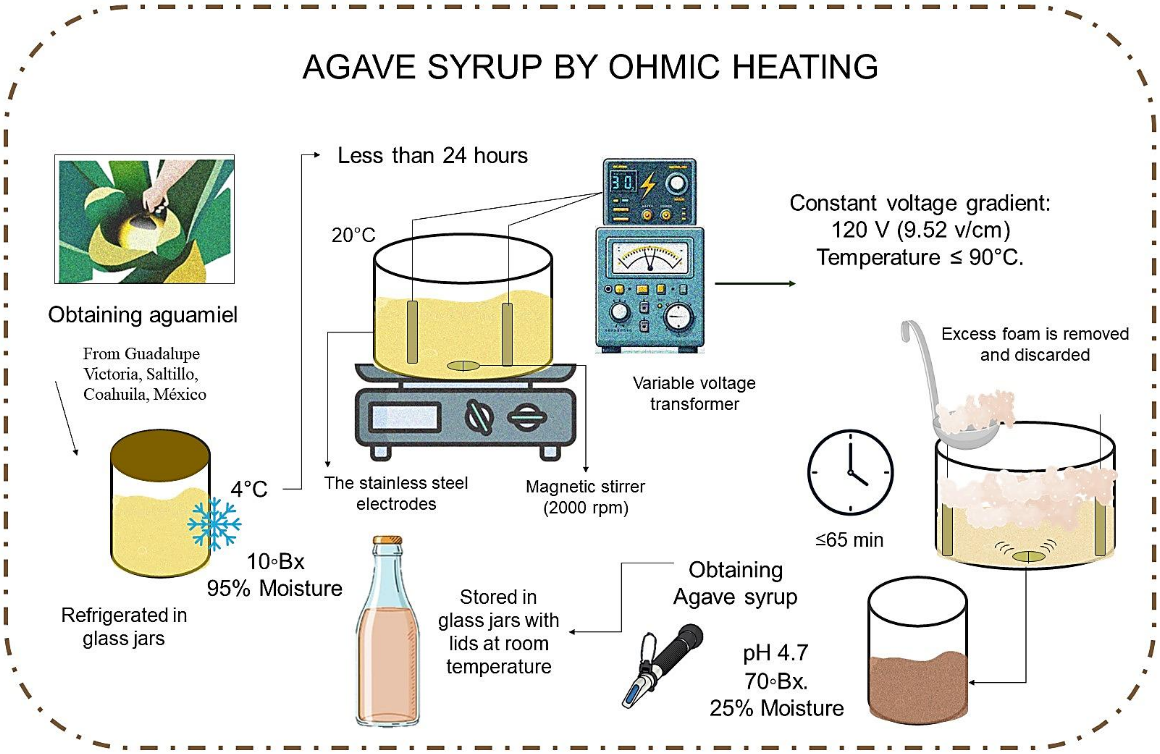

Figure 1. Agave syrup by ohmic heating

Carbohydrate content

The fructose, sucralose and fructooligosaccharides content in agave syrup were analyzed using an HPLC system (Brand: Jasco, Model: LC4000, USA) with a refractive index detector and an autosampler. The analysis involved a separation process using an ASAHIPAK NH2P-50 4E protective column (Dimension: 4.6 × 250 mm, Particle size 5 µm, Brand: Shodex, Japan) bonded to an ASAHIPAK NH2P-50G 4A protective column (Dimensions: 4.6 × 10 mm, Brand: Shodex, Japan). Sample elution (20 µL) was performed using a mixture of acetonitrile (HPLC grade, Brand: Fisher Chemicals, USA) in Mili-Q water (68:32 v/v) and 0.04% ammonium hydroxide in water (HPLC grade, Brand: Sigma-Aldrich, Germany). The elution was performed at a flow rate of 1 mL/min with the column temperature maintained at 30 °C. The chromatographic signal was captured and processed using Star Chromatography Workstation Software Version 6.3 (Varian, USA) [5].

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Ethics committee

The project was approved by the scientific ethics committee of the Faculty of Chemical Sciences of the Autonomous University of Coahuila, under the registration TDCYTA-20-10-22-2. All volunteers signed an informed consent form, ensuring their understanding of the study's objectives, risks and benefits, in compliance with ethical regulations of the Sanitary Control Regulations for Products and Services, the Regulations of the General Health Law on Health Research, and the ISO 26642:2012 standard referring to the protocol for the determination of the glycemic index [13–15].

Volunteers

A call was made to recruit volunteers between the ages of 20 and 40 without food allergies. The protocol described by Martínez et al. (2024)[16] was followed, as shown in **Figure 2,** for the collection of ABCD data (Anthropometric, Biochemical, Clinical and Dietary) and final selection of those who were healthy according to the Mexican standards. The information was collected through personal and private interviews: A) the same measuring equipment was used for all volunteers to reduce variability; B) an SD Lipid Care® analyzer (brand: SD Biosensor, INC, manufactured by: Kana Undesa SA de CV, Mexico) was used to determine the lipid profile, as well as fasting glucose using capillary blood; C) the absence of any pre-existing illnesses or conditions, as well as food allergies, was verified through basic personal medical history; and D) a brief questionnaire on habits, lifestyle, and physical activity was administered. Finally, to standardize carbohydrate consumption prior to the test, volunteers were asked to follow a 2000 kcal meal plan for five days, which was approved and adapted to each volunteer. On three separate occasions, volunteers were asked to fast for 6 to 8 hours before each analysis.

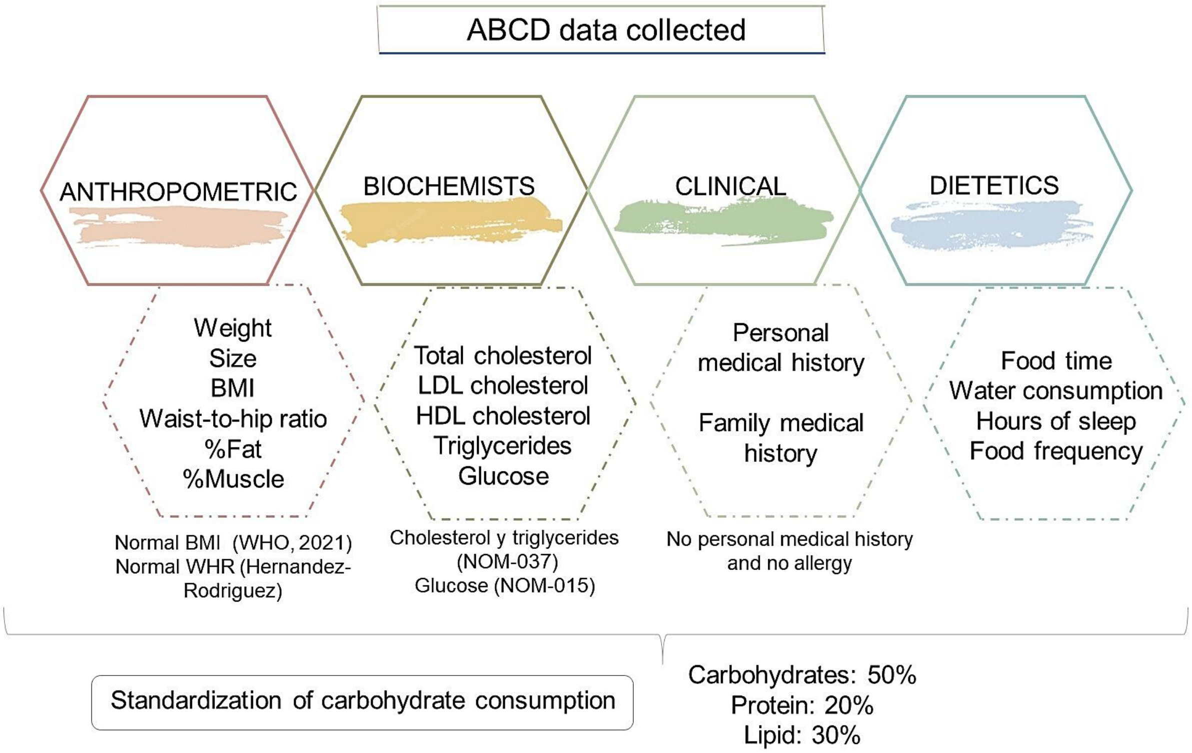

Figure 2. ABCD data collected for the selection of apparently healthy volunteers. Inspired by Martínez et al. (2024) [16].

Glycemic index evaluation

Following official international protocols (ISO 26642:2010(E), 2010), glucose tolerance curves were performed using a standardized and stable oral solution (Brand: Glucox, Manufacturer: Materiales y Abastos Especializados SA de CV, Mexico) with a 50 g carbohydrate load. Capillary punctures to measure glucose levels were performed at 0, 15, 30, 45, 60, 90, and 120 minutes after ingestion. If glucose response curves with normal reactions are obtained according to the official Mexican standard[17], the curves for the food under study, agave syrup obtained by ohmic heating, are continued. Using 71.4 ± 0.2 g of syrup (corresponding to 50 g of carbohydrates), the response curves were plotted at the puncture points mentioned above. After obtaining the average Area Under the Curve of both curves, the glycemic index (GI) is calculated using the corresponding equation. [1]:

$$GI\ \%: \frac{AUC\ Agave\ syrup}{AUC\ glucose} * 100 \qquad \text{Eq [1]}$$

Statistical analysis

The collected data are presented as mean values with their respective standard deviations (SD). To assess significant differences between the Areas Under the response Curves, a comparison of means was used using Tukey's test ($p < 0.05$). OriginPro 2025-2026 student version (OriginLab Corporation, USA, https://www.originlab.com/) and Microsoft 365 Excel® 2022-2025 (Microsoft Windows, USA) were used for data analysis.

RESULTS

Agave syrup obtained by ohmic heating with a final concentration of 70°Brix composed of 45% fructose, 35% sucrose and 19% fructooligosaccharides, presented values that are appropriate under the protocol explained in Martínez-Zavala et al. (2023)[5]. Enough syrup was produced from the same batch of Aguamiel to be used throughout the experiment.

On the other hand, based on the established ABCD data collection standards, only 15 volunteers were classified as healthy, with an age range of 21–33 years. **Table 1** shows the Anthropometrics and Biochemical average data collected from these volunteers. Regarding clinical data: in the personal medical history section, 12 volunteers reported having had COVID-19, and only 2 volunteers reported anxiety disorder. In the family medical history section, volunteers reported having a total of 13 family members with type 2 diabetes mellitus, 12 family members with hypertension, 7 family members who had or currently have cancer, and 4 family members with hypothyroidism. Dietary data revealed an average of 8 hours of sleep for both men and women, with 3 meals a day, and an average consumption of 2 liters of water. Similarly, dietary frequency shows, on average, a high consumption of recommended foods such as leafy green vegetables and fruits, and at the same time, a high consumption of foods not recommended such as baked goods and salty snacks, according to the classification ENSANUT (2022) [18], which denotes this awareness of healthy foods that is matched by the craving for these sweet or salty snacks

Table 1. Anthropometrics and Biochemical data of the selected volunteers

Data	Analysis	Result
Anthropometrics	Weight	64.71 ± 11.3 kg
	BMI	24.08 ± 3.53 kg/m^2
	WHR*	0.85 ± 0.03 M** 0.77 ± 0.05 F***
	%Visceral Fat	4.13 ± 1.46
	%Muscle	37.4 ± 3.39 M 25.92 ± 2.52 F
Biochemistry	Triglycerides	91.93 ± 51.68 mg/dL
	Total cholesterol	150.47 ± 32.63 mg/dL
	LDL cholesterol	90.4 ± 29.45 mg/dL
	HDL cholesterol	41.4 ± 8.63 mg/dL
	Glucose	87.6 ± 5.75 mg/dL

Data are expressed as mean ± standard deviation; *WHR Waist-to-hip ratio; **M: Male average; *** F: Female average.

Primarily, the results of the *In vivo* clinical trial demonstrated that agave syrup obtained by ohmic heating is a low-glycemic index product. As shown in **Figure 3**, this is due to the fact that the glucose peaks were significantly lower compared to the control curve. The highest peaks in both the glucose and syrup curves occurred at 30 minutes; however, the glucose peak reached 146.36 mg/dL, while the peak for the ohmic heating agave syrup was 119.64 mg/dL. Subsequently, both curves showed a decrease over time, more pronounced in the syrup than in the glucose. This behavior resulted in an average glycemic index of 35 mg/dL, considered low, as shown in **Table 2.**

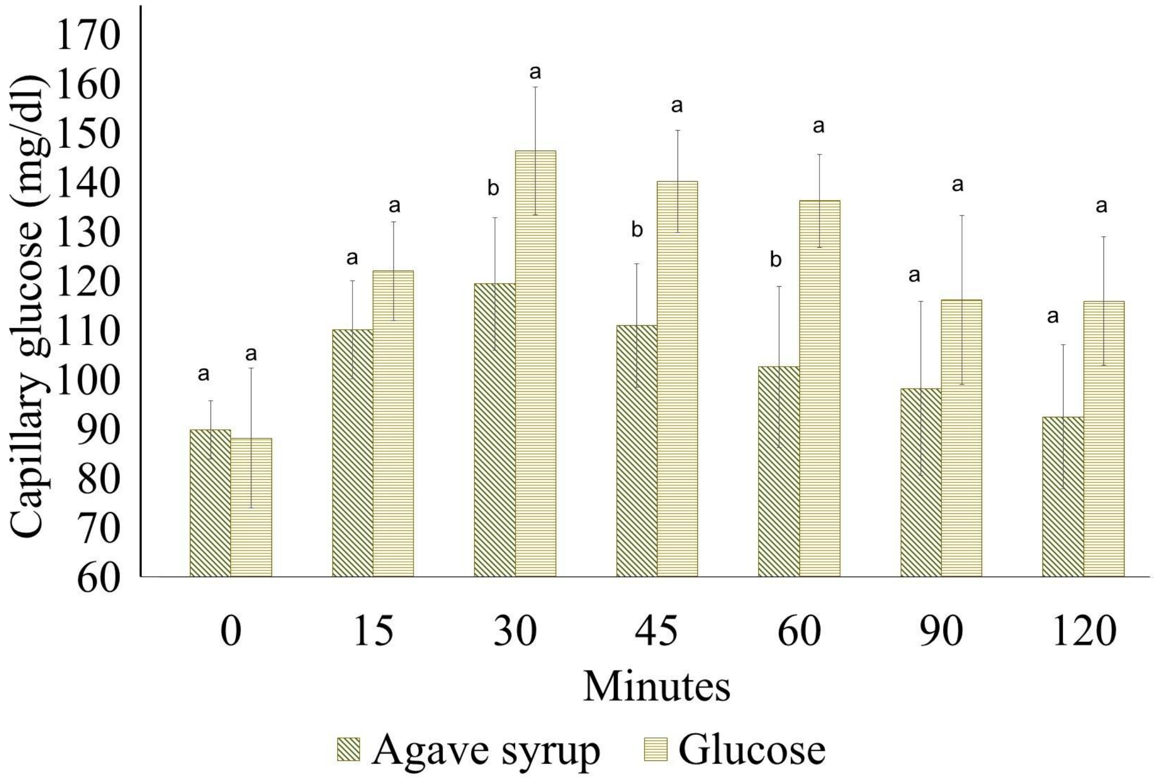

Figure 3. Response curves: Glucose vs Agave syrup

Table 2. Area under the glucose curve and glycemic index

	n	AUC Glucose (mg/dl)	AUC Agave syrup(mg/dl)	GI (mg/dl)
Male	5	5845.5	2078.5	41.6 ± 19.2
Female	10	5342.6	1635.4	30.2 ± 10.7
Total	15	5552.1	1820.0	34.9 ±15.2

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DISCUSSION

The average data collected falls within the expected range: a Waist-to-hip ratio indicating low risk, a high prevalence of respiratory diseases in personal history and a high prevalence of chronic non-communicable metabolic diseases among the volunteers' relatives. The volunteers presented a high prevalence of type 2 diabetes and hypertension in their immediate family history. This seemed to raise awareness among the volunteers about the importance of proper nutrition throughout the different stages of life, as their eating patterns (despite consuming salty and sweet snacks four times a week), show daily consumption of vegetables and almost daily consumption of fruits, legumes, and low-fat cereals. Likewise, the volunteers report doing daily physical activity with a maximum of two rest days per week for general health reasons.

It is important to mention that the glycemic index is a controversial tool, as several factors can interfere with this response. These can be dependent factors (age, gender, gut microbiota) or independent factors (type of food, cooking method) that can introduce bias into the research. In addition, many reported glycemic indexes are obtained through *In vitro* evaluation, which generates reliable results but in "ideal" environments that may differ from the *In vivo* reaction in humans. Despite this, there is clear evidence of the relationship between high glycemic index foods and the prevalence of chronic non-communicable metabolic diseases. Likewise, the relationship has been noted between the composition of nutrients and beneficial bioactive compounds in the food matrix that interfere with glucose absorption or provide sweetness without containing glucose [19].

In this study, agave syrup presented an Index of 35; although in recent reviews indices of 10 to 19 are claimed and even research articles mention an index of 11[20]. The carbohydrate profile varies from syrup to syrup, generating these variations in the glycemic impact. A clear example is sucrose, which is usually presented at 16-22% in commercial agave syrup, while in this study a higher content (35%) was presented [21]. Although the internationally reported and accepted values are maintained at the Index of 11 with 90% fructose agave syrup[22]. Most of these studies use commercial syrups, without a carbohydrate profile and do not show a clear methodology, since studies have been found that report index estimates of 36 measuring agave syrup obtained by vacuum rotary evaporation but do not mention the fructose content [23].

Agave syrup typically contains 70 to 90% fructose; this carbohydrate being responsible for its low glycemic impact. The syrup, obtained by ohmic heating, had a 70° Brix with a fructose content of 45%, notably lower than commercial syrups. Agave syrups from the *A. tequilana* and *A. salmiana* species have been reported with 76° Brix and contain up to 84% fructose [24]; in contrast, recent studies using *A. salmiana* and *A. atrovirens* species to obtain a syrup with 65° Brix show only 24% fructose [21]. These inconsistencies generate controversy among specialists, although all reported ranges are low, which has favored its commercialization and use as a natural sweetener with functional properties.

The content of compounds and nutrients varies from one species of agave to another, harvest time, growing region, and other factors that will affect the final products and byproducts. Primarily, the considerable fructose content has been linked to an increased prevalence of diseases such as metabolic syndrome, liver disease, and increased blood lipids [11]. There are even reviews that conclude that there is a direct relationship between excessive fructose consumption and the pathogenesis of ischemia-reperfusion injury, resulting from the accelerated production of reactive oxygen species (highly reactive molecules that generate irreversible chemical changes in macronutrients such as proteins or fats, as well as deep cellular dysfunction and cytotoxicity) [25].

Other authors argue that this concern is unrealistic, as these adverse effects occur when fructose is consumed in isolation, in unreasonable quantities and compared to glucose in isolation [26]. It is worth mentioning that another relevant compound in the syrup, belonging to the prebiotic dietary fiber group is agave inulin. Inulins are a polysaccharide formed by numerous fructose units. Their β configuration makes them resistant to enzymatic hydrolysis in the digestive tract, with partial and variable digestion in the colon, allowing them to be fermented by the gut microbiota. This substrate for probiotic bacteria offers broad health benefits such as reducing blood lipids, modulating appetite, positively influencing the glycemic index and improving the gut microbiota [27].

Moreover, other fructans and fructooligosaccharides in agave have prebiotic properties, help regulate blood sugar and oxidative stress, provide sweetness and can reduce blood lipid levels [28]. Mellado-Mojica & López, (2015) [29] analyzed the carbohydrate profile of various syrups from different agave species, reporting mainly fructose and fructooligosaccharides in most of the syrups; however, specifically the syrup from *A. salmiana* presented similar proportions of sucrose and fructose with small portions of FOS such as 1-kestose, inulotriose, 6-kestose, and neokestosa, a profile similar to that presented in this research.

Among the most common syrups, we can find that: Corn syrup presents very varied ranges, depending on its production process, however it usually contains glucose or maltooligosaccharides as its main carbohydrate; Honey (with an index of 55) can be widely recommended with adequate consumption, its composition is mostly glucose and fructose: Sugarcane syrup shows a high glucose profile, followed by fructose and sucrose [29]. These profiles generate a greater glycemic impact, while the profile of agave syrup containing a wide range of bioactive compounds and micronutrients such as fructans, flavonoids, and tannins that other fructose-based sweeteners de not contain, shows a more varied carbohydrates profile with a lower glycemic impact.

Likewise, as mentioned, the production process is relevant: traditional production involves cooking over an open fire, typically using firewood, where temperatures can exceed 100°C for hours, causing significant changes in its composition, emphasizing the loss of key functional components [5]. Although commercial syrups contain higher amounts of fructose and a lower glycemic index, ohmic heating produces syrups that are also low in this index, in addition to offering both economic and efficiency advantages, as the cost and time invested are lower than their traditional counterparts, with a fructooligosaccharides content higher than the average commercial syrups [29].

On the other hand, although the average glycemic index is low (35), significant differences can be observed by gender, with average indices in males and low indices in females. As mentioned, the factors that affect the glycemic index are numerous; however, some gain relevance due to supporting evidence, such as the volunteer's gender: Eldakhakhny et al. (2021)[30] concluded that there are undeniable differences between men and women when measuring the glycemic index, even for the same food; this is observed in the area under the glycemic response curves by gender. However, it should be noted that the sample size in this research is limited and asymmetrical, which may cause bias in the interpretation of the results.

CONCLUSION

The glycemic index values reported by other authors are quite low; however, these syrups are primarily commercial, lack clear methodologies, and generally only represent predictions of the index. Agave syrup obtained by ohmic heating has a slightly higher in vivo glycemic index than that widely reported by other authors, although it still falls into the low category. In appropriate portions, the numerous nutrients and compounds present in agave syrup make it a viable sweetener option.

LIMITATIONS

This study has a sample of 15 volunteers, without an equal distribution between genders. This could result in low statistical robustness. Furthermore, the suggested serving of agave syrup according to the Mexican System of Equivalent Foods (SMAE) is 12 g, while 71 g was used in this study. Therefore, the prebiotic potential of agave syrup can be evaluated based on the suggested serving in a balanced diet. It is important to note that this *In vivo* study was conducted on apparently healthy young volunteers, so the response may vary depending on the study group.

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AUTHORS' CONTRIBUTIONS

R.MM-H was the main researcher, an important part of sample collection and management of biological waste, interpretation of results and the main writer of this article. RB and MC contributed to the creation and design of the study, supervised compliance with the established protocol and performed data analysis with the work team as well as providing financing from the Universidad Autónoma de Coahuila and Universidad Autónoma Agraria Antonio Narro. MMZ contributed to the collection of data for the approval of the protocol before the ethics committee and performed data analysis with the work team. HAR-L, RMR-J, JCC and AAN-V contributed to the creation of the statistical design and interpretation of results. All authors critically reviewed this and previous versions of the document.

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CONFLICTS OF INTEREST

The authors express that there are no conflicts of interest when writing the manuscript.

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