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Review

Agave By-Products: An Overview of Their Nutraceutical Value, Current Applications, and Processing Methods

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Abstract: Agave, commonly known as "maguey" is an important part of the Mexican tradition and economy, and is mainly used for the production of alcoholic beverages, such as tequila. Industrial exploitation generates by-products, including leaves, bagasse, and fibers, that can be re-valorized. Agave is composed of cellulose, hemicellulose, lignin, fructans, and pectin, as well as simple carbohydrates. Regarding functional properties, fructans content makes agave a potential source of prebiotics with the capability to lower blood glucose and enhance lipid homeostasis when it is incorporated as a prebiotic ingredient in cookies and granola bars. Agave also has phytochemicals, such as saponins and flavonoids, conferring anti-inflammatory, antioxidant, antimicrobial, and anticancer properties, among other benefits. Agave fibers are used for polymer-based composite reinforcement and elaboration, due to their thermo-mechanical properties. Agave bagasse is considered a promising biofuel feedstock, attributed to its high-water efficiency and biomass productivity, as well as its high carbohydrate content. The optimization of physical and chemical pretreatments, enzymatic saccharification and fermentation are key for biofuel production. Emerging technologies, such as ultrasound, can provide an alternative to current pretreatment processes. In conclusion, agaves are a rich source of by-products with a wide range of potential industrial applications, therefore novel processing methods are being explored for a sustainable re-valorization of these residues.

Keywords: *Agave* spp.; composites; emerging technologies; biofuels; fibers; functional foods; by-products

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1. Introduction

The Agaves are plants commonly known as "magueys", since ancient times they have had a strong relationship with Mexican culture and history [1]. They were sacred to the Aztec population of prehispanic Mexico and in the Nahuatl language, were named *Metl*. Agave (Agavaceae) is endemic to the American continent, with a distribution extending from the southern United States (with two disjunct species in Florida) to northern South America, including the Caribbean islands [2]. The genus contains approximately 210 species: 159 are present in Mexico (75% of the total) and 129 are endemic to the Mexican territory, representing 61% of the world's species [3]. These data are constantly changing as new species are discovered.

Agave is mainly used for the production of distilled (spirits) and non-distilled alcoholic beverages, including Tequila, Mezcal, Bacanora, Raicilla, Sotol and Pulque, all of which have special connections to Mexican history and culture, and contribute to the Mexican economy [4]. Four main species dominate the agave market, namely *Agave tequilana*, *Agave salmiana*, *Agave angustifolia*, and *Agave fourcroydes* [1]. Their cultivation has been exponentially expanded because of their minimal water and maintenance requirements [5].

Currently, the agave has several alternative uses, including the whole plant and certain by-products. The whole plant is used as a living fence, improving the soil pH, increasing the concentration of phosphorus and potassium, and avoiding erosion, whereas the different by-products have a wide range of applications. In this review, we summarize the main uses for the re-valorization of leaves, bagasse, and fibers that otherwise are considered waste for the agave industry. Particular emphasis is placed on the processing methods for agave by-products and their transformation into high-value products.

2. Agave By-Products

Figure 1 depicts the most important agave by-products and their different known applications. As previously mentioned, beverages made with agave predominate as the most popular application, followed by foods and agricultural purposes. Interestingly, some unconventional uses, such as toys, needles, and bleaching products, have been reported. Additionally, most of the by-products are of great interest for biofuels and composites fabrication.

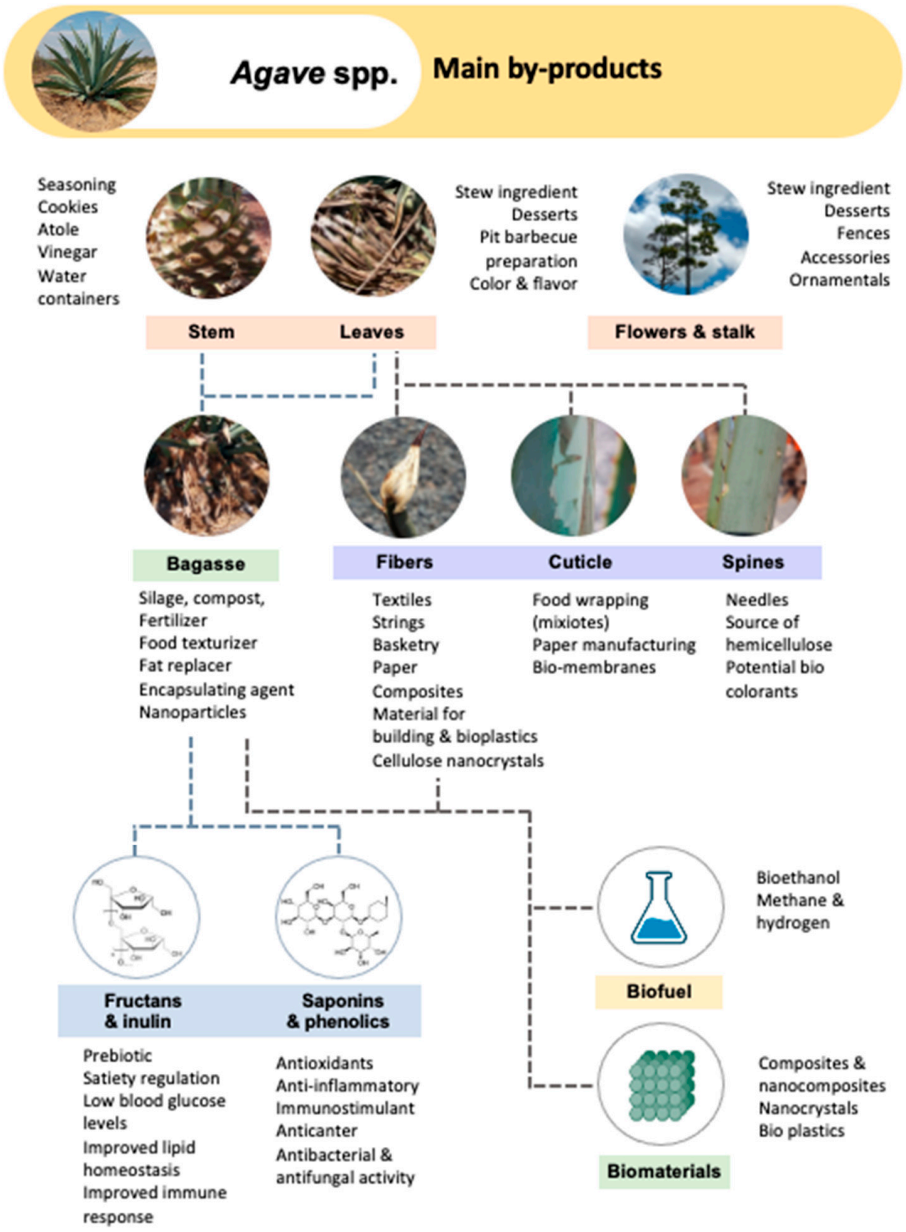

Figure 1. Most relevant by-products obtained from the agave plant and some reported uses.

Leaves, bagasse, and fibers (from leaves and stems) are the main by-products or residues generated by the agave industry; however, the spines, cuticle, and stalks are being explored for their important content of cellulose and some bioactive compounds.

Fresh leaves account for 25–50% of the plant [4,6]; approximately 5,168,200 tons of leave waste were produced from 2015 to 2019 and were derived from the tequila industry [4]. Leaves are lignocellulosic material mainly composed of strong and coarse fibers that have a traditional application for textile and fabric elaboration. Mainly, the *Agave sisalana* (sisal), *Agave mapisaga*, and *Agave salmiana* varieties provide hard fibers appreciated for their durability for ethnic clothing and strings [7]. Recent studies have shown that leaf inclusion in composites and bioplastics enhances some thermal and mechanical properties [8–16] and renders a significant yield of bioethanol [17–19]. Bagasse is the fibrous residue obtained after the stem is used either for tequila and mezcal elaboration or agave sap extraction. In the former, the stem is cooked and compressed to obtain the juice for further fermentation and distillation. The remaining material known as bagasse, represents approximately 40% of the original stem weight and is composed of cellulose and lignin. In the latter process, the stems are scrapped to obtain the sap and the fibers (bagasse) that come out and are considered waste and discarded. An estimated amount of 7,710,520 tons of residual bagasse were generated from 1995 to 2019 [4], which could have applications as compost [20], silage [21], and bioethanol and methane production [19,22–28], among other uses. Furthermore, bagasse is an excellent source of fibers, bioactive compounds (saponins, fructans, and phenolic compounds), sugars, and other valuable biomolecules that can be recovered from this residue [24,29–34].

Spines and stalks, although exploited to a lesser extent, represent an interesting source of bioactive compounds, bio colorants, and fibers for the textile industry, and could be a substrate for saccharification [35,36].

The cuticle, also denominated "mixiote", is the outer waxy membrane that covers the surface of agave leaves (Figure 1), protecting the plant from environmental conditions. Traditionally, the cuticle is used for wrapping meat preparations in Mexican cuisine and for paper manufacturing [37,38]. A recent publication has shown the value of the cuticular material as a proton-exchange bio-membrane integrated into an electrolyzer and fuel cells [38].

In summary, by-products from the agave plant have great applications in the energy and environmental sectors, as well as potential as functional ingredients for food products.

3. Chemical Composition of the Main Agave By-Products

The overall composition of agave is cellulose, hemicellulose, lignin, and pectin in fluctuating amounts according to the variety. The plant is a source of other molecules, such as glucose, sucrose, and fructose, fructans, gums, saponins, and phenolic compounds. Table 1 summarizes the chemical composition of several by-products from agaves. Given the importance of the tequila industry in Mexico, the predominance of *A. tequilana* by-products and information about their composition is found in the literature. Cellulose, mainly comprising glucans, is the main component of agaves, followed by hemicellulose (mainly xylans), and lignin. Cellulose is a valuable material since it can be hydrolyzed to obtain simple sugars (monosaccharides) that serve as substrates for fermentation and bioethanol production. Nanoparticles and bioplastics made with cellulose have also been obtained from leaves and bagasse [39,40]. Crystallinity is an important feature of cellulose that might be undesirable depending upon the application. A high crystallinity index makes cellulose less digestible and hard to convert into simple sugars by enzymatic or chemical methods [22]. Cellulose from leaves seems to have higher crystallinity than other tissues [39], although nanocrystals with a high crystallinity degree have been obtained from bagasse using an adequate pretreatment [39,40]. Fourier transform infrared spectroscopy (FTIR) experiments have shown that the leaves have a degree of crystallinity of 50.1% [41], proportional to the hydrogen bond formation. Moreover, the leaves have slightly higher concentrations of lignin than other by-products, increasing the recalcitrance of these tissues,

which necessitates a pretreatment for delignification before utilization as substrates for biofuel of methane production.

Table 1. Chemical composition of agave by-products.

Component (%)	*Agave tequilana*		*Agave salmiana*		*Agave durangensis*	*Agave americana*		*Agave fourcroydes*	*Agave angustifolia*	*Agave tequilana*
	Bagasse [1]	**Leaves** [2]	**Bagasse** [3]	**Leaves** [4]	**Leaves** [5]	**Leaves** [6]	**Stalk** [7]	**Leaves** [8]	**Spines** [8]	**Bagasse** [9]
Moisture	6.44–8.5	-	-	-	6.9	9.3	-	-	-	7.78
Protein	3.7–3.8	6.6–8.35	2.5–4.4	4.8	-	-	-	-	-	-
Lipids	0.3	-	-	-	-	-	-	-	-	-
Ashes	2.0–7.4	7.5	2.1–6.2	7.6	16.6	3.3	-	-	-	1.3
Holocellulose	-	-	-	-	20.4	-	-	-	-	-
Hemicellulose	4.4–20	15.2 -19.7	4.6	3.7	-	5.6–18.4	-	24	45	34.1
Cellulose	41.8–42.0	38.9–54.5	35.0	20.7	-	65.2–68.5	-	72	52	48.0
Lignin	7.1–20.1	9.8–16.3	13.0–19.1	9.5–26.1	14.5	2.7–9.1	18	14	14	20.7
Xylan	13.0–19.9	9.5–18.3	12.0	9.7	-	-	13.6	-	-	-
Glucan	30.9–45.6	35.0–38.8	34.1	35.2	-	-	-	-	-	-
Arabinan	0.5–0.9	1.5–2.1	1.0	2.4	-	-	1.0	-	-	-

[1] [10,26,42–45], [2] [18,26], [3] [26,46], [4] [26,47], [5] [34], [6] [41,48,49], [7] [36], [8] [35], [9] [50].

There are some differences in the composition of leaves and bagasse; it is known that bagasse contains higher amounts of simple sugars and xylan [26], whereas leaves and stalks are richer in lignin. Spines represent an interesting source of hemicellulose, since their content is comparable to that of cellulose [35]; other valuable compounds identified in spines include flavonoids, condensed tannins, and monolignol subunits. Although scarce studies are published on the chemical composition of foliar cuticle, all reported water, cutin and cutan as its main components [51].

4. Main Applications of Agave By-Products

The main properties and applications of agave by-products are summarized in Table 2.

Table 2. Functional properties and main applications of agave by-products.

Application	By-Product	Compound/Material	Functionality	References
Bioactive compound	Leaves, bagasse, and stem (*A. Americana*, *A. salmiana*, and *A. tequilana*)	- Fructans and inulin	- Prebiotics, stimulate microbiota growth. - Enhance lipid homeostasis. - Increase the production of anorectic hormones (GLP-1) and improves the immune response. - Low glycemic index. - Fermentable carbohydrate, stimulate the production of SCFA. - Reduces blood glucose and lipids levels.	[52–58]
	Leaves and bagasse (*A. americana*, *A. angustifolia*, *A. salmiana*, *A. tequilana*)	- Phenolic compounds: Flavonoids (such as glycosylated kaempferol and quercetin, hecogenin, diosgenin, chlorogenin, kammogenin, and gentrogenin) - Phenolic acids: ferulic acid, p-coumaric acid, caffeic acid, p-hydroxybenzoic acid, syringic acid	- Found in the bagasse after pulque and tequila processing. - Identified in leaves of several agave species. - Antioxidant and anti-inflammatory effect when tested by in vitro methods. - Antimicrobial activity.	[26,28,51,52,55,56,59]
	Bagasse (*A. tequilana*, *A. salmiana*)	- Pyranones and pyrazynes.	- Antioxidant capacity.	[31,59]
	Agave extracts	-Terpenes	- Antifungal activity.	[5,60]
	Leaves and bagasse (*A. salmiana*, *A. tequilana*, *A. sisalana*, *A. attenuata*, and *A. shevrei*)	- Steroidal aponins (Kammogenin glycosides and aglycones, manogenin glycosides) - Sapogenins (hecogenin, thiogenin and canthalasaponin 1).	- Hypocholesterolemic, antiobesity, immunostimulant and parasitic activities. - Anti-inflammatory activity tested in a membrane permeability induced by acetic acid model. - Antimicrobial activity. - Anti-inflammatory and antioxidant activity.	[5,29–31,61–64]
Food ingredient	Residual leaves Cuticle	The whole material	- Roasted or baked for enhanced flavor and color of culinary preparations, boiled to make soup. - Used as wrapping material for barbecue and steamed preparations (mixiote)	[37]
	Bagasse	Whole bagasse	- Added to oat cookies to improve the fructooligosaccharides content and some techno-functional properties.	[52]
	Stem (*A. tequilana*)	Fructans	- Increased the fiber content and reduced the glycemic index in granola bars. After in vitro fermentation, the production of SCFA was observed.	[56,65]

Table 2. *Cont.*

Application	By-Product	Compound/Material	Functionality	References
	Leaves (*A. americana*)	Powder of the whole material	- Used to improve texture, viscosity, and color of steamed yoghurt.	[66]
	Fibers (*A. angustifolia*)	Fructans	- Fat replacer in cookies. Fructans addition lowered the calorie content, increased the soluble fiber, and improved the sensory and techno-functional properties of cookies.	[67,68]
	Not specified	Fructans	- Encapsulation of *Eugenia uniflora* L. with fructans prolonged anthocyanins and color stability and prevented loss of antioxidant capacity. - Encapsulation of proteolytic extracts of *Bromelia pinguin* and *Bromelia karatas* with fructans by electrospraying minimized variability of antioxidant capacity during storage.	[69,70]
	Not specified	Fructans	- Mixtures of fructans and whey protein were effective to reduce hygroscopicity and to increase thermal stability of *Coccoloba uvifera* L. leaf extracts.	[71]
	Bagasse	Polysaccharides	- Used as encapsulating material for indomethacin nanoemulsions stabilization and dermal uptake.	[72]
Biofuel feedstock	Bagasse (*A. tequilana*)	Bioethanol	- The bioethanol recovery was 98 and 2% higher than ethanol obtained from corn and sugarcane, respectively.	[73]
	Bagasse (*A. tequilana* Weber)	Bioethanol	- Several pretreatments were used to improve saccharification and obtain 87–91% bioethanol yield	[74]
	Leaves (*A. tequilana*)	Bioethanol	- Fermentation of juice extracted from leaves with *Saccharomyces cerevisiae* rendered 13.8 g/L bioethanol.	[75]
	Leaves (*A. salmiana*)	Bioethanol	- Acid-alkaline pretreatment of leaves was effective to achieve 93% bioethanol yield.	[17]
	Bagasse (*A. tequilana*)	Bioethanol	- Yield of 61–68% bioethanol was reached after 7–13 h fermentation with *Saccharomyces cerevisiae.*	[18]
	Bagasse (*A. tequilana*)	Hydrogen	- Hydrolysates from bagasse subjected to enzymatic pretreatment and dark digestion, yield 3.4 mol hydrogen/mol.	[24]
	Bagasse (*A. tequilana*)	Hydrogen and methane	- Bagasse pretreated with alkaline hydrogen peroxide increased 1.5 and 3.6 times the hydrogen and methane production, respectively.	[25]
	Bagasse (*A. tequilana*)	Methane	- Anaerobic digestion of the bagasse rendered 0.26 L methane/g COD (chemical oxygen demand).	[23]
	Bagasse (*A. tequilana*)	Methane	- Methane yield was increased 1.5 times by ozone-assisted hydrolysis and dilute acid pretreatment.	[76]

Table 2. *Cont.*

Application	By-Product	Compound/Material	Functionality	References
Biomaterials	Fibers (*A. americana*)	Composites	- Fibers combined with HDPE are suitable for composites with application for roof panels manufacturing. This combination enhanced the tensile strength and thermal stability of composites.	[8]
	Fibers (*A. tequilana)*	Reinforcement material for composites	- Agave-polypropylene composites were obtained from heated fibers. Thermal stability did not change, whereas the mechanical properties were negatively affected.	[9]
	Fibers (*A. tequilana*)	Composites	- Agave fiber-filled polypropylene composites improved their mechanical properties and had better compatibility between matrices after fibers addition.	[10]
	Bagasse (*A. tequilana*)	Composites	- Extruded polylactic acid-based composites were added with 20–40% bagasse for plates manufacturing. Higher tensile and flexural strength was achieved with 40 % bagasse.	[12]
	Fibers (*A. tequilana*)	Composites	- Low weight polylactic acid-agave fiber composites were made by rotational molding. However, this method negatively affected the mechanical properties, and produced a material with high porosity.	[13]
	Fibers (*A. tequilana*)	Composites	- The addition of 15–30% agave fiber enhanced the mechanical properties of extruded PHB-bagasse composites and reinforced their toughness. A more thermostable material was produced when a compatibilizer was added (organic peroxide).	[14,15]
	Fibers (*A. tequilana*)	Bioplastic	- Hybrid composites made of pine fiber, agave fiber and HDPE showed higher tensile and flexural strength with 20/80 pine/agave ratio.	[16]
	Leaves and bagasse (*A. tequilana*)	Hybrid composites	- Sonochemical hydrolysis of bagasse and leaves significantly increased the nanocrystals yield. The highest production yield was obtained with leaves (93%).	[40]
	Leaves (*Agave pulquero*)	Nanoparticles	- Alkaline pretreatment of leaves and high-energy planetary micro milling were effective to obtain cellulose nanoparticles (3 nm) with a high degree of crystallinity.	[54]
	Leaf fibers (*A. americana*)	Cellulose nanocrystals	- The chemical pretreatment of fibers improved the composites compatibility with hydrophobic materials and mechanical properties.	[77]

SCFA-short chain fatty acids, COD-chemical oxygen demand, HDPE-high-density polyethylene, PHB-poly(3-hydroxybutyrate).

4.1. Functional Potential

As previously described, agave by-products are a rich source of bioactive compounds, with varying concentrations depending on the agave variety, age, environmental conditions, and the extraction method used to measure their content [78]. Recent reports have highlighted the role of different metabolites, such as fructans and saponins, in the bioactive properties of agave by-products [31,52]. Fructans are considered prebiotics because they are resistant to human digestion and reach the colon intact, where they can stimulate the growth of beneficial microbiota (e.g., *Bifidobacteria* and *Lactobacilli*) [52,53]. Fructans have been related to other health benefits, such as lower blood glucose levels, enhanced lipid homeostasis, increased mineral absorption and modulation of immune functions [52,54]. Recently, the bioactive composition of residual leaves from the mezcal industry were analyzed [79], and an important fructan content (37%) was found in mature leaves. Other publications have reported that agave fructans induce satiety by increasing the secretion of anorectic hormones (GLP-1) [55].

Recently, agave fructans have been employed as functional ingredients for granola bars [56], and encapsulating material for bioactive compounds, given their high degree of polymerization [80].

Inulin, a type of fructans and another constituent of agave by-products, has been extracted from *A. americana* leaves [57]. Inulin is a fermentable substrate for the colonic microbiota to produce short-chain fatty acids (SCFAs). There is plenty of evidence of the role of inulin as a prebiotic, which reduces blood lipids and glucose levels for the prevention of metabolic disorders [58].

Among the primary phytochemicals found in agave by-products, saponins, flavonoids and terpenes are the main compounds responsible for their biological effect. Some of the beneficial health properties attributed to agave saponins comprise hypocholesterolemia, anti-inflammatory, immunostimulant, antiobesity, and antiparasitic activities [5]. Several by-products, including leaves and bagasse, have a significant saponins content. Steroidal saponins have been isolated from *A. salmiana* bagasse by ultrasound-assisted (US) extraction and US-supercritical fluids [31,61]. Kammogenin glycosides and their aglycone, manogenin glycosides, and antioxidant compounds (pyranones and pyrazines) were identified in these residues. When a micellar extraction method was used, 38% ofsaponins were recovered from the *A. sisalana* waste [30]. Some other phytochemicals, such as phenolic compounds, including kaempferol, quercetin in various glycosylated forms, hecogenin, diosgenin, chlorogenin, kammogenin, and gentrogenin have been identified in agave and its by-products [59,81].

4.1.1. Antioxidant and Anti-Inflammatory Activity

The antioxidant potential of agave by-products is associated with the presence of flavonoids (such as kaempferol and quercetin) found in the leaves [82], as well as pyranones and pyrazynes identified in the bagasse [31], which have shown an antioxidant effect when tested by in vitro methods [59]. In general, the agave genus has anti-inflammatory properties, attributed to the presence of saponins, phenolic compounds and terpenes. Steroidal saponins isolated from *A. attenuata* and *A. shevrei*, exhibit anti-inflammatory activity in a membrane permeability induced by acetic acid models [62,63]. It has been reported that *A. angustifolia* Haw, *A. tequilana* Weber, and *A. americana* contain a mixture of sapogenins, such as hecogenin and thiogenin and canthalasaponin 1, that confer the anti-inflammatory activity [63]. Saponins, flavonoids and terpenes have been identified in the bagasse of *A. tequilana* and *A. salmiana* after tequila or pulque production [31,83]. A recent metabolomic profiling study analyzed the leaves of *A. americana* L., *A. americana* var. marginata Trel, *A. angustifolia* Haw. cv. marginata, *A. desmettiana* Jacobi, and *A. pygmaea* Gentry, identifying 56 metabolites [84]. Steroidal saponins, sapogenins, flavonols, homoisoflavonoids, phenolic acids (ferulic acid, p-coumaric acid, caffeic acid, p-hydroxybenzoic acid, syringic acid), and fatty acids were the most abundant phytochemicals of these by-products. Agave

leaf extracts showed inhibitory activity against acute inflammation due to the presence of spirostane saponins.

As a result of the antioxidant and anti-inflammatory activity, agave by-products are potential candidates for anticancer and anti-hypertensive activity [5]. However, other potential biological effects must be explored in depth. Several studies have focused on identifying and quantifying bioactive compounds; thus, the next step must include the validation of the biological effects of agave by-products.

4.1.2. Antibacterial and Antifungal Activity

The antimicrobial and antifungal effects of agave are also related to phenolic compounds and saponins, which interact with bacterial membrane proteins and lipids [59,81]. These compounds induce changes in the hydrophobicity, surface charge and membrane integrity causing leakage of intracellular components of Gram-positive and Gram-negative bacteria, resulting in cell death [85]. Previous studies have shown that agave extracts have an inhibitory effect on microorganisms, such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *B. subtilis*, *Serratia marcescens*, *Listeria monocytogenes*, *Clostridium perfringens*, and *Shigella dysenteria*. This effect is attributed to saponins glycosides, terpenoids, steroids, flavonoids, and tannins identified in this material [29,64]. The antifungal mechanisms of terpenes are mainly associated with the disruption of membranes and cell walls, resulting in leakage of intracellular components [5,60], whereas saponins produce complexes with sterols and cause sterol-dependent membrane permeabilization [64]. Extracts obtained from Agavaceas (*A. scabra, A. striata*, *A. Victoria*, and *A. bracteosa*) have inhibitory effects on the growth of *Aspergillus flavus* and *Aspergillus parasiticus*, specifically, while *A. americana* has the ability to inhibit conidiogenesis, causing mycelial morphological changes in fungi [86].

4.1.3. By-Products as Food Ingredients

Agave is of great importance in Mexican cuisine, especially for producing traditional beverages (Tequila, Mezcal, Pulque, Bacanora). For hundreds of years the plant has had a variety of culinary and medicinal uses. The entire agave plant can be used in different Mexican dishes as described below. The specific uses of the different by-products are focused on texture improvement, microencapsulation, or inclusion as a prebiotic ingredient in food products.

Residual leaves from the tequila, mezcal, and pulque fabrication, are traditionally roasted or baked for a rich caramel color and flavor, boiled to make a bitter soup, or used as a wrapping material for pit-barbecued meat [37]. The cuticle (mixiote) is carefully separated from the plant to obtain a broad, translucent, and thin membrane that is used in a variety of preparations made with different meats, sauces, and other ingredients, which are wrapped in the mixiote and steamed; the resulting stew is also called mixiote.

In the food industry, the techno-functional properties of some food products have been improved by adding leaves and bagasse extracts. For instance, *A. americana* leaves have been incorporated as powder in the formulation of steamed yoghurt, and a significant improvement of color, texture and viscosity of the product was observed [66]. Moreover, fructans isolated from *A. angustifolia* were used as fat replacers in cookies [67,68]. The incorporation of fructans produced a significant reduction in the calorie and fat content, and an increment of soluble fiber. Besides, the water and oil holding capacity was superior to the control cookies without the addition of fructans, which rendered a higher yield (bigger cookies). The sensory and texture properties of the cookies were also enhanced, such as color and hardness. Moreover, a sensory analysis did not show differences in the overall preference in formulations that contained 10% and 20% fructans as fat replacers [68].

Despite the well-known low glycemic index of agave fructans that are isolated from the syrup, there is scarce literature assessing this feature for agave by-products. In this sense, one study has evaluated the effect of *A. tequilana* fructans supplementation in a mouse model [55]. After 5 weeks of feeding a diet with 10% fructans, the glycemic response

improved, and some satiety hormones increased as well. In another study, the feasibility of incorporating *A. tequilana* fructans in the formulation of low glycemic index oat-based granola bars was demonstrated. The content of soluble fiber increased to 23% after the fructans' addition, resulting in a moderate glycemic index product [56]. In a further work, the in vitro fermentation of granola bars confirmed their prebiotic effect by producing SCFA with known health benefits [65]. Interestingly, when the agave bagasse was added to the oat cookies, the fructooligosaccharides content and oil holding capacity increased without altering the sensory and textural properties [52].

Agave fructans have also shown good potential as an encapsulating material, protecting food ingredients from degradation. For instance, fructans sprayed on *Eugenia uniflora* L., conferred stability for bioactive molecules (anthocyanins) and color, and prevented the loss of the antioxidant capacity of the encapsulated material [69]. The same type of fructans were used for encapsulating proteolytic extracts of *Bromelia pinguin* and *Bromelia karatas* by electrospraying [70]. The encapsulated material had a minimal loss of proteolytic activity during storage. Although an initial reduction of the antioxidant capacity and anti-inflammatory ability was observed after electrospraying, the variability during storage was minimized. Furthermore, *Coccoloba uvifera* L. leaf extract was encapsulated by electrospraying with mixtures of whey protein and agave fructans [71]. The resulting material had a lower hygroscopicity, a higher thermal stability and exhibited an in vitro controlled release of the extract.

Regarding agave bagasse, isolated polysaccharides from an *A. salmiana* bagasse were used to elaborate and stabilize indomethacin nanoemulsions, and to improve the cellular uptake in a human dermal fibroblast model [72].

4.2. Agave as a Potential Biofuel Feedstock

Agave could be a promising bioenergy feedstock due to its high productivity, high capacity to thrive in semi-arid regions, and efficiency in the use of water [87]. Commercial agave crops have annual biomass productivities ranging from 8.5 ton $\times$ a^{-1} $\times$ $year^{-1}$ to 22 ton $\times$ ha^{-1} $\times$ $year^{-1}$ [88], and their remarkable sugar content and lower lignin levels (compared with their cellulose content), make agave by-products an attractive material from a bioprocessing perspective.

4.2.1. Bioethanol

The bioethanol production capacity of agave by-products would be comparable (or superior) to other ethanol feedstocks, such as maize, switchgrass, and sugarcane, in terms of life cycle energy, water use, and greenhouse gas (GHG) balances [73]. The readily fermentable, water-soluble carbohydrate (WSC) fraction of the agave leaves was comparable to that of conventional lignocellulosic feedstocks, such as sugarcane bagasse and corn stover [89]. Some studies indicate that the cost for biofuel production from agave stems is approximately 0.5–9 USD per liter. This cost could be greatly reduced if the by-products (considered waste) are also used, for example the leaves and bagasse [90]. A more recent study reported a calculated cost of USD 1.68 per gallon of bioethanol produced from *A. tequilana* bagasse, which is lower than the theoretical value reported by the U.S. Department of Energy, and the cost of bioethanol production from sugarcane bagasse [91].

Bioethanol can be produced from different sources and is classified by generations. The second-generation (2G) of bioethanol is obtained from lignocellulosic materials (LCM), such as agro-industrial residues [74,92]. For 2G bioethanol production there are three fundamental stages. The pretreatment step is required to decrease the recalcitrance of the lignocellulosic material and increase the accessibility of simple sugars. The next step is an enzymatic saccharification or hydrolysis, where the enzymes transform cellulose into their monomeric form to obtain fermentable sugars. Finally, a fermentation step is carried out with a microbial inoculum to convert sugars into ethanol [74].

Both bagasse, and residual leaves have been used for bioethanol production. *A. tequilana*, *A. atrovirens*, and *A. salmiana* are frequent substrates for this purpose. An adequate pre-

treatment for delignification is crucial for a high ethanol yield, along with an efficient saccharification step. Yields up to 38.39–55.02 g/L ethanol (87–91%) have been achieved with different methods with *A. tequilana* Weber bagasse [74]. For instance, a 93% saccharification yield was obtained in an improved process of enzymatic saccharification on *A. salmiana* leaves [17]. The bioethanol conversion was higher when *Kluyveromyces marxianus* was used (93%), compared with a *Saccharomyces cerevisiae* strain (87%). Carbohydrates from *A. tequilana* juice and bagasse were fermented with *Saccharomyces cerevisiae*, during 7 h and 13 h respectively, achieving ethanol concentrations of 12.4 g/L and 38.6 g/L, equivalent to a 68% and 61% yield, respectively [18], whereas the fermented juice from the *A. tequilana* leaves with *Saccharomyces cerevisiae* rendered ethanol concentrations of 13.8 g/L [75].

4.2.2. Hydrogen and Methane Production

In recent years, agave bagasse has been used for hydrogen and methane production. Hydrogen is produced by dark fermentation, while methane is obtained by anaerobic digestion. Dark fermentation refers to the partial oxidation of carbohydrates to produce volatile fatty acids (VFA), mainly acetate and butyrate, with the concomitant production of molecular hydrogen (H_2). This process is carried out by acidogenic microorganisms (e.g., *Clostridiaceae* and *Enterobacteriaceae* families). A subsequent anaerobic digestion is performed with the consumption of H_2 and CO_2 by hydrogenotrophic and acetate by acetoclastic methanogens, respectively, to produce methane [93].

Hydrogen production by dark fermentation is considered a promising biotechnological process towards the establishment of lignocellulosic biorefineries due to its high-efficient conversion to electricity. Agave bagasse hydrolysates have demonstrated to be a feasible feedstock for hydrogen production [76]. In this sense, acid and enzymatic hydrolysates of *A. tequilana* bagasse were digested at different concentrations 20–100% *v/v* to produce hydrogen [24]. The enzymatic pretreatment was the most effective method to obtain a high yield of hydrogen (3.4 mol H_2/mol hexose).

For methane, previous studies have used an *A. tequilana* bagasse as a raw material to produce biogas, obtaining a constant rate of methane production of 0.26 L CH_4 per g COD [23]. Other pretreatments have also shown to be efficient for methane production, among these, dilute acid and ozone-assisted hydrolysis had the highest methane yield when compared with enzymatic techniques [76]. Alkaline hydrogen peroxide (AHP) pretreatment followed by enzymatic hydrolysis (cellulases + hemicellulases) also improved the hydrogen and methane production from the *A. tequilana* bagasse. The resulted yield was 1.5 and 3.6-times (215.14 $\pm$ 13 L H_2 and 393.4 $\pm$ 13 L CH_4 per kg bagasse, respectively) superior to those obtained with the non-pretreated bagasse [25].

Biogas production does not depend on the climate or geographical conditions; thus, it can be produced anywhere, representing an important advantage over other biofuels.

4.3. Nanocomposites and Nanocrystals

Agave by-products have been used as biomaterials for composite fabrication or as reinforcements for polymer-based composites. Particularly, the remaining fibers from the stem left after tequila and mezcal processing, or fibers obtained from leaves, have shown remarkable thermo-mechanical properties, including tensile strength and thermal stability.

An important drawback of natural fibers is their low thermal stability and weak bonding with hydrophobic polymers. Certain chemical pretreatments cause surface modifications and remove non-cellulosic components from agave by-products, providing strength and compatibility with hydrophobic polymers, and thus, enhancing their properties as composite materials [77].

An interesting work showed that *A. americana* fibers subjected to mercerization (alkali treatment) and silane treatment, are low-cost materials that, combined with recycled high-density polyethylene (HDPE), are suitable for the fabrication of roofing panels [8]. The mercerization treatment improves the fibers' tensile strength; thus, the material has a higher resistance to breakage, and increases the interfacial bonding between the fibers and HDPE.

Cellulose crystallinity is another important feature of agave fibers, in this regard, a higher crystallinity index has been related to better mechanical properties for composites, although some studies have not found an improvement of these properties when fibers crystallinity is increased [9].

Heating or the application of chemical pretreatments has been successfully used to decrease the amorphous components of *A. americana* leaf fibers for an improved compatibility with hydrophobic materials [77], for enhancing thermal resistance and the crystallinity index of *A. tequilana* fibers-polypropylene composites [9], and to increase the stiffness and flexural strength of these composites [10]. For thermal treatments, washing at 85 °C with water resulted in a better compatibility and fiber adhesion to artificial polymers (polyethylene) than steam heating [10].

Agave (rambans) fibers are good reinforcements for other materials, such as polyester resins, to obtain light-weight composites by mold forming [11]. Interestingly, polylactic acid-based composites obtained by extrusion and press molding with a 20–40% addition of *A. tequilana* bagasse fibers were used for plates elaboration [12]. An increase in tensile and flexural strength was observed with the addition of up to 40% of agave fibers, although brittleness and water absorption also increased. In another study, composites made from agave fibers and polylactic acid were obtained by rotational molding [13]. The composites showed a lower bulk density with an increasing addition of agave fibers, which translates into lower weight materials. Compared with press molding, this method resulted in high porosity composites caused by the poor adhesion between polylactic acid and the fibers, moreover, the thermal and mechanical properties were slightly better for press molding except for hardness, that was improved by the rotational molding process.

Bioplastics are increasingly produced given their biodegradable nature. For this reason, the use of natural polymer composites, such as poly(3-hydroxybutyrate) (PHB), has become popular. Two recent works have explored the use of PHB and *A. tequilana* bagasse to make extruded composites [14,15]. The inclusion of 25–30% bagasse significantly improved the mechanical properties of the composites, mainly the tensile and flexural strength, reducing the brittleness of the blend.

Lastly, hybrid composites containing pine sawdust, agave bagasse and HDPE were fabricated in a twin-screw extruder [16]. Results showed that the inclusion of agave bagasse increased the flexural and tensile strength, whereas the pine was helpful in reducing water absorption and conferring stability to the composites.

Another potential application of agave by-products includes the production of nanoparticles. Given the abundance of cellulose in agave, nanomaterials from this biopolymer may have wide applications in the material and pharmaceutical industry. In this sense, cellulose isolated from *A. tequilana* leaves and bagasse was subjected to sonochemical acid hydrolysis for nanocrystal formation, while mechanical defibrillation was used to obtain cellulose nanofibers [40]. Results showed that the bagasse was easier to convert into nanocrystals and fibers, given the previous pre-processing during tequila making, although the highest yield (93% and 60.84 for crystals and fibers, respectively) was attributed to the leaves.

Nanoparticles have also been obtained from residues of the "pulque" production. Leaves of the agave "pulquero" were pretreated with an alkaline solution (5% NaOH) and then milled using a high-energy planetary micro mill [48]. An average size of approximately 3 nm was achieved with this process, and the agave cellulose had a high degree of crystallinity, which had a great influence on the nanoparticle size.

Overall, the information gathered from the aforementioned studies points to the potential of agave by-products as an important source of biomolecules, and as low-cost biomaterials that could be used to produce compostable and biodegradable composites and renewable biofuels.

5. Processes and Technologies for the Treatment of Agave By-Products

There are several processes applied to agave by-products to exploit their potential as bioactive ingredients, biofuels or biomaterials. The most relevant processing techniques are described in Table 3.

5.1. Chemical Pretreatments

The use of acids and alkali as pretreatments of agave by-products is common. In this step, fibers are extracted by hydrolysis of the lignin and hemicellulose. Acid hydrolysis depolymerizes the lignocellulosic material, increasing the amorphous cellulose, which favors the release of sugars and improves their digestibility and fermentability [19,76,94]. Diluted acid is the most effective way to achieve high efficiency in hemicellulose depolymerization, but also to degrade the amorphous regions and to obtain cellulose nanocrystals [39]. Acetic, formic, phosphoric, and sulfuric acids (>95 wt%) are widely used in the pretreatment step. *Agave salmiana* leaves were pyrolyzed and treated with phosphoric acid at three concentrations (30, 60, 85% *w/v*) to obtain activated carbon. The highest yield was achieved with a mild concentration. In another study, hydrochloric acid (2.7%) was used for the pretreatment of *Agave tequilana* bagasse subjected to anaerobic digestion [24]. There was a higher yield for simple sugars and valuable compounds (i.e., phenolic compounds) compared with enzymatic hydrolysis, however, toxic compounds, such as formic acid, hydroxymethylfurfural (HMF) and furfural were also formed during the activation process. To improve the sugar recovery in this material, sulfuric acid was used at different concentrations (1–4% *v/v*) and tested for monosaccharides [18]. The sugar composition varied with concentrations and at 2% sulfuric acid the highest monosaccharide recovery was obtained with a predominance of xylose and glucose.

The leaves of *A. atrovirens* Karw were used to obtain cellulose of high purity with formic–peroxyformic acid that was produced by the combination of formic acid (80, 87.5, and 95%) hydrogen peroxide (2, 3, and 4%) [95]. Organic solvents (organosolv), such as formic acid, have been used for delignification due to the high affinity of lignin for these compounds. With this process, the highest pulp yield was reached with 80% formic acid, 2% hydrogen peroxide. Under these conditions a low content of lignin (3.6%) was obtained, which indicates high cellulose abundance and purity.

Hydrolysis with alkali allows the separation of the lignin and cellulose, modifies the degree of polymerization, as well as some structural properties [8,47]. For instance, mercerization, consisting of an alkali treatment, has been used to improve the fiber's tensile strength for composites production [8]. For this purpose, leaves from *Agave americana* were boiled in 1N NaOH for 24 h; authors observed enhanced interfacial strength between the fibers and thermoplastic polymers, as well as thermal stability after the mercerization step. Delignification is another pretreatment to make cellulose and hemicellulose available for fermentation. This step is essential to improving cellulose breakdown into simple sugars (saccharification), and if not performed, the saccharification yield is remarkably lower [24,25].

Alkaline delignification with dilute solutions of NaOH (2%) has been tested on agave bagasse prior to the enzymatic step for saccharification [19]. A higher amount of reducing sugars was obtained with the addition of alkali, specifically, the glucose content was significantly increased compared with an acid pretreatment.

Ammonia fiber expansion (AFEX) is another chemical treatment applied to agave waste [26,42]. The treatment consists of exposing the material to liquid or gaseous ammonia at moderate temperatures (90–100 °C) and pressures (250–300 psi) to disrupt and solubilize lignin and other recalcitrant components. The advantage of this method is the minimal water requirements and the absence of effluents. Moreover, approximately 97% of the ammonia can be recovered and re-used [26,42]. The bagasse and leaves from *A. salmiana* and *A. tequilana* were treated with AFEX in a biorefinery process with high glucans and xylans conversion to simple sugars [26]. Moreover, a considerable yield of bioethanol was produced, >19% for the leaves, and 15–17% for the bagasse.

Table 3. Technologies used for agave by-products processing.

By-Product	Process	Application	Main Outcomes	Reference
Agave americana leaves	For mercerization, leaves were boiled in 1N NaOH for 24 h at 70 °C.	Mercerization was used to obtain fibers with quality to produce composites.	The alkaline treatment increased tensile strength and interfacial strength of fibers. Thermal stability was also enhanced with this process.	[8]
Agave tequilana leaves and bagasse	Pretreatment with sulfuric acid (1–4%) for 30, 60, and 90 min at 115, 120, and 130 °C. Enzymatic saccharification with Cellic® CTec2 at a dose of 3, 6, 10, and 15%. Fermentation was performed with *Saccharomyces cerevisiae*.	Bioethanol production from bagasse after enzymatic saccharification.	The highest sugar recovery (117.9 mg/g) was obtained with 2% H_2SO_4/60 min/120 °C with the lowest amount of degradation products, such as acetate and HMF. A maximum saccharification value of 69% and 38.6 g/L bioethanol was achieved.	[18]
Agave atrovirens bagasse (metzal and metzontete)	Pretreatment by acid hydrolysis with HCl (1.2% *v/v*) or combined alkaline pretreatment with NaOH (2%) followed by enzymatic hydrolysis (Viscozyme, Celluclast, Novozyme, Cellubrix and Pulpzyme). Fermentation with *Saccharomyces cerevisiae* at 30 °C for 48 h.	Delignification and enzymatic saccharification for bioethanol production.	A higher yield of glucose was reached with the alkaline-enzymatic pretreatment of metzal (56%). Although higher bioethanol production was obtained by acid hydrolysis of metzal (29.81%) and mezontete (33.42%).	[19]
Agave tequilana bagasse	Cooked and uncooked bagasse were used. Dilute acid pretreatment with HCl 5% *w/v* at 90 °C for 2 h. Anaerobic digestion was performed in a lab-scale PVC reactor. A loading of 5.8 suspended volatile solids/L from a granular sludge, and 5 g/L COD/L bagasse hydrolysate was used at 32 °C, 2N NaOH.	Acid pretreatment, followed by anerobic digestion to obtain methane.	Uncooked bagasse was more suitable for methane production (0.26 CH_4/g COD). Acetate (136 mg/L) and propionate (109 mg/L) were obtained as by-products.	[23]
Agave salmiana leaves	Dilute acid treatment. HCl 2.7% *v/v*	Pretreatment for saccharification of fibers obtained from leaves.	Higher monosaccharides and phenolic compounds yield compared with enzymatic hydrolysis. Formation of hydroxymethylfurfural and furfural.	[24]
Agave tequilana bagasse	Pretreatment with 2% (*w/v*) alkaline hydrogen peroxide (AHP) at 50 °C for 1.5–6 h. Saccharification was performed with Celluclast and Viscozyme at 40 °C, 120 rpm, 12 h. Anaerobic digestion was performed with a granular sludge loading 10 volatile solids/L and 5 bagasse hydrolysate/L.	Oxidative delignification with AHP to enhance methane production.	Higher delignification was achieved at 1.5 h after AHP pretreatment. The sequential saccharification with Celluclast first and then Viscozyme was more effective (35%). The hydrogen production was 215.14 ± 13 L H_2/kg of agave bagasse after 64 h and for methane, the yield was 0.20 ± 0.02 L CH_4/g COD.	[25]
Agave tequilana and *Agave salmiana* bagasse and leaves	Pretreatment with ammonia 0.5–2 g/dry matter (DM) for bagasse and 1–3 g/DM for leaves. Saccharification was performed with a mixture of Cellic® CTec3 and HTec3 (Novozymes) and Multifect® Pectinase. Fermentation was conducted with *S. cerevisiae* 424A (LNH-ST) at 30 °C/150 rpm/72 h.	Pretreatment for bio-ethanol production.	A high xylans and glucan conversion (>85%) was observed at the optimized conditions that differed for the agave residues. Leaves produced greater yield in the saccharification process and during fermentation. Up to 19.8% and 15–17% ethanol yield was achieved for leaves and bagasse, respectively.	[26]
Agave tequilana bagasse	IL-10% bagasse/[C2mim][OAc]) autoclaved at 120 °C for 3 h. Organosolv–50 g bagasse, 4.5% water, 25% ethanol and 0.5% H_2SO_4. Processed at 160 °C, 138 psi and 10 min in a high-pressure chemical reactor. Saccharification (50 °C/48 h) with cellulase CTec2® and enxylanase HTec2® (Novozyme), respectively and fermentation (48 h) at 37 °C/60 rpm.	Pretreatment of bagasse to improve saccharification and bioethanol production.	IL–86% xylan removal, 45% lignin reduction. Organosolv–50% xylan removal, 45% lignin reduction. High glucan (>90%) and xylan (>84%) conversion into simple sugars. Ethanol yield was similar, 82% for IL and 85% for organosolv.	[27]

Table 3. *Cont.*

By-Product	Process	Application	Main Outcomes	Reference
Agave tequilana bagasse	The autohydrolysis was carried out in a bioreactor at 140–200 °C for 15–60 min, loaded with 83.33 g bagasse and 500 mL water. Saccharification was performed with Cellic®, then a fermentation with *S. cerevisiae* was conducted at 32 °C, 100 rpm and 12 h.	A pretreatment using autohydrolysis was optimized to improve saccharification and bioethanol production.	The optimal conditions that increased the bagasse digestibility were: 90 °C, 30 min. The saccharification yield was 74.3% that allowed to obtain 98% yield of bioethanol (65.6 g/L) after 10 h.	[28]
Agave atrovirens Karw, fibers from leaves	Peroxyformic treatment: formic acid (80, 87.5, and 95%) hydrogen peroxide (2, 3, and 4%). Processing conditions: 60, 120, and 180 min at 60, 70, and 80 °C.	Organosolv pulping to obtain high purity fibers.	The best yield was obtained with 80% formic acid, 2% hydrogen peroxide, 180 °C for 70 min. A high purity was achieved since very low lignin content was found (k = 3.6).	[94]
Agave salmiana leaves	Pretreatment with 1–4% (*v*/*v*) sulfuric acid at 121 °C and 30–60 min. A subsequent treatment with 1–4% NaOH under the same conditions. Saccharification was performed with Celluclast at 50 °C, 150 rpm for 48 h.	A sequential acid-alkaline pretreatment was used to improve saccharification.	Delignification (91%) and saccharification (93.1%) was successfully achieved with the sequential pretreatment. The best conditions were 1% sulfuric acid (90 °C) and 3.4% NaOH (70 °C).	[95]
Agave tequilana bagasse	Autohydrolysis—1:10 bagasse to water at 180 °C, heated in a pressurized batch reactor (140 psi). AFEX-Anhydrous liquid ammonia was added to bagasse (2:1) and heated in a high-pressure stainless-steel Parr reactor at 102 °C for 30 min. IL-10% bagasse/[C2mim][OAc]) autoclaved at 120 °C for 3 h. Saccharification (50 °C/72 h) with cellulase CTec2 and enxylanase HTec2, (Novozyme).	Comparison between pretreatments of bagasse to reduce recalcitrance and improve saccharification.	Autohydrolysis and IL had the highest lignin and xylan removal. Autohydrolysis: Residual content 5.9% xylan, 34.3% lignin, 14.4 g/L of simple sugars. AFEX: Residual content 15.3% xylan, 18.2% lignin, 21.4 g/L IL: Residual content 14.6% xylan, 13.8% lignin, 25.5 g/L of simple sugars. Untreated bagasse: Residual content 4.1 g/L of simple sugars	[47]
Agave tequilana bagasse	IL-15% bagasse/[C2mim][OAc]) autoclaved at 120–160 °C for 3 h. Enzymatic saccharification (55 °C for 72 h) with cellulase CTec2 and enxylanase HTec2, (Novozyme).	Application of IL pretreatment to improve saccharification.	The use of IL at 160 °C allowed 45 % delignification, and saccharification efficiency of 85% (7.64 mg/mL of simple sugars).	[42]
Agave tequilana bagasse	IL-10% bagasse/[C2mim][OAc]) autoclaved at 40–160 °C for 3 h.	IL pretreatment for enhanced delignification, morphological and structural properties.	Pretreatment at 120 °C removed the highest amount of lignin, glucans, and xylans. A decrease in cellulose crystallinity was also observed.	[43]
Agave durangensis leaves	Ultrasound pretreatment (42 kHz, 132 W, 60 min) or autoclaving at 120 °C for 60 min. Phenolic compounds were analyzed by HPLC. Additionally, inulinase, β-fructofuranosidase and cellulase were produced by submerged fermentation with spores of *A. niger* isolated from *A. durangensis.*	US pretreatment for modification of the leaves' structure and enzyme production.	The US treatment increased the lignin content up to 43% and holocellulose up to 45%, but decreased fructans, fructooligosaccharides, and simple sugars. Moreover, certain phenolics. such as quercetin glucuronide, rutin, and procyanidin B2. The enzymatic activity was also enhanced, specifically the inulase and cellulase activity.	[34]
Agave americana biomass (by-product non specified)	Pretreatment with 72% sulfuric acid at 121 °C for 1 h. Glucosidase 49291–1G, cellulase Onozuka R-10, pectinase Guojiaomei, and Cellulase Celluclast were used for saccharification at 50 °C, 150 rpm, 72 h. Lignocellulosic enzymes were produced from spores of *Aspergillus niger* Gyx086 fermented for 8 days at 30 °C.	Biomass saccharification.	The sugar content after acid pretreatment was 29.1%. The combination of glucosidase and pectinase or pectinase alone showed the highest saccharification activity. Lignocellulosic enzymes had mainly PG and xylanase activity. This allowed to obtain reducing sugars at 35 °C for 72 h.	[44]

Table 3. *Cont.*

By-Product	Process	Application	Main Outcomes	Reference
Agave sisalana fibers (sisal waste)	Ultrasound was used under the following conditions: US power 50–70 W, temperature 40–60 °C, time 10–30 min, and solid-liquid ratio 20–40 g/mL.	US-assisted extraction of pectin from sisal waste.	The best conditions for pectin extraction were 61 W, 50 °C, and 26 min with 30 % yield.	[96]
Agave sisalana fibers (sisal waste)	Ultrasound at 400 W and 24 kHz. Sisal fibers and a 1–6% (*m/v*) NaOH solution, 10–50 min, 30–90% amplitude.	US pretreatment for delignification and cellulose solubilization	The optimal values were 27 min, 4.1% (*m/v*) NaOH, and 50% amplitude. An 82% removal of hemicellulose and 86% of lignin was achieved.	[97]
Agave lechuguilla leaves	Ultrasound were applied at 750 W, 20 kHz, and 60 °C. The microwave treatment was applied at 1000 W. The conditions tested were: 0.5–1.5% (*v/v*) H_2SO_4; 5–15 min, 60–140 °C, amplitude 40–100%, solid to liquid ratio 1:12–1:36 (*w/v*)	Comparison between US and microwave treatment to remove lignin	The H_2SO_4 concentration was the most important factor that improved the enzymatic hydrolysis. US allowed a higher removal of lignin (67%), whereas microwaving resulted in a lower hemicellulose content and higher sugar content (74%)	[98]
Agave sisalana waste	Optimization for saponins extraction was performed with 0–100 ethanol, 30–70 °C, 100–300 rpm, 0.17–0.33 waste/solvent ratio for 4 h. Micellar extraction was conducted with 5% (*v/v*) Triton-X.	Extraction of bioactive compounds.	Micellar extraction with Triton-X showed a higher saponins recovery than other methods (US and US-assisted micellar extraction). Low yield for phenolic compounds was obtained (22–27%).	[30]
Agave salmiana bagasse	Supercritical extraction was carried out at 700 bars and 70 °C. Ultrasound were applied at 60 W and 30 kHz. A mass load of 5 and 10 g was used with pressure 150–450 bar, 40–60 °C, and 5–10% co-solvent.	Ultrasonically-assisted supercritical fluid treatment to extract saponins.	An increase (25%) in the saponin content and antioxidant capacity was observed after US application with the lower mass load. Higher pressure and temperature increased the antioxidant capacity (300 bar and 60 °C with 10% co-solvent).	[31]
Agave salmiana bagasse	Control without US application: 60 °C, 58% ethanol, and S/M 20 under mechanical agitation for 60 min. US treatment: 60 °C, 0% ethanol (100% water), and solvent to mass ratio (S/M) of 20.	Steroidal saponins extraction.	Optimal conditions for US-assisted extraction were 60 °C, 0% ethanol (100% water), and S/M 20. The highest saponins recovery was 24.41 mg/g for US and 22.48 for control samples.	[59]
Agave salmiana leaves	Leaves were pyrolyzed and activated with H_3PO_4 (1:2 *w/v*). To obtain activated carbon (AC), microwaves were applied at 200–600 W, 2–6 min, 30–85% H_3PO_4, and nitrogen flow rate of 100–200 mL/min.	Microwaves were used as pretreatment to obtain activated carbon for removal of methylene blue.	The optimal conditions were 200 W, 4 min, 60% H_3PO_4, and nitrogen flow rate of 200 mL/min. AC yield ranged from 73 to 81%, that removed 71.41% of methylene blue.	[99]
Agave angustifolia stem	Microwaves were applied at 300 W and 2450 MHz, for 5–15 s. An ethanolic solution of 0.2 *n* KOH was used for the extraction.	Microwaves were applied for extraction of phytosterols.	The lowest extraction time (5 s) had the highest yield of 124.76 mg b-Sitosterol b-d-Glucoside/g.	[100]
Agave angustifolia Haw	Organosolv assisted by microwaves application—40–60% ethanol and 1–2 h, 0.1 % HCl	Organosolv assisted with microwaves for bagasse fractionation and bioetanol production.	The highest extraction yield (70.39%) was achieved at 50% ethanol for 1.5 h.	[101]
Agave atrovirens leaves (branches)	A WR90 straight rectangular waveguide was used to measure the absorption coefficient.	The microwaves' absorption capacity of leaves was measured.	Agave leaves were better microwaves absorbers than other plant materials, being a good alternative to polyurethane, a material commonly used as absorber.	[102]

k number = level of lignin remaining in the pulp, PVC-Polyvinyl chloride, COD-Chemical oxygen demand.

Ionic liquids (IL), especially 1-ethyl-3-methylimidazolium acetate ([C2mim][OAc]), have also been used as pretreatments for delignification and xylan removal. With this method, the cellulose from the agave bagasse becomes less crystalline, facilitating the further saccharification process [43] due to the lower content of lignin and xylan that reduce the recalcitrance. Waste from the tequila industry, mainly agave bagasse, has been pretreated with [C2mim][OAc] in varying concentrations [27,42–44]. Temperature was within the main factors affecting the lignin reduction: up to 45% at 160 °C (15% bagasse/[C2mim][OAc]) [43], and up to 16% at 120 °C (10% bagasse/[C2mim][OAc]) [44], both for 3 h.

Compared with other methods, IL has shown a better performance to reduce waste recalcitrance. A comparative study between chemical pretreatments for agave bagasse showed that autohydrolysis (bagasse + hot water) was the most efficient method to remove xylans and IL the best method to reduce the lignin content, whereas AFEX did not significantly change the composition of the agave bagasse [42]. An improvement in the conversion of the remaining xylans and glucans to simple sugars was also observed with IL, which is crucial for the further fermentation process to obtain biofuels. When compared with organosolv, IL showed a lower efficacy to reduce lignin, but a greater capacity to reduce xylans in a process to obtain bioethanol from bagasse [27]. When it comes to the fermentation step, a similar ethanol yield was found for IL and organosolv, but the volumetric productivity was slightly superior with the organosolv method at 24 h fermentation (1.20 gEtOH/Lh). A disadvantage of IL is the high amount of water used for the removal of residual [C2mim][OAc], approximately 9000 kg per 100 kg bagasse [27], although part of this stream might be recycled if the appropriate strategy is used.

Other non-chemical approaches, such as autohydrolysis and steam explosion methods, have been explored with good performance for enhancing delignification, xylan reduction, and saccharification [19,28].

5.2. Enzymatic Saccharification and Fermentation

Regarding enzymatic hydrolysis, this method is useful for releasing simple sugars after the chemical pretreatment. The saccharification process is driven by the enzymatic action on the agave fibers to break down complex carbohydrates into simple sugars, which in turn, serve as a substrate for fermentation to produce biofuels such as bioethanol, or anaerobic digestion to produce methane and hydrogen.

A wide array of enzymes has been used for this phase with varying efficacies; typically, cellulases are mandatory for simple sugars production, which can be significantly increased by adding xylanases, hemicellulases, and pectinases to the enzymatic mixture. The pretreatment is key to achieving an adequate conversion of xylans and glucans to simple sugars, particularly glucose and xylose. A comparative study using acid, enzymatic, or a combination of alkaline (2% NaOH) and enzymatic hydrolysis showed that the alkali-enzyme pretreatment rendered the highest saccharification yield [19]. Different agave by-products were tested, being bagasse the substrate with the best yield (up to 58%) compared with the stem and leaves (12–36%). Viscozyme®, a mixture of β-glucanase, hemicellulase, cellulase and xylanase, as well as Celluclast® (cellulase) were the enzymes that allowed the maximum reducing sugars production, predominantly glucose rather than xylose. Another study showed that glucosidases and pectinases are critical for fiber degradation, being remarkable the activity of pectinase for agave biomass saccharification [96]. Sequential acid and alkaline pretreatments have also shown to be effective in removing hemicellulose (>90%), and lignin (79%), while a significant degree of saccharification was achieved with different alkali conditions and the use of cellulose (7–93 %) [47].

Recent studies have found that pretreatment with IL and AFEX achieves >80% saccharification from *A. tequilana* leaves, which is highly dependent on the initial glucan content (and loading) [26,27,42]. This yield was achieved with CTec2 (cellulase) and HTec2 (endoxylanase) at 40 mg protein/g glucan and 4 mg protein/g xylan [27,42], or with Cellic® CTec3 (Novozymes A/S, Bagsværd, Denmark), a commercial enzyme cocktail comprised of endo- and exo-cellobiohydrolases, beta-glucosidase, and hemicellulose, as well as Cellic® HTec3

(xylanase and xylosidase), and Multifect® Pectinase (arabinofuranosidase, xylan esterase, pectinase, pectin lyase, alpha galactosidase, mannanase, and mannosidase) (Genencor, Palo Alto, CA, USA) [26].

Biological pretreatment is another way to decrease recalcitrance in agave by-products. Although this method is considered economical and eco-friendly, the process takes a long time, usually more than two weeks [96]. In that study, the use of *Aspergillus niger* Gyx086 was also explored as a source of low-cost enzymes.

Regarding fermentation, conditions for this process depend on the final application. For biofuels production, the reducing sugars obtained after saccharification are fermented by different bacterial strains, mainly *Saccharomyces cerevisiae*. This yeast was used to carry out fermentation of *A. atrovirens* bagasse, leaves, and stem at 30 °C for 48 h [19]. Findings showed that acid pretreatment (1.2% HCl) rendered a higher ethanol production: 33.42% for leaves and stem, and 29.81% for bagasse, equivalent to 6.5 and 7.4 g EtOH/L. Similarly, *A. tequilana* leaves subjected to fermentation with *S. cerevisiae* at 30 °C, had a maximum ethanol yield of 38.6 g/L (68%) after 13 h and pretreatment with 2% sulfuric acid [18].

As previously mentioned, the use of IL, AFEX, and organosolv improved not only saccharification, but also the amount of ethanol produced. Yields from 20 g/L to 40 g/L were observed when these pretreatments were used [26,27]. Autohydrolysis of *A. tequilana* bagasse enhanced bioethanol production with a total yield of 65.26 g/L (98% conversion from sugars) [28], which represents a significant advantage since only water is used.

Variety and the by-product type are key factors to obtaining a high ethanol conversion. In this sense, *A. tequilana* leaves have shown to produce higher ethanol yields (19.8 kg/100 kg raw material), compared with the bagasse (154 kg/100 kg raw material) [26]. Moreover, bagasse from *A. salmiana* was a better substrate than the leaves from this variety, obtaining 176 kg ethanol/100 kg raw material, whereas the leaves could not be fermented.

5.3. Ultrasound

The use of ultrasound (US) technology is growing due to several advantages, such as lower energy consumption and the rapid disruption of vegetable tissue due to the collapse of nearby cavitation bubbles. With this method, a high degree of delignification is obtained, reducing the recalcitrance of agave by-products. *Agave durangensis* leaves, the remaining by-product after mezcal production, were subjected to ultrasound-assisted pretreatment for 30 and 60 min at 42 kHz/132 W [34]. In this study, an important effect of time was observed; the 30 min treatment increased the lignin and holocellulose content, whereas after 60 min both components were reduced. US favored the extraction of fructans and phenolic compounds from the leaves due to the breakdown of cellular structures and the release of soluble compounds to the aqueous phase. Interestingly, certain phenolics, such as quercetin glucuronide, rutin, and procyanidin B2 increased in the US-treated samples. In another study, pectin was recovered from sisal (*Agave sisalana*) waste and the yield values ranged from 14% to 30% depending on the ultrasonic power applied [97]. The best conditions were set at 61 W, 50 °C, and 26 min, and allowed the maximum recovery of pectin; beyond 65 W, the yield dropped off due to an excessive bubble formation in the solvent during cavitation, which diminished the efficiency of the US. In a further study, the combination of alkali and US pretreatments at optimal conditions (4.1% NaOH, 27 min, 60 °C, and 50% sonication amplitude) allowed 82% and 86% removal of hemicellulose and lignin from the sisal, respectively [98]. Enhanced enzymatic saccharification of the leaves of *Agave lechugilla* was observed after US application [99]. Hemicellulose and lignin removal was higher in samples treated with US, compared with a microwave pretreatment at milder conditions, whereas microwaves enhanced the enzymatic hydrolysis. Thus, a higher content of simple sugars was found for the microwaved samples.

US application is especially useful for the extraction of lipophilic bioactive molecules. In this regard, the effect of solvent type and US intensity was evaluated for the extraction of steroidal saponins from *A. salmiana* bagasse [59]. Results showed that water allowed

the optimal extraction of saponins (24.41 mg/g dry matter) for US at 400 W/24 kHz, 60 °C, and a solvent to mass ratio equal to 20. Extensive damage on the bagasse morphology was observed by SEM in the US-treated samples, and the water exhibited a more intense power density and cavitation than ethanol, which enhanced the saponin extraction. By contrast, ultrasonically-assisted supercritical fluid extraction did not show a significant improvement on the saponin's recovery from *A. salmiana* bagasse [61]. According to the authors, the propagation of ultrasound was limited by the sample swelling, hindering its intensification effect.

5.4. Microwaves

Microwave technology has been considered an environmentally friendly method and suitable for scaling up for industrial applications. With this technique, by-products from different agave varieties have been processed to obtain activated carbon, bioactive compounds, and simple sugars [99–102]. For instance, *A. salmiana* leaves were used to obtain activated carbon (AC) that can be applied to eliminate contaminants, such as methylene blue, from water streams [100]. Through the use of microwaves under acid conditions, an AC yield up to 81% was obtained, as well as a 71.4% removal of the colorant. Microwaves are useful to extract valuable biomolecules from agave, such as phytosterols. β-Sitosterol-β-*D*-glucoside was efficiently extracted from the stem of *A. angustifolia* with a microwave treatment at 300 W and 2450 MHz for 5 s [101]. The recovery percentage reached with this method extracted 5 times more phytosterol than a traditional maceration process in only 5 s, compared with the 48 h process that traditional maceration necessitates.

Dilute acid pretreatment assisted by microwaves allowed the selective increase of cellulose, while it decreased the hemicellulose and lignin content of *A. lechuguilla* leaves [99]. Microwaving was more effective than the ultrasound treatment to reduce the leaves' recalcitrance and improve saccharification.

Finally, agave leaves have proven to be good microwave absorbers for anechoic chambers, blocking electromagnetic interferences [103]. Usually, polyurethane is used for this purpose, but it may release toxic gases and represents an environmental problem when discarded. Agave leaves are better absorbers than other wastes tested for this purpose, which makes this by-product a low-cost and eco-friendly material.

5.5. Other Processes

The supercritical fluids (SCF) technology has been explored as a pretreatment for the saccharification of agave bagasse and for bioactive molecules extraction, as previously mentioned. Increments up to 40% in the simple sugars content were observed with SCF applied to *A. tequilana* bagasse [104]. The saccharification yield was highly dependent on the hydration level; at low values of CO_2 the diffusivity was higher, resulting in higher damage to the agave structure. Furthermore, the combined treatment of US and SCF had a good potential for the extraction of saponins from *A. salmiana* bagasse, preserving the antioxidant capacity of these bioactive molecules [59,61]. Micellar extraction with ionic surfactants has been reported as an alternative method for saponin extraction [30] with high yield (89%), compared with other techniques such as US, but with low recovery for phenolic compounds.

6. Conclusions

Agaves are a rich source of by-products with a wide range of potential applications. Rather than considering these by-products as waste, current reports have shown their value as biofuels, materials for nanocomposites, and functional ingredients. Particularly, the leaves and bagasse from the agave industry are lignocellulosic materials that can be converted into digestible sugar for bioethanol, methane, and hydrogen production. Using by-products as a source of biofuels can be an attractive source of energy for industrial processes. Furthermore, most of the by-products have a significant content of saponins, that are valuable biomolecules with applications in the food industry (foaming, emulsification,

bitterness) and in the pharmaceutical sector (antimicrobial, anti-cancer and anti-obesity activity). Considering these points, it would be pertinent to design processes that allow us to make a comprehensive utilization of agave by-products for distinct applications. Fructans and saponins stand out as bioactive ingredients given their multiple health benefits, such as low glycemic index, antilipidemic, and anticancer activity. Fructans application in the food industry as texturizer, fat replacer, and encapsulating agents is also of great interest at present. An increasing number of processing methods, including emergent technologies, are being explored for the re-valorization of these residues. US and microwaves are promising and eco-friendly methods for the efficient saccharification and increased digestibility of agave, that can eventually replace chemical processing, reducing waste generation. Throughout this review, we have highlighted the current potential of agave by-products as low-cost and natural materials, yet, in some cases, the processing of these materials is still expensive. In this regard, future studies are required concerning accessible, low-cost, and more efficient technologies as a more attractive way for the industry to make a sustainable utilization of this by-product.

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