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2 **Natural sweeteners: Sources, extraction and current uses in foods and food**
3 **industries**

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22 **Abstract**

23 Food producers have leaned towards alternative natural and synthetic sweeteners in food
24 formulations to satisfy market demands. Even so, several synthetic sweeteners (e.g.,
25 aspartame, saccharin, sucralose) are becoming less popular due to health-related
26 concerns, lower nutritional values, and controversies around their safety. Conversely,
27 natural sweeteners confer favourable customer perceptions due to their association to a
28 healthier lifestyle and higher nutritional values. This article discusses the evidence of
29 natural sweeteners in the available commercial products. A comprehensive review of
30 natural sweeteners is presented, which includes their resources, properties and extraction
31 methods, as well as a discussion on several emerging technologies that offer
32 improvements to the traditional extraction methods. Finally, the progress of natural
33 sweeteners in the food industry is assessed, and the commercial food products containing
34 these natural sweeteners are mentioned.

35

36 **Keywords:** *Natural sweeteners; food industry; steviol glycosides; novel extraction*
37 *methods; honey; commercial products.*

38

39 **Nomenclature**

40 ADME: Absorption, Distribution, Metabolism and Excretion

41 ATP: Adenosine triphosphate

42 CPCRI: Central Plantation Crops Research Institute

43 DNL: *De novo lipogenesis*

- 44 FDA: Food and Drug Administration
- 45 F&B: Food & beverage
- 46 EFSA: European Food and Safety Authority
- 47 FAO: Food and Agriculture Organization
- 48 FE: fructose equivalent
- 49 FOS: fructo-oligosaccharides
- 50 GLP-1: glucagon-like peptide-1
- 51 GRAS: generally recognized as safe
- 52 HHP: High-Pressure Processing
- 53 MWCO: Molecular weight cut-off
- 54 MF: Microfiltration
- 55 NF: Nanofiltration
- 56 RE: Rotary Evaporator
- 57 Reb-A: *rebaudioside A*
- 58 SGs: Steviol glycosides
- 59 SSB: Sugar-Sweetened Beverages
- 60 UF: Ultrafiltration
- 61 WHO: World Health Organization
- 62 5-HMF: 5-hydroxymethylfurfural

63

64 **1. Introduction**

65 Among macronutrients, carbohydrates provide 40-80% of the total energy intake. In
66 foods, carbohydrates can be found as free and non-free sugars; non-free sugars are

67 naturally present within the cell structure, e.g., sugar in fruit and vegetables, starchy
68 carbohydrates in grains, lactose in dairy products, etc. In contrast, the free sugars do not
69 present naturally but are often added to food, such as monosaccharides (glucose,
70 fructose) and disaccharides. However, excess energy intake is associated with the
71 accumulation of body fat (Onaolapo et al., 2020). More specifically, excess intakes of free
72 sugars not only result in fat accumulation but also compromises micronutrient density and
73 increased the risk of other adverse health conditions, such as diabetes and
74 cardiovascular diseases (Hagger et al., 2017).

75 Sucrose (common sugar), composed of fructose and glucose in equal ratio, is
76 fundamental in our diet since glucose metabolism is needed to produce adenosine
77 triphosphate (ATP), synthesis of different biomolecules and more importantly, for cellular
78 respiration function (Archer, 2018). Unfavourably, fructose causes dysregulation of
79 carbohydrate and lipid metabolism. This is because fructose metabolism is not regulated
80 by hepatic energy requirements, resulting in excess fructose uptake by the liver and an
81 increase in *De novo lipogenesis* (DNL) (Stanhope, 2016). Consequently, even though
82 sugar is a significant energy source in the human diet, it may also promote dysmetabolic
83 conditions (Lee et al., 2018).

84 The main disadvantage of common sugar made out of sugarcane juice is that the refined
85 product lacks additional beneficial compounds (e.g., bioactive molecules) that could
86 enhance its nutritional value. In the sugarcane juice refining process, non-centrifugal cane
87 sugars, brown sugar and molasses are produced as by-products. These by-products
88 were proven to contain several bioactive molecules, including flavonoid glycosides and
89 phenolic acids (Singh et al., 2015), which later led several other authors to recommend

90 the non-centrifugal sugars to substitute the refined sugars due to phenols and flavonoids'
91 favourable dietary effects (Cervera-Chiner et al., 2021; Lee et al., 2018). For the same
92 reason, natural sweetening alternatives are becoming more attractive to consumers.

93 Although the global sugar demand has generally declined due to the rising concerns
94 around the potential health effects caused by elevated sugar intakes, the Food and
95 Agriculture Organization (FAO) data suggest that the growth in sugar intake will remain
96 strong in developing countries. By 2028, it is estimated to increase by 32 Mt, compared
97 to the approximated value of 203 Mt in 2008 (OECD/FAO, 2019).

98 Currently, processed foods play a substantial role in excess sugar intake. Steele et al.
99 (2016) reported that ~90% of the total average sugar intake comes from ultra-processed
100 foods (e.g., fruit juices), concentrated syrups, soft and sports drinks, bakery products,
101 among others, which often consist of elevated sucrose indexes ranged from 50 to 1000
102 g/L (Raganati et al., 2015). In addition, the most popular drinks (e.g., energy drinks, soda,
103 fruit juices), depending on their beverage type, contain sugar contents of between 100-
104 135 g/L (Health, 2014). Past studies have pointed out that a greater intake of sugar-
105 sweetened beverages (SSBs) is related to a 30% increase in developing type 2 diabetes
106 (Wang et al., 2015), and one SSB serving (250 mL) per day increased the type 2 diabetes
107 incidence by 18% (Imamura et al., 2015).

108 The increasing demand has led researchers to explore new natural and synthetic
109 sweeteners as sucrose alternatives (Saraiva et al., 2020). When extracted with the
110 beneficial compounds from their sources, the natural sweeteners (glucose, fructose, and
111 sucrose) are classified as nutritional choices. New possibilities have been proposed and
112 studied to cater to the demand, including honey, xylitol, erythritol, maltose, maltodextrin,



113 stevia, molasses, maple syrup, coconut sugar, agave nectar and date sugar (Valle et al.,
114 2020).

115 Artificial sweeteners, also known as sugar substitutes or non-sugar sweeteners, are
116 synthetic substances used to replace sugar during the sweetening process of several
117 products. These additives are identified as intensive sweeteners because they display a
118 higher sweetening power than conventional sugar. The use of synthetic sweeteners has
119 become popular in the last 40 years. Currently, they are used in a wide range of
120 processed foods, such as sweets, preserves, dairy products and beverages. Six of these
121 high-intensity sweeteners have been approved as food additives by the Food and Drug
122 Administration (FDA), including aspartame, neotame, saccharin, acesulfame-k, sucralose
123 and advantame (Mooradian et al., 2017).

124 Aspartame, the first FDA-approved sweetener, was used in solid foods since 1981, in
125 beverages since 1983, and it currently has no usage restriction in food and beverage
126 (F&B) preparation since 1996. However, the use of aspartame is still controversial.

127 Aspartame consumption generates concerns among health representatives and the
128 general consumers since it is widely used by big F&B companies such as *Coca-Cola* in
129 many of its products (FDA, 2019). The consumption has been associated with different
130 health effects, including oxidative stress in blood cells (even at the recommended dose
131 of 40 mg/kg per day), interference of neuronal cell function, hepatotoxicity and kidney
132 dysfunction (Ardalan et al., 2017; Choudhary & Pretorius, 2017). Moreover, its
133 consumption is not recommended for people suffering from phenylketonuria since they
134 cannot metabolize phenylalanine (a chemical component used in the aspartame
135 synthesis). On the contrary, some evidence support that aspartame consumption does



136 not influence blood pressure, glucose and lipid profiles, suggesting that aspartame has
137 no adverse effects on human metabolism and constitutes a safe option for type 2 diabetes
138 patients (Choudhary, 2018; Gupta, 2018), particularly neotame, an isomer of aspartame.
139 Similar advantages also claimed for advantame, an N-substituted derivative of aspartame
140 and vanillin (B. Chen et al., 2020; Dhartiben & Aparnathi, 2017). Both neotame and
141 advantame are sweeter than aspartame, ~13,000 and ~20,000 times sweeter than
142 common sugar, respectively. The neotame was FDA-approved as a general-purpose
143 sweetener in 2002 (Gupta, 2018), whereas the advantame was approved in 2014 (Khan
144 & Aroulmoji, 2018). Despite their relationship with phenylalanine, neither neotame nor
145 advantame present a bitter taste (Carocho et al., 2017).

146 Another artificial sweetener, sucralose, is also a non-caloric sweetener (does not break
147 down in the body) and ~600 times sweeter than sugar. Sucralose is stable at different
148 temperature and pH conditions, making it an optimal option for many industrial
149 applications. However, some studies pointed out that sucralose interferes with digestive
150 processes and may increase glucose and insulin levels in the organism, boosting the risk
151 of weight gain and diabetes (Khamise et al., 2020). Regardless of this evidence, ADME
152 (i.e., absorption, distribution, metabolism, and excretion) research on sucralose shows
153 that it is mainly eliminated through faecal excretion and is not absorbed or digested in the
154 human body (Magnuson et al., 2017)

155 As for saccharin, its usage in Canada had been banned since 1977. The USA FDA had
156 also considered its prohibition since evidence suggests its role in inducing bladder cancer
157 in rats. However, in several subsequent studies, the relationship between saccharin and
158 bladder cancer in humans was not demonstrated. After that, the use of this product has



159 been approved by the FDA and the European Food and Safety Authority, EFSA (Ansari
160 et al., 2015). Another common synthetic sweetener is acesulfame-K, which is recognized
161 as a thermostable component and used in cooking. Acesulfame-K is 120 times sweeter
162 than sugar but bears a bitter taste, and thus, it is commonly used with other sweeteners.
163 It is worth mentioning that acesulfame-K cannot be metabolized, implying a zero caloric
164 intake. Acetoacetamide, a breakdown product of acesulfame-K, may be toxic at high
165 concentrations; nonetheless, the human exposure at low concentration is negligible.
166 (Krishnasamy, 2020).

167 In general, artificial sweeteners have very low-calorie content and intense sweetness,
168 making them attractive to both consumers and food manufacturers. However, these non-
169 caloric sweeteners have minimal nutritional value. They also may result in incomplete
170 activation of food reward pathways, leading to sweet cravings and food-seeking, which
171 may cause excessive caloric intake and weight gain (Mooradian et al., 2017).
172 Furthermore, some experimentation in animals has demonstrated that these artificial
173 sweeteners influence specific metabolic syndromes. For example, glucose tolerance may
174 be reduced in response to the changes in the microbiome after a moderately prolonged
175 artificial sweeteners consumption (Green & Syn, 2019). Although notable field authorities,
176 including FDA and EFSA, have approved some artificial sweeteners, the available
177 evidence to support their industrial use and consumption is still inconclusive. Moreover,
178 many contradictory findings on safety and health implications were reported, making their
179 use is controversial.

180 Presently, both sucrose and artificial sweeteners can be replaced by natural sweeteners.
181 Prevailing market trends suggest that natural food products are more appealing to



182 consumers, who identify natural products as healthier options. The current trend indicates
183 that consumers are willing to try natural sucrose alternatives (Mora & Dando, 2021). For
184 example, Stevia-sweetened beverages have more positive consumer perceptions than
185 the common SSBs (Olivo, 2019). Thus, natural sweetener usage may represent a new
186 and substantial commercial opportunity for many companies. Natural sweeteners also
187 present positive consumption effects, such as improving metabolic health, preventing
188 weight gain and lowering blood glucose. Other advantages are; (1) low glycemic potency,
189 as presented in honey and agave nectar, could be advantageous for people on low
190 glycemic index diets, (2) low fructose contents, as found in maple syrup (Edwards et al.,
191 2016), and (3) containing biomolecules with nutritional and health benefits (e.g., vitamins,
192 phytohormones and minerals) (Valle et al., 2020). It was reported that the general
193 composition of honey, maple and agave syrup consists of at least 3% dietary fibre, 1.4%
194 proteins, <2% minerals (potassium, calcium, and magnesium), and polyphenols with
195 potential antioxidant activity (Edwards et al., 2016). Similarly, dark molasses and
196 blackstrap molasses consist of high antioxidant activities, at 4.89 and 4.56 mmol/100g,
197 respectively. The substitution of 130g refined sugar with 337g blackstrap molasses in
198 viable products would increase its antioxidant content by ~10.7 mmol (Eggleston, 2019).
199 Stevia, another important natural sweetener that constitutes mainly steviol glycosides,
200 has been widely used in Paraguay and Japan. Stevia has low-calorie with a zero glycemic
201 index; it helps lower blood glucose levels and maintains dental health since it does not
202 contain carbohydrates. It has been widely used in bakery products due to its stability at
203 elevated temperatures (Singh et al., 2020). Because of its attractive benefits, *Cargill Inc.*,
204 in collaboration with *Coca-Cola*, *Whole Earth Sweetener Co.* and *PepsiCo*, requested



205 stevia to be FDA-approved as a Generally Recognized As Safe (GRAS) ingredient. After
206 exhaustive attempts, stevia was authorized in 2004 by the United Nation's FAO and the
207 World Health Organization (WHO) Committee on Food Additives (Merillon & Ramawat,
208 2017). Not until 2008, the US FDA granted GRAS recognition for the purified steviol
209 glycosides (Perrier et al., 2018). Ever since, this sweetener has been employed in several
210 commercial beverages, such as Sprite®, produced by the *Coca-Cola* company (Ismail et
211 al., 2020).

212 Polyols, including erythritol, mannitol, and xylitol, are natural sweeteners since they occur
213 naturally in fruits and vegetables, but they also can be synthesized from mono or
214 disaccharides (Edwards et al., 2016). Xylitol is a five-carbon polyol, obtained from xylose
215 hydrogenation and the sweetest polyol but only provides 2.4 kcal per gram (making it 5%
216 less sweet than sugar). Xylitol is beneficial to promote salivation, as well as the reduction
217 of bacterial load and cavities. It is estimated that the xylitol market is worth 670 million
218 USD and continues to increase, as it is being used in several commercial product
219 formulations, including soft drinks, gum, candy, and baked products (Carocho et al.,
220 2017). However, consumers are still not familiar with xylitol as much as stevia or other
221 artificial sweeteners (sucralose, aspartame and saccharin); thus, making xylitol a less
222 preferred sucrose-alternative (Mora & Dando, 2021).

223 Considering the benefits and current attention given to the natural sweeteners in the
224 sweetened food market, this review aims to provide evidence related to their
225 implementations and recent uses. Traditional natural sweeteners discussed are the
226 common nutritive alternatives and possess promising commercial potentials, including
227 honey, molasses and blackstrap molasses, maple syrup, coconut sugar, agave nectar,



228 date syrup and steviol glycosides and sorghum syrup. Most of these sweeteners are
229 readily introduced in several commercial products, frequently marketed as healthier
230 choices and accepted by the general consumers for their positive attributes. This review
231 also compiles the information on the natural sweeteners' main extraction routes,
232 production and purification. Equally important, their physicochemical properties are
233 presented. Finally, an overview of the current uses and applications in the food industry
234 is provided.

235

236 **2. An overview of sources, properties, current and promising extraction** 237 **methods of the natural sweeteners**

238 **2.1. Honey**

239 *2.1.1. Physicochemical and bioactive properties*

240 Honey is likely to be the most consumed natural sweetener worldwide. Its composition
241 and properties may vary depending on the botanical source, harvest reason, climate and
242 the geographical region where it is produced. Nonetheless, its typical chemical
243 composition consists of 60–85% carbohydrates, 12–23% water, minerals, vitamins,
244 proteins, organic and amino acids, enzymes and several bioactive substances (e.g.,
245 phenols and flavonoids) (Machado De-Melo et al., 2018). Due to its unique properties,
246 honey is useful in both nutritional and therapeutic applications. Its moisture content,
247 typically ranging from 13 to 25%, is the primary determinant for honey shelf-life and the
248 optimal value for preservation is 17%. The water content heavily influences the
249 proliferation of microorganisms in honey. Honey with high water content is susceptible to
250 osmophilic yeast spawning, whereas not in common sugar due to its osmotic pressure



251 (unsuitable for yeast growth). Moisture also affects other properties, such as colour,
252 crystallization and viscosity (Machado De-Melo et al., 2018). In theory, honey with low
253 water content tends to be difficult to be processed. Water availability for microbial growth
254 is defined by water activity, a_w (Reid, 2020). In honey, sugar binds with water molecules
255 making it unavailable for microorganism growth (Machado De-Melo et al., 2018). Hence,
256 it is a better-quality criterion than water content (Reid, 2020); e.g., a_w values of honey is
257 $\sim 0.49\text{--}0.65$ and bacterial growth requires an a_w of ~ 0.9 , while yeast and moulds need an
258 a_w of ~ 0.7 and ~ 0.8 , respectively. Therefore, honey generally inhibits self-fermentation
259 and self-spoilage (Machado De-Melo et al., 2018).

260 A small number of enzymes, including glucose-oxidase, diastase and invertase, are
261 present in honey chemical composition. *Glucose-oxidase* is predominantly produced in
262 the bees' hypopharyngeal glands. This enzyme degrades glucose into gluconolactone,
263 which turns into gluconic acid and after converted into hydrogen peroxide. Hydrogen
264 peroxide gives honey its microbial resistance. Elevated concentrations of hydrogen
265 peroxide protect honey from bacterial degradation until the sugar concentration is high
266 enough to preserve the honey through osmotic pressure. *Glucose-oxidase* is light-
267 sensitive and deactivated at only $60\text{ }^\circ\text{C}$ (Machado De-Melo et al., 2018). On the contrary,
268 *diastase* or *amylase* is the most thermal resistant enzyme; used to determine the honey's
269 freshness. *Diastase* is responsible for starch and dextrans hydrolysis; however, its function
270 in honey has yet to be understood since the nectar does not contain starch. Nonetheless,
271 it is believed that *diastase* participates in pollen digestion (Machado De-Melo et al., 2018).
272 Another important enzyme is *invertase* (α -*glucosidase*), which is crucial in transforming
273 honeydew into honey. *Invertase* hydrolyzes sucrose into fructose and glucose, and its



274 activity is maintained even after the extraction. Unfortunately, due to the fructose inhibition
275 property, the activity is lowered over time in storage. Furthermore, since *invertase* has a
276 higher sensitivity to thermal processes, it has been proposed to be the primary indicator
277 for honey quality control (Machado De-Melo et al., 2018).

278 Unlike the other natural sweeteners, honey presents various functional properties, the
279 most important being antioxidant and antimicrobial activities (Dzugan et al., 2018). As
280 discussed, honey is proven to delay or prevent food spoilage caused by its oxidative
281 reactions. Moreover, *in vitro* studies have shown that honey ingestion inhibits the
282 oxidation of human serum lipoproteins. Many honey components are classified as
283 antioxidants, such as glucose oxidase, catalase, organic acids, carotenoids, ascorbic
284 acid, amino acids, proteins, phenolic compounds and melanoidins (Maillard reaction
285 products). Different pathways have been suggested to describe the honey's antioxidant
286 activity, such as reducing reactive oxygen and nitrogen species, and inhibiting super
287 oxidant production enzymes. Based on the presence of multiple bioactive compounds in
288 its composition, a recent research has suggested that honey can also be used as a
289 protective agent against pathologies that cause liver damage, radiation and inflammation
290 (Machado De-Melo et al., 2018).

291 *2.1.2. Extraction and promising methods for honey processing*

292 Honey is extracted from either combs or apiaries. Initially, a beekeeper must place the
293 beehive (by brushing the frames or using a fume board, bee blower, or escape board) in
294 another apiary. The hive is then cleared for the extraction to begin. Several traditional
295 honey extraction methods are available, but they often reduce the honey quality. The
296 most common commercial honey production uses an extractor method (radial or



297 tangential), which essentially applies centrifugal force to the uncapped frames. Here, the
298 wax-sealed honey is removed with a knife (MacFawn, 2018). After the extraction, honey
299 may still contain undesirable compounds such as beeswax and pollen, which are
300 removed to increase its quality and shelf-life (MacFawn, 2018). This process variation
301 depends on the operation scale, but the general schematic is described in **Figure 1**.

302

303 **Figure 1.** Traditional honey production process.

304

305 The conventional extraction method starts with the removal of suspended particles. This
306 step is combined with a preheating process (up to 40 °C) in a large scale production. The
307 strained honey is filtered with pressure filters (e.g., polypropylene microfilter, a pore size
308 of 80 µm) at 50-55 °C and followed by indirect heating in a tubular heat exchanger
309 between 60–65 °C for 25–30 min. In the final stage, rapid cooling is applied to preserve
310 its natural colour, flavour, enzyme content, and other biological substances.

311 Microwave heating and infrared processing, well-known methods in the food industry,
312 have been proposed as promising extraction practices to meet better-quality honey
313 demand. Water and dissolved sugars in honey are highly receptive to microwave
314 interactions (Reynolds, 2019) and exhibit good absorption in the thermal radiation region.
315 This will reduce the heating periods in both methodologies, thus bringing several added
316 advantages over the conventional method.

317 *i) Thermosonication* combines both thermal treatment and ultrasonication,
318 two methods that have been proven successful in honey treatment. For

319 instance, Chong et al. (2017) demonstrated that enhanced honey quality
320 (water activity, viscosity and colour intensity) was obtained using an
321 ultrasonication bath with heating.

322 *ii) Membrane processing* is a non-thermal alternative technique. To date,
323 membrane-based technologies have been successfully applied in many
324 food sectors (Castro-Muñoz et al., 2020). In honey processing, different
325 ultrafiltration (UF) membranes, characterized by MWCO of 20, 25, 50, and
326 100 kDa, could eliminate microorganisms; however, the desirable enzymes
327 were also removed. This ultrafiltered honey is typically used in the
328 pharmaceutical industry.

329 *iii) High-pressure processing (HPP)* is another non-thermal processing
330 alternative. Akhmazillah and co-workers reported a Manuka honey, HPP-
331 processed at ambient temperature, presented higher total phenolics content
332 than its thermally processed counterpart. This method proved to improve
333 the honey's nutritional value (Akhmazillah et al., 2013).

335 **2.2. Molasses and blackstrap molasses**

336 *2.2.1. Physicochemical and bioactive properties*

337 Molasses is a broad term that refers to the concentrated sugarcane or sugar beet juice.
338 This sweetener is derived from the sucrose crystallization process, where crystallization
339 inhibitors are accumulated in the residual syrups. These syrups are called molasse and
340 are mainly used as livestock feed and substrate for ethanol production (Palmonari et al.,
341 2020). Although their composition depends on the syrup's recovery stage, the molasses'



342 approximate compositions correspond to 17-25% water, 30-40% sucrose, 4-9% glucose
343 and 5-12% fructose. Several compounds, such as amino acids and vitamins, are also
344 present in small percentages. These molasses are acidic, with pH values ranging from
345 ~5 to ~7 for cane molasses and pH of ~5.8 for blackstrap molasses. Also, salt content in
346 these molasses can prevent hydrolysis, thus providing buffering capacity to stabilize the
347 flavours (Mordenti et al., 2021).

348 Molasses are classified depending on their source and constituents. For example,
349 blackstrap molasses, heavy and dark viscous syrups, are the residual when sucrose
350 recovery is not feasible by the standard physical methods in a sugarcane manufacturing
351 process or raw sugar refinery (Mangwanda et al., 2021; Mordenti et al., 2021). Similarly,
352 high-test molasses are the resulting high concentration syrups from the clarified cane
353 juice and have approximately 85° Brix. In contrast to the blackstrap molasses, high-test
354 molasses are intentionally produced and not as a by-product. It has several advantages
355 over the blackstrap molasses, such as (1) containing fewer sugar decomposition sub-
356 products, (2) higher sugar contents as it is only subjected to a lower temperature process,
357 and (3) possesses an intense aromatic flavour. Another molasses type is sulfured
358 molasses, in which sulfur dioxide is added to bleach out the initial dark colouration.
359 Sulfured molasses present higher ash contents (Mordenti et al., 2021); however, their
360 physicochemical properties differ considerably since the compositions are highly varied.

361 Additionally, molasses have interesting properties for food processing, e.g., masking
362 undesired flavours. These sweeteners may present a caramel flavour (in high-test
363 molasses) and heavy liquorice-like nuances in the other forms. Molasses are valued as
364 a colouring agent, especially in baked goods, where it gives golden, dark and brown



365 colours, enhancing the goods' visual presentation. Molasses also exhibit humectant and
366 colligative properties, reducing water activity and extending the shelf-life of baked
367 products. Since molasses retain the high-added-value components (e.g., phenolics) from
368 their sources, it has been reported that the extracts promote resistance to infections and
369 auto-inflammatory activities. Furthermore, molasses are considered a non-expensive
370 antioxidant source (Chen et al., 2015).

371 *2.2.2. Extraction and promising methods for molasses and blackstrap molasses* 372 *processing*

373 It is estimated that ~70% of fructose production is obtained from sugarcane (grown in
374 tropical climates), while ~30% is produced from sugar beet (grown in relatively temperate
375 zones). As the process by-product, 35 million tons of molasses are produced annually.
376 **Figure 2** illustrates the sugar production process and its derived blackstrap molasses.

377

378 **Figure 2.** Sugar production process and its derived blackstrap molasses.

379

380 In the process, the sugar canes are first washed before the juice is extracted. The
381 extracted juice goes through a purification stage, where it is clarified to remove the
382 contaminants before undergoing an evaporation process (concentration step) to reduce
383 the water content, becoming a syrup. In the crystallization step, the goal is to maximize
384 the sucrose recovery from the syrup, and due to the process limitation, ~80% sucrose is
385 retained in a large quantity of molasses. Even though the crystallization process involves
386 recirculation steps to decrease the purity of the mother liquor (syrup), it still contains high

387 sucrose levels. When the mother liquor purity cannot be lowered further, it is separated
388 from the process to prevent non-sucrose compounds accumulation and referred to as
389 blackstrap molasses (Mangwanda et al., 2021).

390 Molasses are desirable for high-value biotechnological processes and auxiliary sucrose
391 extraction. Unfortunately, molasses contain many contaminants, making the extraction
392 process challenging and requiring purification before further processing (Sjölin et al.,
393 2019). To date, several alternative methods for further molasses processing have been
394 implemented, such as:

395 *i) Carbonation:* The increased sucrose recovery is obtained by ion-exclusion
396 chromatography. Nonetheless, the obtained molasses cannot be used for other
397 applications due to the impurities bound irreversibly to the compound, rendering it
398 useless. Thus, the molasses are first diluted (to decrease its viscosity) and
399 subsequently carbonated at high pH to precipitate the divalent cations, the major
400 impurities. This procedure can be accomplished with different salts (e.g.,
401 potassium carbonate and sodium carbonate) or carbon dioxide. The resulting
402 mixture is further diluted with an organic solvent, which precipitates the organic
403 polymers, polysaccharides and proteins. Afterwards, the supernatant and
404 precipitated fractions are separated using filtration or centrifugation, and the sugar
405 is recovered from the supernatant solution using ion-exclusion chromatography.

406 *ii) Ceramic nanofiltration (NF) and ultrafiltration (UF) membranes:* Membrane-
407 based process is often seen as a reliable separation technique. For sugar beet
408 molasses, 10 kDa UF and 200 Da NF ceramic membranes have been selected to
409 remove the high-molecular-mass compounds, including polyphenols and the



410 small-molecular-mass contaminants (like salts). Purified sucrose is collected as
411 the permeate in UF and as the retentate in NF. The main drawback is the severe
412 membrane fouling caused by starch, pectin and proteins (Sjölin et al., 2019). This
413 phenomenon is widely acknowledged as one of the main limitations when
414 processing complex mixtures (Pichardo-Romero et al., 2020).

415 *iii) Bentonite addition:* This process is an additional filtration step that decreases
416 the non-sugar compounds in molasses, easing the sucrose extraction process. In
417 principle, bentonite-based solutions are added to the molasses with citric acid to
418 adjust the pH. The blends are heated in a water bath at 60 °C and mixed for 30
419 min before being filtered. This method was reported to yield a reduction in turbidity
420 and colour whilst minimally decreased the sucrose content (Djordjević et al., 2018).

421 **2.3. Maple syrup**

422 *2.3.1. Physicochemical and bioactive properties*

423 Maple syrup is a natural sweetener produced from different Canadian maple tree species,
424 and the most common is *Acer saccharum Marsh* (Garcia et al., 2020). Maple syrup
425 contains phenolic compounds that account for its antioxidant, anti-mutagenic and human
426 cancer anti-proliferative properties. The properties of maple syrup, such as pH, colour,
427 sugars, depending on the cultivation area. Maple syrup can be categorized into four types:
428 golden, amber, dark and very dark (Brochu et al., 2019). Recent studies addressed that
429 a darker colour is related to higher phenolic compounds (e.g., protocatechuic acid,
430 coniferyl alcohol, vanillin) and mineral contents (Nimalaratne et al., 2020).

431 The inhibition of the carbohydrate hydrolyzing enzymes, such as α -glucosidase, is
432 beneficial for type 2 diabetes personnel since α -glucosidase has shown inhibitory activity
433 against glucose absorption in the intestine (Wan et al., 2012). Additionally, ethyl acetate-
434 based extracts from this sweetener have the potential to aid in treating Alzheimer's
435 disease. Notably, the extracts decrease the oligomerization and aggregation of the
436 primary peptides (e.g., β -amyloid and T-peptides) that cause Alzheimer (Hawco et al.,
437 2015). Besides that, the acetate-based extracts also exhibit anti-inflammatory effects.

438 2.3.2. *Extraction and promising methods for maple syrup processing*

439 The traditional maple syrup production techniques involve changing sap into syrup.
440 Usually, the sap is collected in buckets, followed by water evaporation using direct heat
441 between 9-56 h. A standardized process tends to follow these steps: extraction, filtration,
442 evaporation and filtration, as described in **Figure 3**. It is estimated that ca. 44-gallons sap
443 are needed to produce 1-gallon syrup (Nimalaratne et al., 2020; Snyder et al., 2019).
444 Alternatively, a vacuum pump and tubing may extract more sap than gravity tubing or sap
445 buckets (Moore et al., 2020).

446 It is also reported that reverse osmosis can enhance the sap concentration process
447 efficiency. For example, using a commercial polyimide membrane (from GE Sepa®)
448 crossflow filtration process increased the sap concentration due to the amplified system's
449 osmotic pressure (Weaver et al., 2020). The method also required a shorter evaporation
450 time. However, reverse osmosis involves maintenance and filter modules replacement,
451 which translated into downtime issues and additional costs (Ali et al., 2021). Reverse
452 osmosis and evaporation coupling method was suggested to overcome the limitation.

453

454 **Figure 3.** Schematic representation of the traditional maple syrup production.

455

456 **2.4. Coconut sugar**

457 *2.4.1. Physicochemical and bioactive properties*

458 Coconut sugar is a natural sweetener rich in carbohydrates. The sugar obtained from
459 Suttiphuan coconut palm or *Cocos nucifera* L comprises ~15% sucrose (Muriel et al.,
460 2019). The sugar provides a better digestion rate and lower glycemic index (GI) of
461 between 35 to 42. The sugar contains ~4 kcal per gram, high in vitamin C, B1, B2, B3,
462 and B6. It also presents a low glass transition temperature (Srikaeo & Thongta, 2015),
463 typically associated with its fructose, glucose and sucrose components (Asghar et al.,
464 2020). It is known that a coconut tree produces sap throughout the year. The sap,
465 collected from unopened spadixes, generally presents a pH value of 7.0–7.3, along with
466 high antioxidant activity and phenolic content (Asghar et al., 2020).

467 *2.4.2. Extraction and promising methods for coconut sugar processing*

468 Conventional coconut sugar processing involves sap collecting and storing at -2 °C. The
469 sap is then heated at 115-120 °C for 3-5 h; before the nectar is crystallized into granules,
470 oven-dried, and subsequently sieved. However, the protocol overcooks sugar, affecting
471 its physical and chemical properties, e.g., reducing the vitamin C and B3 concentrations
472 (Asghar et al., 2020). The temperature also affects the colour quality and pH, directly
473 influences its antioxidant activity (Karseno et al., 2018). The standard process involves



474 collection and filtration, evaporation, crystallization, sieving/filtration and drying (as
475 illustrated in **Figure 4**).

476

477 **Figure 4.** Schematic representation of the traditional coconut sugar production.

478

479 The sap collection is commonly performed by tapping the palm's spathe (for
480 approximately ten-day) and collected into pots. The collected sap is filtered with
481 deodorizing aids at 2-8 °C before being pasteurized at 90-95 °C (Karseno et al., 2018).
482 Additionally, the sap is heated up to 140 °C for 30 min to increase the sugar crystalline
483 properties. The product is then cooled, producing a varied mass and mixed for 10 min to
484 dissolve the remaining lumps before drying at room temperature (Muriel et al., 2019). The
485 steps are described below:

486 *i) Collection techniques:* Tapping and coconut sap collection are conducted at
487 atmospheric temperature for 8-12 h using a container on the palm tree. Sap
488 fermentation is a critical issue, where it turns white and the pH decreases from 7
489 to 6. The sap is preferred to be collected every hour at a steadily low temperature
490 to avoid the occurrence. Insects and other particles could also contaminate the
491 sap. To prevent the issues, Kasaragod-based Central Plantation Crops Research
492 Institute (CPCRI) designed and built a coco-sap chiller, a closed system that
493 regulates the temperature between 2-3 °C, maintaining its hygienic and freshness.
494 The system can collect coconut sap with a zero fermentation possibility (Hebbar
495 et al., 2015).



496 *ii) Filtration:* A filter funnel and chiffon fabric have been used in the traditional
497 process to extend the utilization of the sap (Muriel et al., 2019).

498 *iii) Evaporation:* Generally, the syrup is concentrated between 100–120 °C for 3-5
499 h (Srikaeo & Thongta, 2015). Coconut sugar producers actively seek new
500 technologies to concentrate and preserve the sugar better; **Table 1** tabulated
501 several alternatives explored to date. The rotary evaporator method (RE) has been
502 promising by providing precise and efficient distillation that conventional methods
503 cannot achieve. RE is conducted under vacuum and allows water to evaporate at
504 a lower temperature (the lowest of all methods). Other techniques are categorized
505 as open heat evaporation, often conducted at high temperatures and consumed
506 more time and energy. Yet, it is the most common technology on an industrial scale
507 (Asghar et al., 2020).

508

509 **Table 1.** Alternative techniques used to concentrate the coconut sugar.

510

511 *iv) Drying:* The concentrated sap is further dehydrated to produce a dried product.
512 Herein, the sap is heated until it crystalized and then gradually cooled. Since
513 stickiness is an issue in the traditional drying processes, several advancements have
514 been proposed, such as spray and vacuum drying (Nurhadi et al., 2018). For example,
515 the lyophilization method can obtain final dried sugar with good functional (e.g., protein
516 solubilities, emulsification and foaming capacity) and physicochemical properties
517 (e.g., colour and sensory characteristics). In comparison, the spray drying method can



518 produce a final product with good product features and functional properties (Nurhadi
519 et al., 2018).

520

521 **2.5. *Agave nectar***

522 *2.5.1. Physicochemical and bioactive properties*

523 Agave nectar or agave syrup is a natural sweetener obtained from its fructan hydrolysis.
524 The nectar, its main carbohydrate reserve in the form of fructans, is stored in the agave
525 core (*piña* in Spanish) of the *Agave* ssp. plant. *Agave* genus, growing in arid and semi-
526 arid regions, follow a crassulacean acid metabolism and a photosynthetic adaptation to
527 the periodic water supply. These plants are commonly found in Northern and Central
528 America; however, nearly 55% of the species are widespread in Mexico and considered
529 agave's diversity core and origin. Due to its high sugar content, agave's consumption and
530 socio-economic impacts are dated back to the pre-Columbian era (Pérez-López &
531 Simpson, 2020). Among all the recorded species, *Agave tequilana* weber var. azul is the
532 main crop for raw material extraction of agave syrup and Tequila production. The
533 standardized extraction techniques and regulations have supported blue agave syrup's
534 increasing popularity and acceptance as a sweetener in Mexico. It is conditioned that the
535 agave syrup must not contain any food additives, glucose, molasses, dextrin, starches,
536 among other compounds (NOM-003-SAGARPA-2016, 2016).

537 As for its composition, fructans are fructose-bound polymers with a dextrose equivalent
538 between 3 and 29, linked to a single glucose molecule. These carbohydrates are
539 classified accordingly to their link formation type, distinguished into three categories:



540 *inulin*, fructans with linear $\beta(2\rightarrow1)$ glycosidic bond structure; *levan-type*, fructans with
541 linear $\beta(2\rightarrow6)$ glycosidic bond structure; and *graminan*, fructans with both $\beta(2\rightarrow1)$ and
542 $\beta(2\rightarrow6)$ glycosidic bond branched structure (Witzel & Matros, 2020). These fructans are
543 involved in the agave syrup production from *Agave tequilana* Weber var. azul, which
544 contains a fructooligosaccharides mixture with branched structures $\beta(2\rightarrow1)$ and $\beta(2\rightarrow6)$
545 glycosidic bonds, identified mainly as graminan type of carbohydrates (Miramontes-
546 Corona et al., 2020). The agave nectar composition presents nearly 95% total soluble
547 solids (TSS), of which 90% corresponds to fructose concentrates, followed by glucose
548 and sucrose (the lowest). The high content in fructose causes the syrup to have a low
549 glycemic index (between 17-27), lower than other sweeteners (e.g., honey = ca. 55).
550 Agave nectar is also sweeter than most syrups with high glucose or sucrose levels (e.g.,
551 honey and maple syrups). Therefore, lower amounts of agave nectar are needed to reach
552 the desired sweetness and translated into a lower caloric intake (Barajas et al., 2017).
553 The benefit favours agave nectar as the alternative to regular refined sugar, suitable for
554 obesity and disease prevention (such as diabetes) (Ozuna et al., 2020).

555 Additionally, the pH values are often close to 4 with and 22% average humidity. This
556 analysis could serve as a potential tool for authenticity analysis and adulterants
557 identification for agave syrup commercialization (Barajas et al., 2017).

558 2.5.2. *Extraction and promising methods for agave nectar processing*

559 The standardized industrial agave syrup production follows Tequila's procedure, except
560 for the additional fermentation process, distillation and purification steps. Approximately
561 10% of the agave harvest is used for syrup production. The main operating units are
562 shown in **Figure 5**. The process begins with harvesting a mature 5-7 years old blue agave



563 plant, which stores high carbohydrate contents in the plant's *piña*, resembles a pineapple
564 after the leaves are removed. High-quality *piña* (weight up to 68 kg) contains about 25-
565 30% w/w sugars. The second stage involves milling and crushing the *piña* until juicy fibres
566 are obtained. Subsequently, the juice is extracted by hot water washing in a diffuser, and
567 the fibres are discarded. A filtration process is later performed to remove solid particle
568 residues from the raw agave juice. The filtered juice is then thermally hydrolyzed by
569 heating at 80 °C for 8-12 h and refiltered; however, the process could last longer (36 – 48
570 h) in a traditional technique that uses brick-wall kilns. After reducing the water content in
571 the second filtration, the juice is vacuum evaporated at 90 °C for glycosidic activities
572 denaturation, resulting in the final syrup product (Maldonado-Guevara et al., 2018).

573

574 **Figure 5.** Major processing operations involved in agave nectar production.

575

576 Over the last decade, many modern and improved production processes have been
577 introduced to cater to demand growth. In agave syrup, enzymatic treatments, which
578 involve hydrolysis and extraction steps, would enhance fructans hydrolysis efficiency,
579 reduce energy waste and simplify the syrup production process. However, enzymatic
580 hydrolysis could only be achieved through microbial enzymes (Barajas et al., 2017). In
581 theory, inulinases, which catalyze the $\beta(2\rightarrow1)$ fructan hydrolysis, can be found in
582 bacterial, yeast, and filamentous fungal strains; thus, significant variability is expected
583 within their physicochemical characteristics. Since the process involves a high
584 temperature, thermostable enzymes are needed. Exo- and endo-inulinase from



585 *Aspergillus niger* with maximal activity at pH 4.3 is suitable for large-scale production. The
586 enzymes are commercially used and display a negligible influence on the flavour and
587 aroma properties of the final product (Ilgin et al., 2020).

588 Agave syrup also possesses prebiotic effects by stimulating colon bacteria growth,
589 making it suitable for developing nutraceutical products. Other benefits may involve
590 enteric infection protection and immune response stimulation (Catry et al., 2018).
591 Therefore, many current investigations are focused on studying new techniques and
592 protocols to extract and characterize the fructans. Ávila-Fernández et al. (2011)
593 developed a partially thermal acid hydrolysis technique. They demonstrated a relationship
594 between agave fructans hydrolysis and the number of fructo-oligosaccharides (FOS) in
595 the mixture through a fructose equivalent (FE) parameter. The FE defines the percentage
596 of total sugars converted into reduced sugars and could characterize the novel
597 bifunctional product containing prebiotic FOS molecules and sweetening features in the
598 syrup. The study also documented the possibility to remove the resulting
599 monosaccharides through *P. pastoris* cultures. This technique was concluded to be an
600 economical and efficient method at a laboratory scale to develop potential sugar-free
601 prebiotic FOS products (Cervantes et al., 2020).

602

603 **2.6. Date syrup**

604 **2.6.1. Physicochemical and bioactive properties**

605 Date syrup, identified as a dark brown substance, is the main derived product from dates,
606 one of the main fruit trees (*Phoenix dactylifera* L.) in the Middle East region. It is mainly

607 produced by Iran (20%), Egypt (17%), Iraq (15%) and Saudi Arabia (14%) (Hashemi et
608 al., 2018). Since ancient times, dates are deemed essential to human nutrition and food
609 preparation in the desert regions. Favouring the rapid market growth, it is estimated that
610 over 8 million tons of dates were harvested in 2017 (Ben Yahmed et al., 2021). The date
611 fruit's significances rely on its rich carbohydrates' composition (70-80% w/w), dietary fibre
612 (8.7%), amino acids, proteins (1.8%), vitamins, salts and minerals. Its main
613 physicochemical compositions are moisture content of 16% and total sugar of 79.5%
614 (94% corresponds to inverted sugar, including glucose and fructose). Due to the complex
615 non-sugar molecules mix, the syrup presents high viscosity (17 P at 20 °C) and 4.1% of
616 colouring matter. Moreover, the inverted sugar compounds influence the product's acidic
617 components (pH 3.8), contributing to its resistance against microorganisms (Ghnimi et
618 al., 2017).

619 The most important properties of date syrup are its potential health benefits, which are
620 related to its high nutritional profiles, i.e., high content of unsaturated fatty acids (such as
621 oleic, linoleic, palmitoleic and linolenic acids) and a combination of 15 minerals, including
622 potassium, iron, magnesium and calcium. The syrup also contains fluorine and selenium,
623 which display good teeth protection against decay and stimulate immune function. It also
624 contains at least six vitamins, including B1 thiamine, B2 riboflavin, nicotinic acid, A and C
625 (Ibrahim et al., 2020).

626 *2.6.2. Extraction and promising methods for date syrup processing*

627 Date syrup extraction is considered one of the oldest practices in sweeteners production,
628 and it is naturally produced while the fruit is stored under hot conditions. Industrial
629 production involves six main steps: pretreatment, extraction, filtration, clarification,



630 purification and concentration (see **Figure 6**). In principle, the pretreatment step consists
631 of soaking, pitting and pulping of the fruit. Once the pulp is obtained, the extraction is
632 done by mixing the pulp with water and heating until the sugars are released. The syrup
633 is then filtered to eliminate the remaining solids, followed by clarification and purification
634 processes before evaporation to concentrate the syrup (Ben Yahmed et al., 2021;
635 Bertuzzi, 2018).

636

637 **Figure 6.** Major processing operations used in date syrup production.

638

639 The applied thermal extraction process contributes to its characteristic dark colour.
640 However, a clear syrup has a better acceptance as a flavouring agent in the food industry
641 (Nasabi et al., 2017). Several different clarification techniques have been explored to
642 eliminate this dark colour characteristic, such as cation/anion exchangers (Nasabi et al.,
643 2017), membrane processes (Zhang et al., 2021), ion exchange adsorption (Ahdno &
644 Jafarizadeh-Malmiri, 2017). Traditionally, thermal extraction usually degrades specific
645 nutritional components and darkens the product, resulting in the need for an additional
646 clarification step and thus increases the production cost. Other new improvement
647 techniques have also been studied, and the sonication method is the most promising.
648 Sonication disrupts cell tissues and organic matter, improving the extraction process by
649 enabling a more efficient solvent penetration into the cells and thus allowing a greater
650 release of the targeted molecules. Another sonication advantage is microorganism
651 inactivation, caused by the disruption of microorganisms' membrane cells (Hamza et al.,



652 2021). For instance, the ultrasonication extraction at high-intensity ultrasonic waves (ca.
653 20 kHz) and low temperature (ca. 15 °C) resulted in the highest extraction efficiency at a
654 shortened extraction time while significantly reduced the total microbial counts. These
655 features play an essential role in producing the highest quality final product (desired
656 colour and nutritional profile preservation) and requiring fewer operation units,
657 consequently reducing the production time and cost.

658

659 **2.7. Stevia (steviol glycosides)**

660 *2.7.1. Physicochemical and bioactive properties*

661 *Rebaudiana* has gained scientific and industrial attention for broad applications as a
662 natural sweetener in commercial food products for its nutritional value and a potential
663 sucrose alternative (Bursać Kovačević, Maras, et al., 2018). *Stevia Rebaudiana* is a
664 perennial herb plant native to South America, notable for its intensely sweet taste and
665 potential pharmaceutical and medicinal applications (Lemus-Mondaca et al., 2015).
666 Diterpene glycosides compounds, specifically stevioside and rebaudioside (shown in
667 **Figure 7**), are responsible for Stevia's sweetness. *Stevia*

668

669 **Figure 7.** Chemical structures of a) *Stevioside* and b) *Rebaudioside A* contained in
670 Stevia plants.

671

672 Among the 230 species of *stevia* plants, *Stevia Rebaudiana* and *Stevia phlebophylla*
673 present a sweet taste, contributes by a high concentration of Steviol glycosides (SGs),
674 representing ~4-20% of the dry *stevia* leaves weight (Bursać Kovačević, Maras, et al.,
675 2018). SGs display 40 to 450 times stronger sweetness intensity than sucrose. The SGs
676 amount in the plant varies depending on the climatic, environmental and growth
677 conditions (Aghighi Shahverdi et al., 2018). As previously mentioned, sucrose
678 consumption is controversial due to health concerns related to long-term weight gain,
679 inducing metabolic syndrome, and in the case of saccharin, glucose intolerance and
680 dysbiosis (Yoneda et al., 2017). Therefore, SGs' sweetness intensity, low-calorie content,
681 cardiogenic, anti-cancer and anti-inflammatory properties increase *stevia*'s commercial
682 value in the food market (Mathur et al., 2017). Today, SGs extraction methodologies must
683 be revisited to improve these biologically active compound extraction yields (Bursać
684 Kovačević, Barba, et al., 2018).

685

686 2.7.2. *Extraction and promising methods for steviol glycosides processing*

687 Conventional SGs extraction methods include solvent, Soxhlet, heating-under-flux and
688 cold extraction. These traditional techniques present several drawbacks, such as time-
689 consuming, lack of thermoregulation and utilize high amounts of organic solvents (e.g.,
690 methanol, ethanol, acetone, chloroform and even petroleum ether) (Castro-Muñoz et al.,
691 2020; Jentzer et al., 2015). Consequently, other potential SGs extraction techniques have
692 been studied (as reported in **Table 2**).

693

694 **Table 2.** SGs extraction yields from *Stevia Rebaudiana* using different methods.

695 Adapted from (Castro-Muñoz et al., 2020).

696

697 The highest extraction yield was achieved using an ultrasonication extraction of
698 *Rebaudioside A*, producing a 35% yield (35g SGs in 100g) (Gasmalla et al., 2017). The
699 extraction required 60% v/v isopropyl alcohol as the solvent and only used 360 Watts for
700 the 12 minutes process. Nonetheless, the volatile organic solvent is a critical concern due
701 to its high volatility, inflammability, toxicity and represents a negative impact on health,
702 safety, environmental and production costs (Yoneda et al., 2017). Solvent-free SGs
703 extraction methods such as pressure-driven membrane processes (MF, UF and NF) are
704 successfully proven to facilitate the extraction of various high-value-added components,
705 such as antioxidants, sugars, carbohydrates, pectin, phenolic compounds. Membrane
706 processes rely on transmembrane pressure as the driving force for selective separation,
707 while the membrane serves as a physical barrier that selectively permeates the targeted
708 compounds (Castro-Muñoz et al., 2016). The classification of pressure-driven membrane
709 processes is based on membrane pore sizes, as specified in **Table 3**.

710

711 **Table 3.** Classification of pressure-driven membrane processes based on the
712 membrane pore size and their application in sweetener extraction.

713

714 Membrane processes constitute a practical option for recovering valuable molecules and
715 present numerous advantages, including no harmful solvent requirement. Water may be

716 used as the main solvent in several membrane processes, directly lowers the
717 environmental impact and simplifies the process conditions (Vian et al., 2017).

718 To date, different authors have evaluated the pressure-driven membrane performances
719 in SGs extraction from *Stevia Rebaudiana* dried leaves (Castro-Muñoz et al., 2020). All
720 the tested membranes have yielded >90% recovery efficiency, where the highest yield
721 (ca. 91 %) was obtained at 6 bar for 90 min using MF membranes with a pore size of 0.2
722 µm (Castro-Muñoz et al., 2020). Integrated membrane processes also acquired the
723 highest recovery, as demonstrated by Díaz-Montes et al. (2020) in purifying *rebaudioside*
724 *A* (Reb-A) from *Stevia rebaudiana* aqueous extracts using a two-step UF process. Each
725 UF membrane has a different molecular weight (100 and 1 kDa, respectively). The results
726 revealed that the 100 kDa membrane removed most of the total solids and carbohydrates
727 (ca. 42% and 41%, respectively). Meanwhile, the 1 kDa membrane was able to retain
728 ~98% of phenolic compounds. The authors concluded that the two-step UF system
729 effectively extracted up to 38 mg Reb-A per 22 g dry leaves. In addition, the process
730 exhibited a 93% Reb-A recovery efficiency from 49 mg presented in the crude extract.
731 Accordingly, it demonstrated that the membrane process could surpass the standard SGs
732 extraction yields and purity.

733

734 **2.8. Sorghum syrup**

735 *2.8.1. Physicochemical and bioactive properties*

736 *Sorghum bicolor* is among the most widely produced cereals globally, with wheat, rice,
737 maize and barley (de Morais Cardoso et al., 2017) and used to produce syrup for



738 beverage and food industries. The physicochemical properties of sorghum syrup are
739 comparable to that of sugarcane syrup since both are extracted from the same *Poaceae*
740 species family (Asikin et al., 2018). This cereal is an essential diet in semi-arid regions,
741 such as Africa and some parts of Asia. Phenolic compounds isolated from the plant
742 displayed therapeutic effects in preventing several conditions, such as cancer, obesity
743 and cardiovascular diseases (de Morais Cardoso et al., 2017). Moreover, sorghum
744 consists of other bioactive compounds, such as carotenoids, proteins and vitamins,
745 extracted from specific parts of the grain and summarized in **Table 4** (de Morais Cardoso
746 et al., 2017).

747

748 **Table 4.** Bioactive compounds contained in *Sorghum bicolor* at the specific anatomic
749 structure (de Morais Cardoso et al., 2017).

750

751 2.8.2. *Extraction and promising methods for sorghum syrup processing*

752 Sorghum syrup is produced by boiling the sweet juice extracted from the Sorghum plant
753 stalks. The conventional steps involved in its production are presented in **Figure 8**:

754

755 **Figure 8.** The conventional process for Sorghum syrup production (Ratnavathi &
756 Chavan, 2016).

757

758 Sorghum canes, composed of 70% water and 10-15% fibre, are milled with a three-roller
759 power mill, where the natural juice is extracted. This juice represents ~50-60% of the
760 stalk's weight (Ratnavathi & Chavan, 2016). The juice is then concentrated in continuous
761 flow evaporators until the temperature reaches the boiling point (~100 °C) and heavy-
762 density syrup is obtained. Additionally, this step may also be performed with batch
763 evaporators. One important note is that the evaporators are preferably be built of stainless
764 steel or copper for an efficient heat transfer. Finally, it is cooled, and the quality of
765 standardized syrup is monitored in this stage. Herein, enzymes such as isomerase are
766 added to prevent crystallization during storage and must be added at a temperature below
767 65 °C to avoid denaturalization (Asikin et al., 2018; Ratnavathi & Chavan, 2016). **Table**
768 **5** reports the standard physicochemical properties of the resulting sorghum syrup, which
769 may vary depending on both extraction and concentration steps' operating conditions.

770

771 **Table 5.** Standard physicochemical composition of sorghum syrup.

772

773 Sorghum syrup contains 5-hydroxymethylfurfural (5-HMF) and should be present in low
774 concentrations, and generally, a higher 5-HMF content is associated with a darker colour,
775 lower density and lower viscosity. During storage, 5-HMF concentration can be evaluated
776 to monitor the syrup quality. More importantly, 5-HMF can produce a negative health
777 impact due to its genotoxic effects. The 5-HMF concentrations and accumulations are
778 commonly influenced by different factors, including production methods, processing
779 temperatures and prolonged storage periods. To improve the extraction and quality of

780 sorghum syrup (to reduce the 5-HMF concentration in the final product), several
781 promising juice extraction methods and optimal storage conditions have been studied,
782 including hydrolysis by amylolytic enzymes, active charcoal purification, centrifugation,
783 filtration, sucrose hydrolysis by invertase and chitosan absorption followed by
784 evaporation. These techniques have been reported to reduce 5-HMF accumulations and
785 prevent crystallization during storage (Ospankulova et al., 2020).

786

787 **3. Current practices of the natural sweeteners in foods**

788 High sugar consumption is regularly related to the prevalence of type II diabetes,
789 obesity, cardiovascular diseases and dental decay (Carocho et al., 2017). More than
790 ever, consumers are more aware and concerned with their sugar intakes and actively
791 seeking for healthier food options, i.e. reduced sugar, sugar of natural origin, and
792 unchanged flavour. Consumer demands have prompted the food industry to make
793 considerable R&D investments to prepare products with natural-based sweeteners
794 (Olivo, 2019). An overview of current applications is presented in **Figure 9**. This section
795 addresses the progress in commercial food manufacturing using natural sweeteners in
796 various companies.

797

798 **Figure 9.** Current applications of natural sweeteners in commercialized food products.

799

800 **3.1. Stevia**

801 As mentioned earlier, stevia was one of the earliest sweeteners to gain industrial interest
802 and consumer trust and was considered safe in Japan and Paraguay by 1970. Stevia was
803 recognized in 1994 as a dietary supplement in the USA, but not until 2008 when the FDA
804 granted the GRAS designation to stevia extract (specifically the Reb-A). This was reached
805 in response to the request made by *Cargill Inc.*, teamed with *Coca-Cola Co.* and *Whole*
806 *Earth Sweetener Co*, a unit company of *PepsiCo* (Chesterton & Yang, 2016). *SweetLeaf*[®]
807 *Stevia sweetener* became the first recognized table sweetener with a safe status by 2008
808 (*SweetLeaf Stevia Sweetener*, <https://www.sweetleaf.com>, Access date: 6 April 2021).
809 Since then, various beverages and food formulations contain stevia, including ice cream,
810 bakery products, and spices (Carocho et al., 2017).

811 *Cargill Inc.* was one of the pioneering companies to launch stevia-based sweeteners,
812 called *Truvia*[®] *Natural Sweetener*, introduced in 2008 as a calorie-free stevia leaf extract
813 (Huber, 2017). The success of the natural sweetener came to such an extent that *Coca-*
814 *Cola* introduced *Sprite Green*[®] a year later (Fusaro, 2015). Certainly, *Truvia*[®], the
815 sweetener developed by *Cargill Inc.* in collaboration with *Coca-Cola Co*, was included in
816 the product formulation (Beaufort, 2015). In 2014, *Pepsico* and *Coca-Cola* released
817 competing stevia-based sodas, where *Pepsico* launched *Pepsi True*[®], claiming a 30%
818 reduced sugar content. In comparison, *Coca-Cola* launched *Coca-Cola Life*[®] that
819 contained both stevia and sugarcane with 35% fewer calories than other carbonated
820 drinks in the market (Ahmad et al., 2020). Additionally, to expand its natural line, *Hansen's*
821 *Beverage Company* announced in 2011 a partnership with *Truvia*[®] to introduce *Natural*
822 *Fruit Sticks* and *Tea Stix*[™], which were marketed as low-calorie and sugar-free
823 beverages, respectively (Newswire, 2020a).



824 *YoCrunch 100*[®] was another product launched with *Truvia*[®], which was part of the
825 reduced-calorie yoghurts *YoBand*[®] line. The original preparation contained ~200 calories
826 per serving but was not considered a healthy snack by consumers. Hence, *YoCrunch*[®]
827 introduced erythritol as a low-calorie sweetener and gained low customer approval. When
828 *Truvia*[®] substituted erythritol in January 2010, the product regained public acceptance.

829 The brand *Freshens* also worked with *Truvia*[®] to develop sugar-free and sugar-reduced
830 products. At this point, *Freshens*, as the biggest frozen yoghurt company in the U.S.,
831 created a new yoghurt-based formula with *Truvia*[®], presenting a lower caloric content
832 and no added sugar (Newswire, 2020b). Furthermore, ice-creams with stevia has been
833 suggested for diabetic consumers. Rodríguez et al. (2016) formulated ice cream with 10%
834 sorbitol and 0.48% stevia, obtaining the recommended sweetness level for diabetic
835 patients.

836 *Ingredion Incorporated* is yet another company that distributes stevia as a table
837 sweetener and has a product line with a specific Reb-A content directed to different food
838 industry applications. *Ingredion's Enliten*[®] *Reb-A* sweeteners are marketed as natural-
839 based and non-transgenic sweeteners. Additionally, *Health CO.* has released *Stevia*
840 *FSE*[™], enzymatically processed to eliminate its characteristic sourness (Olivo, 2019).
841 Another company associated with stevia as a low-calorie sweetener is HIP gastroplex,
842 which developed *SUGARLESSe*[™]. This commercial product contains a debittered stevia
843 and other plant-extracted sweeteners, often used in chocolates and marshmallows. Its
844 contributions to the final products are consistency, colour, and sweetness (Bosshardt,
845 2020).

846



847 3.2. *Honey*

848 Honey, for a long time, was the only sweetener available for human consumption. Its
849 application was not restricted to dietary purposes but also promoted in various
850 applications, including drug in-home treatment for infections and burns. Compared to the
851 other natural sweeteners, honey is self-preservative when it is not diluted. Besides being
852 a natural carbohydrate source, it contains vitamins, proteins, amino acids, and
853 polyphenols with high overall nutritional value (Muhammad & Sarbon, 2021; Vara et al.,
854 2019).

855 The consumer familiarity with honey has positioned it as an ideal option for consumers
856 who expect minimally processed products. While most natural sweeteners (stevia, cane
857 sugar, agave nectar, maple syrup, etc.) need to be further processed (extraction and
858 purification) to obtain their sweet components, honey is not typically processed after its
859 initial collection (NHB, 2021). The National Honey Board encourages food manufacturers
860 to use honey in their formulations to meet customer demands for a clean label and
861 sustainable ingredients. Current honey applications in food products include bakery and
862 snacks, cereals, beverages, and brewing.

863 Honey is popularly regarded as a natural energy booster due to its high caloric content.
864 This feature is attractive to cereal and bar manufacturers, where honey serves not only
865 as a sweetness and taste enhancer but also for its functionality (Vara et al., 2019). Its
866 viscosity characteristic acts as a binder that helps maintaining the cereal mixture
867 compactness in cereal bars. Despite the promising attributes provided to cereal and bar
868 products, sugar is still the most used sweetener in the new products. By 2016, 78% of the

869 cereal and bars released in the market were formulated with sugar, while only 21%
870 contained honey (NHB, 2021).

871 Several recently released snack bar products include *Oats'n Honey* granola bars from
872 *Nature Valley*[™], part of the *Crunchy bars* line. *Nature Valley*[™] philosophy is directed by
873 the use of natural products without artificial sweeteners or high fructose syrup; thus,
874 honey is marketed as the preferred ingredient. However, honey is combined with common
875 sugar in other *Nature Valley* products, such as *Oats'n Dark* chocolate and *Coconut*
876 *crunchy granola bars* (Nature Valley, <https://www.naturevalley.com/all-products/>, Access
877 date: 1 October 2020). *Kind LLC* has a strong reputation for honey-sweetened snacks
878 and has introduced cereal bars with 65% honey content (Nature Valley, 2021). *Honey*
879 *Oat Breakfast Bars*, *Oats and Honey with Toasted Coconut* and *Oats and Honey* snack
880 bars stand out among their products (Kind Snacks, 2021).

881 The cereal industry is yet another sector that has widely featured honey in new
882 formulations. Over the last decade, criticism about the elevated sugar content in cereals,
883 particularly those directed to children, raised health concerns associated with their
884 overconsumption. In response, big cereal companies, like *Kellogg's*[®], have been actively
885 highlighting honey usages in their products as a strategy to regain customer acceptance
886 (Askew, 2015; Garcia et al., 2020). Examples of honey-sweetened cereals include *Honey*
887 *Monster by Halo foods* and *Honey Bunches of Oats*[®], a part of the Post Consumer Brands
888 products introduced in 1989 and a top seller in the US. More recently, *Honey Bunches of*
889 *Oats* released a new version of its traditional recipe: *Honey Bunches of Oats*[®] *Frosted*
890 *cereal* with frosted flavours and granola clusters (PR Newswire, 2020a).



891 Interestingly, the brewing industry also uses honey in the fermentation process for
892 centuries. There is evidence of fermented beverages prepared with wild grapes,
893 hawthorn, rice and honey dated back to 7000 years ago in Northern China; however,
894 honey brewing lost its popularity when wine was introduced during the rise of the Roman
895 Empire (Cabras & Higgins, 2016). In recent years, brewing with honey has recaptured
896 commercial interest with increased consumer demands for craft beer. Honey in brewing
897 presents several advantages to producers, including a 90-98% fermentability and an
898 increased polyphenols level relevant to the beer taste and improves its overall antioxidant
899 activity. Among the latest products, barley-malted and honey-enriched *Salmon Fly Honey*
900 *Rye*[®] was launched in 2014 by *Madison River Brewing Company, Inc.* The company's
901 website states that honey is used to confer a mild sweet taste that serves to attenuate the
902 flavours of rye and hop (Madison River Brewing Industry, 2014). Moreover, *Genesee*
903 *Brewing Company*, the Dundee Honey Brown Lager trademark owner, has produced
904 beers with natural honey for over 20 years (Genesee Brewing Company, 2021). Another
905 company, *Apex Predator Brewing*, also recently released *Honey Oat Blonde Ale*[®] in
906 Canada, brewed with local honey and marketed as a clean product (Trade et al., 2019).

907

908 3.3. Molasses

909 Molasses have been traditionally related to a bitter taste and low sweetness, although
910 molasses are most often used in home cooking (in sauces and bakeries) (Bosshardt,
911 2020). The fundamental research in this field aims to produce lighter versions that display
912 a subtle honey taste whilst the manufacturers focus on wider colour varieties and
913 consistencies for different food applications. Unlike other sweeteners, blackstrap



914 molasses show a lower glycemic index and contain many antioxidant compounds. It was
915 reported that the molasses exhibited a higher polyphenol content (ca. 17 mg gallic acid
916 equivalent (GAE)/g) compared to the other natural sweeteners. It also presents a broader
917 variety of nutraceutical compounds (Deseo et al., 2020; Valle et al., 2020) and various
918 minerals (Senthilkumar et al., 2016).

919 Molasses can be used as sweeteners in dairy products, like yoghurts (Noureldin et al.,
920 2020). In 2006, *Danone S.A.* announced a partnership with the Bangladeshi bank
921 Grameen to create a new yoghurt formulation that was nutritious and affordable for low-
922 income households. Since the initial product was not sweet enough for the consumers,
923 the company decided to use locally produced date molasses (Peerally et al., 2019).

924 As a carbon source, molasses are also used for industrial-scale fermentation, such as in
925 the *Saccharomyces cerevisiae* yeast fermentation for bread and food-grade ethanol
926 production (Wu et al., 2020). Molasses represent a suitable fermentation substrate since
927 they are cost-effective and constitute a good energy source for yeasts, mold and bacteria
928 (UM Group, 2021). The *Lesaffre Group*, specialising in yeast, ethanol, and beverage
929 production through fermentation, uses sugar cane molasses in their yeast propagation
930 (Lesaffre, 2021). Moreover, the fermentation of molasses obtained from sugar cane
931 bagasse, has been effectively conducted via *Aspergillus niger* (Bakhiet & Al-Mokhtar,
932 2015).

933 Apart from the food, other molasses application includes the foundry industry
934 (manufacturing of casting molds) (Pribulova et al., 2016). Cheap molasses has effective
935 binding properties that allow them to trap fine dust at a large manufacturing scale. Another



936 advantage is that the molasses are non-pollutant, which means they do not produce toxic
937 emissions during combustion and deemed safer.

938

939 3.4. *Date sugar*

940 Date fruit has a high nutritional value and is a good mineral source. It has an elevated
941 antioxidant content, a low glycemic index, and potentially a suitable sweetener for diabetic
942 individuals (Farahnaky et al., 2016). Date sugar market was valued at \$553.6 million in
943 2018 and is expected to reach \$850.3 million by 2027. Despite being minimally
944 processed, more cost-effective options have similar qualities, such as coconut sugar, thus
945 limiting the date sugar market growth (PR Newswire, 2020b).

946 Although date sugar contains high fibres, vitamins and minerals, it has only 50-70% sugar
947 compounds that directly influence some of the desired physical characteristics (e.g.,
948 solubility), limiting its applicability in the bakery industry (Kumar et al., 2020). On the other
949 hand, date syrup is considered a good sugar substitute in ice cream and yoghurts since
950 it helps maintain good organoleptic properties. A 10% date syrup in yoghurt exhibited
951 higher scores for the sensory evaluation (taste, sweetness, flavour and acceptability).
952 Additionally, date-syrup-enriched yoghurt showed a higher overall antioxidant activity,
953 hydrochloric acid-soluble minerals and folate concentration. Meanwhile, date-syrup-
954 sweetened bakery products have exhibited “good” and “high” in acceptability rating and
955 a “high” for texture (Kumar et al., 2020).

956

957 3.5. *Agave nectar*



958 Agave nectar has been recognized as a low-glycemic alternative sweetener, a slow-
959 release carbohydrate with a high prebiotic capacity (Ozuna et al., 2020). Agave also has
960 a high fructose content (~90%) and lower glucose levels, which may be a disadvantage
961 since long-term fructose consumption might lead to insulin resistance and the risk of
962 developing type 2 diabetes (Gardner, 2017). Even so, there have been arguments that
963 the agave composition is safe for diabetic patients (Carranza et al., 2015). For example,
964 Hooshmand et al. (2014) monitored the blood glucose levels, insulin and weight gain of
965 sucrose-fed and agave-nectar-fed mice. They observed a slower weight gain, a decrease
966 in insulin and glucose levels in agave-nectar-fed mice and suggested the agave syrup as
967 a sucrose substitute. The reduced glucose level causes a higher glucagon-like peptide-1
968 (GLP-1) level, which induces insulin secretion from the pancreas and is responsible for
969 appetite suppression.

970 Agave nectar is a promising sugar substitute due to its minimal bitterness that does not
971 increase with concentration (McCain et al., 2018). Moreover, agavins or agave fructans
972 (a natural form of fructose with a decreased caloric content) may also be used to replace
973 fat. Cookies prepared with *Agave angustifolia*'s fructans exhibited high water-absorption
974 and water-holding capacities, resulting in an increased water volume and thus expected
975 to induce rapid satiety. Furthermore, fructan addition to the formulation increased the total
976 sugar content, aided in the moisture preservation and produced a better crust colour
977 (Santiago-García et al., 2017).

978 Previously, agave syrup and native agave dietary fibre were proposed to substitute honey
979 and wheat flour in granola bars. The optimum granola bar formulation, containing a ratio
980 of 3:7 (agave fructans to agave fibre), showed a balance of soluble and insoluble fractions

981 and exhibited a 72% glycemic index, classified as a moderate level. Meanwhile, agave
982 syrup helped reduce the sugar content in the final product while maintaining its
983 organoleptic properties preferred by the consumers. Since agave products are generated
984 surplus by-products from the tequila industry, their usages in the food industry improved
985 its benefits (Zamora-Gasga et al., 2014).

986 It is worth mentioning that the non-caloric sweeteners (e.g., stevia) display an increased
987 sweetening power, but their contribution to the overall product consistency is deficient.
988 This is contrary to the synthetic sweeteners that enhance the texture and structure of a
989 pastry but generally lack flavour. A 75% substitution of sucrose with agave syrup,
990 combined with xanthan gum and leavening agents, produced a lower viscosity and
991 increased the thermosetting temperature of batters in muffins. However, higher levels of
992 sucrose substitution with agave syrup produced a darker crust and a paler crumb (Ozuna
993 et al., 2020).

994

995 3.6. *Maple Syrup*

996 Maple syrup is yet another natural sugar suggested as a healthier sweetener substitute.
997 Sucrose is the main carbohydrate, representing ~97% of its composition. It exhibits a high
998 lignans content, including lariciresinol, secoisolariciresinol and phlorizin, which are not
999 found in brown rice syrup, agave syrup, corn syrup, or honey. Maple syrup also presents
L000 high phytohormone abscisic acid content, responsible for antidiabetic activity (Mellado-
L001 Mojica et al., 2016). Additionally, maple syrup consumption can lower glycemic and
L002 insulinemic responses as a result of α -glucosidase activity inhibition, which limits glucose

1003 absorption in the intestine (Mora & Dando, 2021). A lower glycemic response is desirable
1004 at the pre-diabetic stages to prevent the transition to an irreversible stage. Besides being
1005 sold as a condiment for bakery products, maple syrup is also used to formulate various
1006 beverages and snacks. For instance, *King's Row Coffee*, the producer of *Maple water*
1007 *cold brew*[®], markets maple syrup as an antioxidant-rich ingredient while conferring a
1008 different taste and aroma in coffees (O'Reilly, 2019). Maple syrup also has been used in
1009 craft soda by the *Soda Folk company*, which formulates *Root Beer Soda* with maple
1010 syrup, vanilla, water, cane sugar and wintergreen (Morton, 2015). Alternatively, maple
1011 syrup is directly sold as a concoction prepared with ginger and sea salt. This product is
1012 branded as a natural carbohydrate source with additional vitamins, minerals and amino
1013 acids, aimed at consumers who practice sports (Slopeside Syrup Unlapped, 2020). Maple
1014 syrup is also included in confectionery products, e.g., *Bixby & Co.* produces *Maine Maple*
1015 *vanilla Bonbons*[®], recognized as the best-selling product in the category (Bixby Co.,
1016 2021).

1017

1018 3.7. Other natural sweeteners

1019 3.7.1. Monk fruit

1020 Monk fruit is a high-intensity natural sweetener that originated in China and Indonesia.
1021 The commercial extract is obtained from *Siraita gosvernori* species, cultivated in the
1022 Chinese province of Guangxi, and it accounts for ~90% of the global production.
1023 Traditionally, monk fruit has been used as a natural sweetener and medicinal product for
1024 pharyngitis treatment (Świąder et al., 2019). Currently, it is commercially available as
1025 table sweeteners. *S. gosvernori* extract is primarily sold in combination with *S. rebaudiana*

1026 and erythritol as a sweetening product (Soejarto et al., 2019). Monk fruit has a GRAS
1027 status in the US; however, due to the lack of evaluation in food products, it cannot be
1028 used as a food ingredient in the EU (Świąder et al., 2019).

1029 The monk fruit's sweetness is contributed by a group of terpene glycoside compounds
1030 called mogrosides. Among the five mogrosides types in monk fruit, mogroside V is found
1031 in higher concentrations with ~256-378 times more sweetness intensity than the common
1032 sugar (Świąder et al., 2019). Mogrosides from *S. gosvernori* also possess bioactive
1033 properties according to the anti-diabetic and anti-cancer effects (Liu et al., 2018; Zhou et
1034 al., 2018). Specifically, mogroside V is responsible for the apoptosis and cell cycle arrest
1035 of pancreatic tumour cells (Liu et al., 2017) and is associated with free radical scavenging
1036 activity (Pandey & Chauhan, 2019). Mogrosides also induce a hypoglycemic response by
1037 increasing insulin secretion, preventing lipid peroxidation, and reducing α -glucosidase
1038 activity (Gong et al., 2020). Furthermore, mogrosides positively impact blood glucose
1039 levels by increasing postprandial insulin levels (Liu et al., 2017). Besides mogrosides,
1040 the fresh fruit contains other bioactive compounds, including rutin, kaempferol and
1041 quercetin, which account for its antioxidant, antimicrobial and anti-inflammatory
1042 properties (Świąder et al., 2019).

1043 Monk fruit is used in a few products, such as sugar, syrup, jam, chocolate and skimmed
1044 milk (Pandey & Chauhan, 2019). Mahato et al. (2021) employed a combination of stevia
1045 and monk fruit extract to reduce the sugar content in chocolate-flavoured milk. The
1046 authors concluded that the combination of 56.27 ppm stevia and 81.90 ppm monk fruit
1047 extract exhibited optimum sensory parameters for the overall liking, appearance, aroma,
1048 sweetness, mouthfeel and aftertaste. Similarly, sugar-free chocolates have been



1049 developed using monk fruit and fibre extract blends, which has resulted in chocolates with
1050 lower fat and complex carbohydrates levels. This new formulation could also be an
1051 alternative for people with diabetes (Pandey & Chauhan, 2019).

1052 3.7.2. Yacon syrup

1053 Yacon (*Smallanthus sonchifolius*) is a perennial plant native to the Andean region in
1054 South America. The tubers from this plant can be processed into juice (or syrup) and
1055 consumed as a sugar substitute. They are mainly composed of fructooligosaccharides
1056 (FOSs) and inulin, constituting ~60% of their dry mass (Kamp et al., 2019). FOSs are
1057 used as low-calorie sweeteners since human digestive enzymes do not hydrolyze them;
1058 thus, they are not metabolized in the gastrointestinal tract (Yan et al., 2019). The FOSs
1059 consumption exhibits prebiotic activity by stimulating bifidobacteria and *Lactobacillus spp.*
1060 growth in the colon, where these bacteria degrade FOS into short-chain fatty acids
1061 (Caetano et al., 2016). At the same time, the inulin has a significant industrial value as a
1062 texture modifier in yoghurt, cheese and milk drinks (Yan et al., 2019). The addition of
1063 Yacon syrup in yoghurt formulations presented the highest scores for sensory
1064 acceptability, appearance, and texture compared to natural yoghurt and yoghurt
1065 supplemented with cashew apple extract (Mendes et al., 2019).

1066 The characterization of Yacon syrup has revealed the presence of chlorogenic acid, a
1067 phenolic and bioactive compound, known for its therapeutic effects, antioxidant,
1068 antibacterial, anti-inflammatory and hepatoprotective activities (Naveed et al., 2018). da
1069 Silva et al. (2017) reported a 1202.25 µg GAE/g total polyphenol and a 175.13 µg/g of
1070 chlorogenic acid content in Yacon syrup, while its total antioxidant activity was estimated
1071 to be ~6.99 µM Trolox/g. Importantly, phenolic compounds in Yacon syrup may be helpful

1072 in chronic disease prevention, including cardiovascular and some cancer types (Yan et
1073 al., 2019). Furthermore, Yacon syrup has been recommended as an alternative natural
1074 sweetener for diabetic patients. Its extracts have demonstrated inhibitory activity against
1075 α -amylase and α -glucosidase, obstructing glucose absorption and, consequently,
1076 decreasing postprandial hyperglycemia (Russo et al., 2015). Similarly, Adriano et al.
1077 (2020) reported that 40g Yacon syrup consumption after breakfast decreased post-
1078 prandial glucose and insulin blood levels in adult women.

1079 Its physicochemical properties show water activity of 0.78, pH of 3.71 and 71° Brix. These
1080 features directly impact the product shelf-life by preventing the proliferation of undesirable
1081 microorganisms (da Silva et al., 2017).

1082 3.7.3. Palm sugar

1083 Palm sugar, a popular natural alternative in Asian countries (Le et al., 2020), is the sap
1084 obtained from the flowers of different palm species, including palmyra palm (*Borassus*
1085 *flabellifer*), nipa palm (*Nypa fruticans Wurmb*) and sugar palm (*Arenga pinnata*) (Saputro
1086 et al., 2019). Palm sugar has been used in various products, such as sweet soy sauce,
1087 beverages and desserts (Saputro et al., 2019). Le et al. (2020) recently studied palmyra
1088 palm granulated sugar's physicochemical properties and chemical composition. They
1089 revealed an overall A_w value between 0.30-0.48, which is optimum for extended storage
1090 times, with a pH value of 6.90. Although the main components of the evaluated samples
1091 were ca. 91% sugar and ca. 5.6% reduced sugars, various minerals (potassium, sodium
1092 and iron) were also detected. In addition, vitamins E, C and D were found in significant
1093 concentrations. Interestingly, palm sugar registered a total phenolic content ranging from



1094 2.77 to 8.94 mg per 100g, depending on the heat treatment used during the sap
1095 processing.

1096 In general, palm sap sugars present lower glycemic indexes (Saputro et al., 2019).
1097 According to Srikaeo et al. (2019), the predicted glycemic values for cane sugar (~91) are
1098 considerably higher than those registered for palm sugars (~70). Furthermore, granulated
1099 palm sugar produced from *B. flabellifer* exhibited cytoprotective activity against NIH3T3
1100 fibroblast cells. Le et al. (2020) showed that cells incubated with *tert*-butyl hydroperoxide
1101 with granulated palm sugar result in increased cell proliferation compared with those
1102 incubated with only *tert*-butyl hydroperoxide (an agent used to induce oxidative stress).

1103

1104 **4. Concluding remarks and future trends**

1105 Throughout this review, recent uses of natural sweeteners in the food industry were
1106 discussed, including current practices in food manufacturing. Their main extraction
1107 routes, as well as the key purification techniques, were analyzed. Although the reviewed
1108 natural sweeteners may present several health-related advantages, major drawbacks are
1109 observed in their extraction processes that directly influence their final physicochemical
1110 characteristics. Several emerging techniques to improve the overall production yield and
1111 quality are discussed. Promising methods like vacuum drying, spray drying, and pressure-
1112 driven membrane processes will likely preserve their natural nutritional properties
1113 effectively. Other techniques, including clarification, enzymatic hydrolysis and sonication,
1114 boost the development further by significantly decreasing the production costs while
1115 satisfying colour, nutritional profile and sterilization parameters. These techniques also
1116 reduced the treatment duration, and it is more imperative as these properties are affected



1117 considerably by thermal processes. Moreover, processing parameters and storage
1118 conditions are essential to ensure the high quality and purity of the final products.

1119 Their applications in food production highly rely on the organoleptic properties, structure
1120 and texture, and the added value they confer to different products. As the food industry
1121 explores more sweetening alternatives to meet consumer demands, large-scale
1122 applications have been expanded to bakery products, beverages, dairy products (mainly
1123 yoghurt) and even fermentation processes. More importantly, the current market trends
1124 signify the need for healthier and minimally processed foods. This represents an
1125 opportunity for the food companies, and natural sweeteners may play a vital role in the
1126 markets. Carob syrup, palm sugar and monk fruit are the rising alternatives that could be
1127 used in the near future. Although their current applications in the food industry are still
1128 limited, several attempts to include them in food formulations, such as in yoghurt, skim
1129 milk and chocolate products, revealed that these sweeteners enhance the final products'
1130 organoleptic properties positively. Further studies concerning the cultivation, extraction
1131 and safety of these minimally explored sweeteners are needed to widen their commercial
1132 use.

1133

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1140

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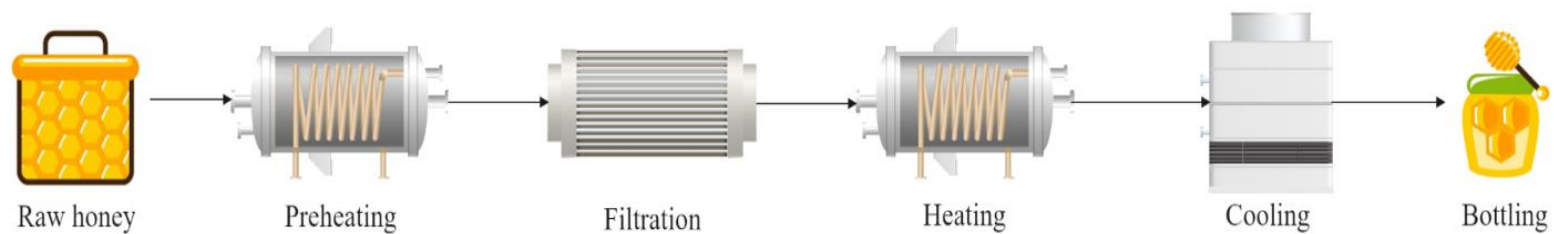


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Figure 1. Traditional honey production process.

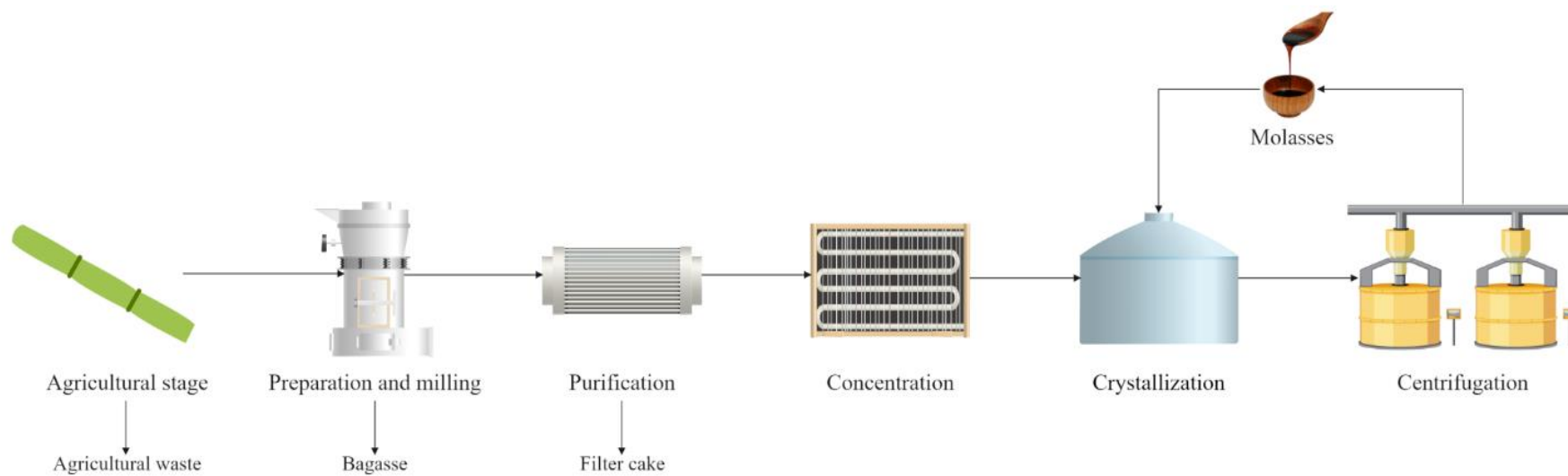


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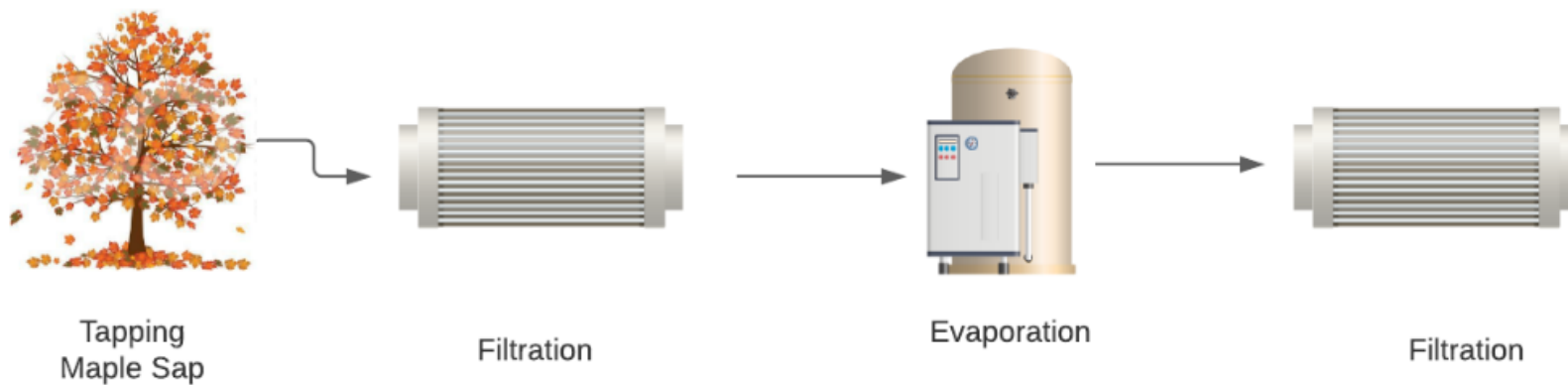
Figure 2. Sugar production process and its derived blackstrap molasses.

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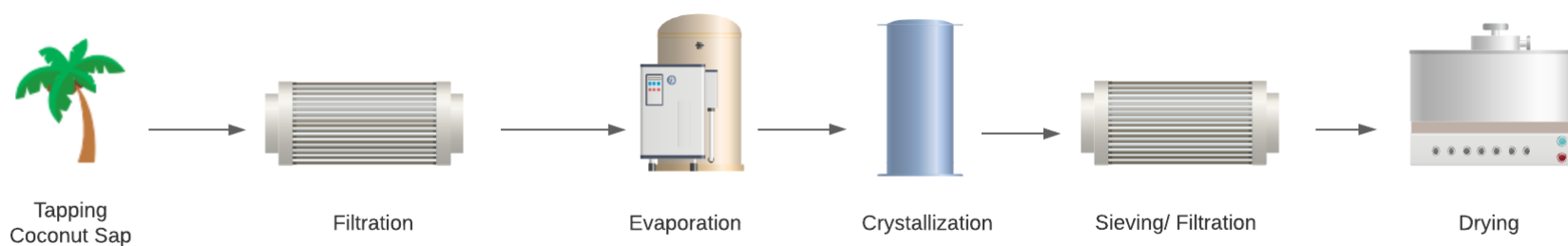
Figure 3. Schematic representation of the traditional maple syrup production.



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Figure 4. Schematic representation of the traditional coconut sugar production.



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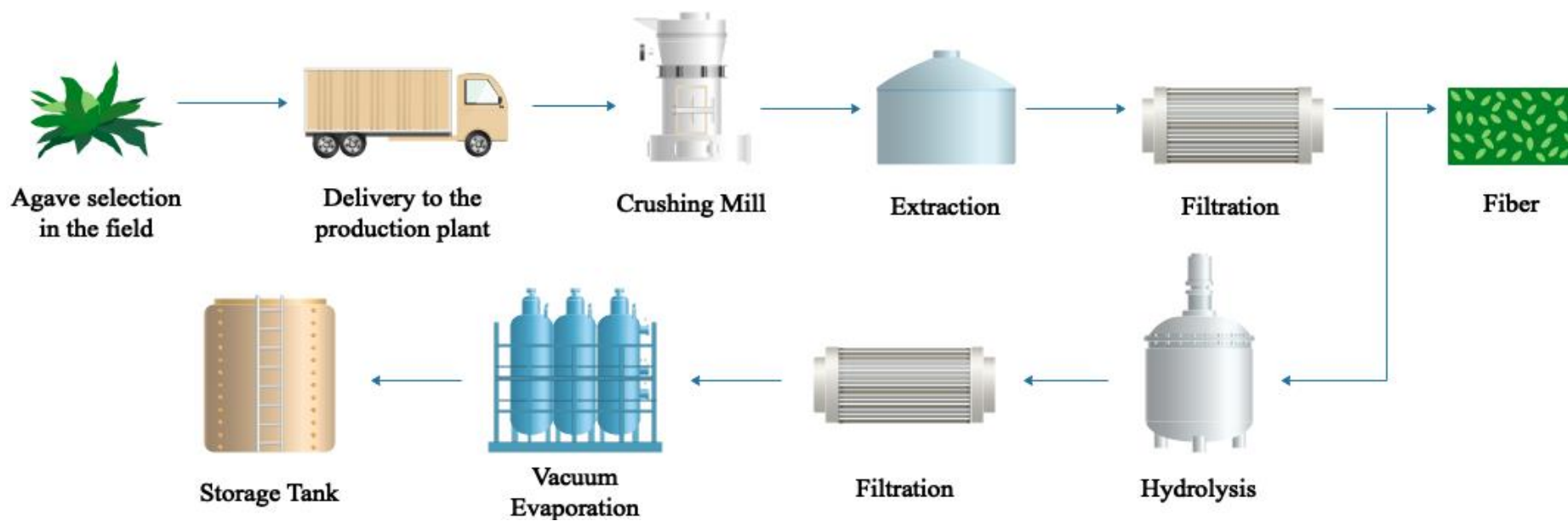


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Figure 5. Major processing operations involved in agave nectar production.

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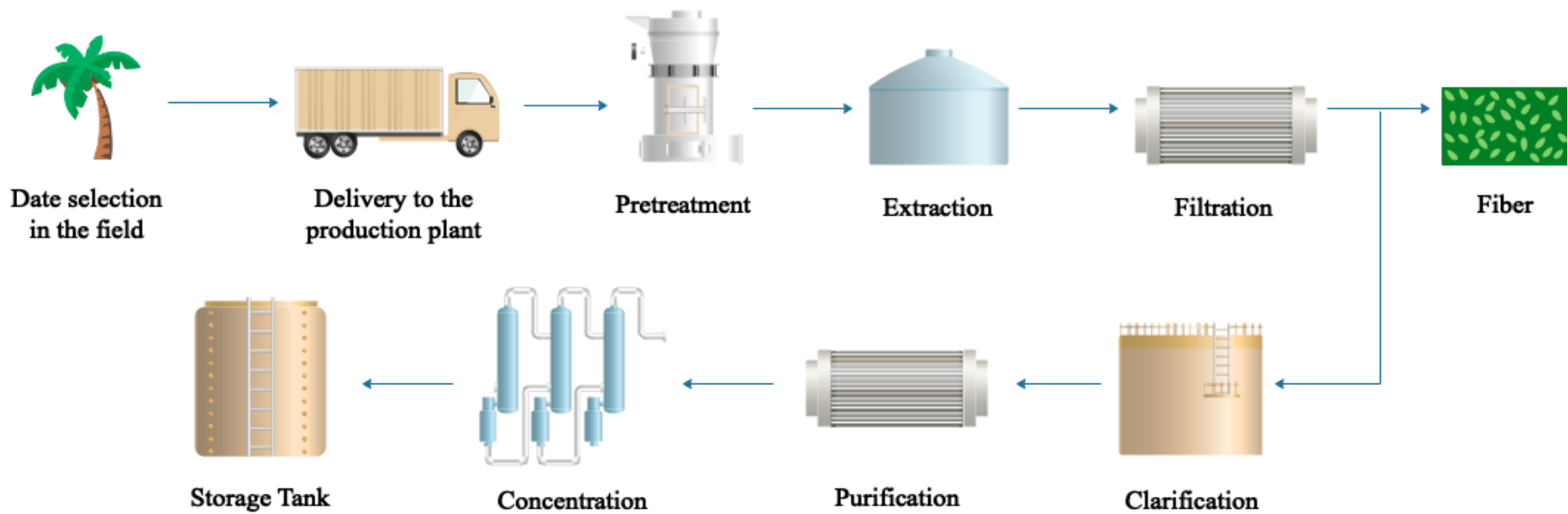
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Figure 6. Major processing operations used in date syrup production.

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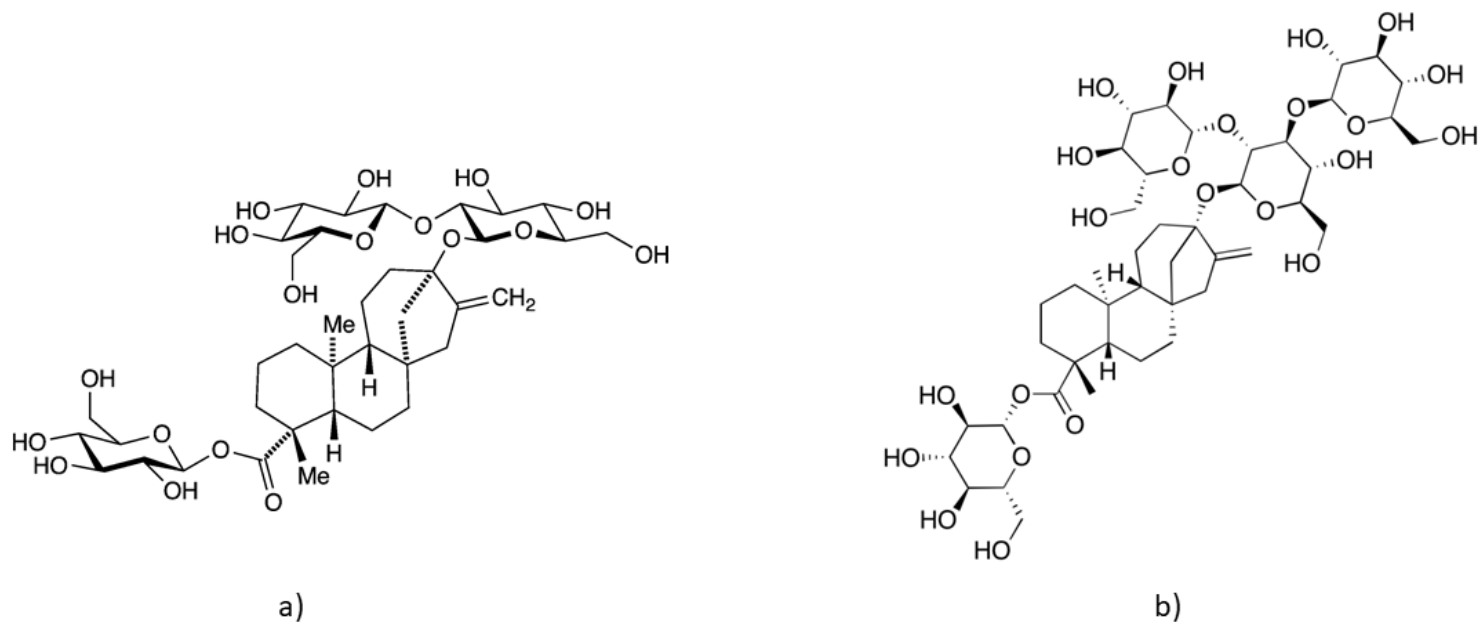
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Figure 7. Chemical structures of a) *Stevioside* and b) *Rebaudioside A* contained in *Stevia* plants.



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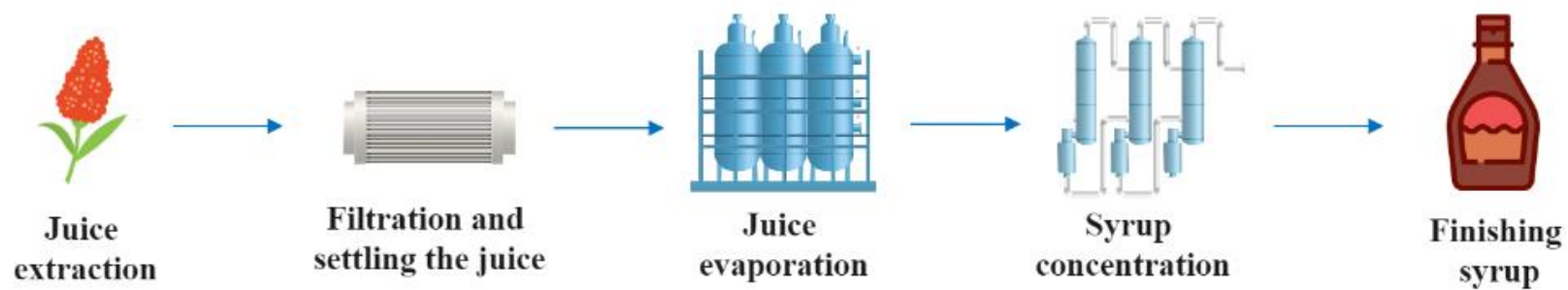
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Figure 8. Conventional process for Sorghum syrup production (Ratnavathi & Chavan, 2016).

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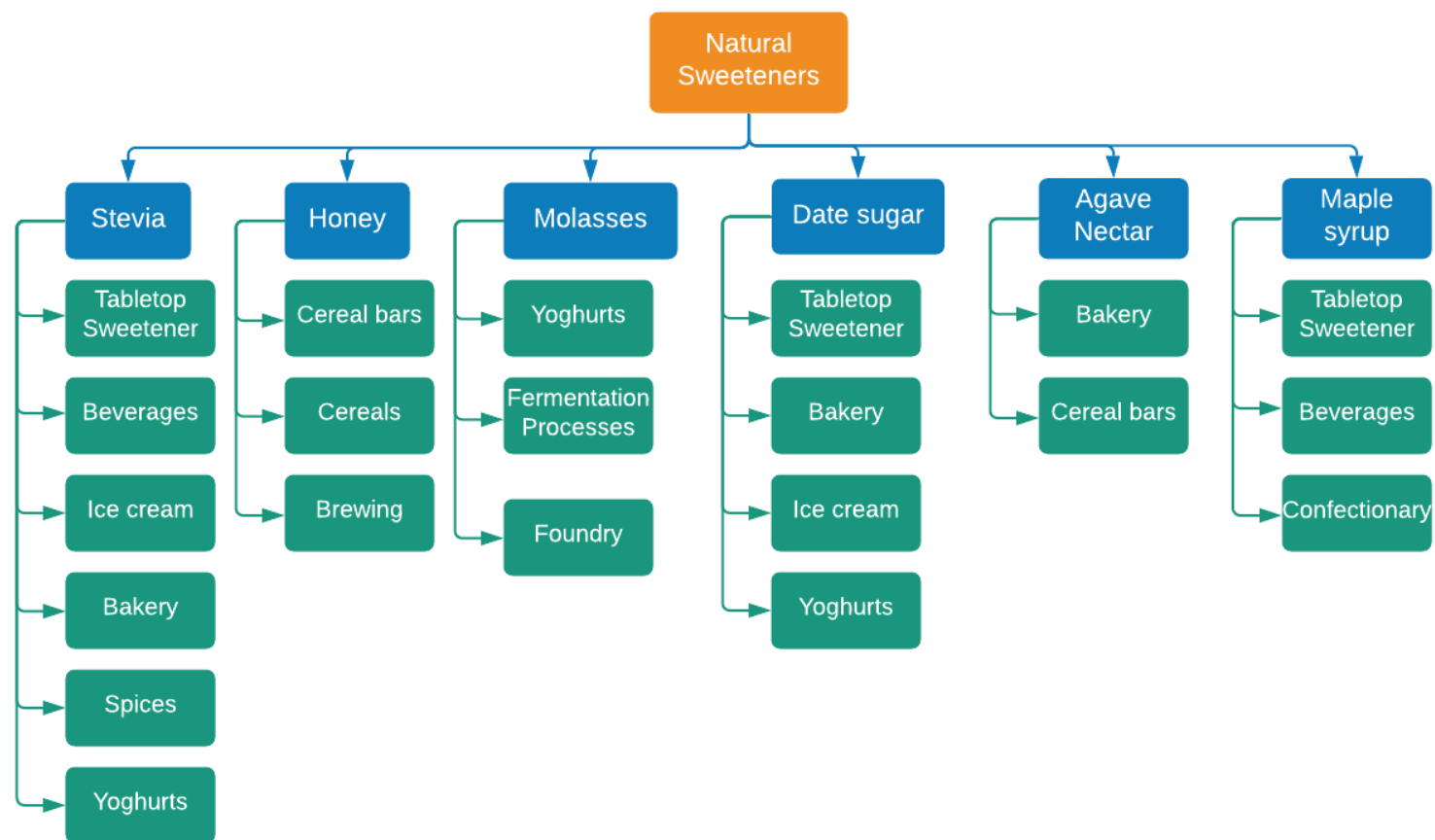
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Figure 9. Current applications of natural sweeteners in commercialized food products.



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Table 1. Alternative techniques used to concentrate the coconut sugar.

Method	Temperature	Time	Energy
Rotary Evaporation (RE) vacuum	54.8°C	12.2 min	0.35 kWh
Microwave Evaporation (ME)	103.2°C	13 min	0.10kWh
Open-heat Evaporation (OHE)	101.6°C	46.8 min	0.83 kWh

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Table 2. SGs extraction yields from *Stevia Rebaudiana* using different methods.

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Adapted from (Castro-Muñoz et al., 2020).

Method	SGs	Extraction yield (%)
Accelerated solvent extraction	Stevioside	7.5-9.9
	Rebaudioside A	2.1-3.2
Assisted by ultrasonication extraction	Rebaudioside A	35
Centrifugal partition chromatography	Stevioside	4.2-24.0
	Rebaudioside A	17.2-22
	Dulcoside	6.8-10
Column-chromatographic technique	Steviolmonoside	4.7
	Stevioside	10.7
	Rebaudioside B	7.3
High Voltage electrical discharges	Stevioside	4.5
	Rebaudioside A	2.0
Pulsed electric fields	Stevioside	27.0
	Rebaudioside A	18.0
	Rebaudioside C	6.5
Microwave-assisted extraction	Stevioside	8.6
	Rebaudioside A	2.3
	Stevioside	2.0
	Rebaudioside A	1.5
	Stevioside	4.5
	Rebaudioside A	2.3
Pulsed electric fields	Stevioside	3.7
	Rebaudioside A	2.1
Pressurized fluid extraction	Stevioside	4.7
Rapid solid liquid dynamic extraction	Stevioside	0.5
	Rebaudioside A	1.4
Supercritical fluid extraction	Stevioside	3.7

	Rebaudioside A	1.8
Ultrasound-assisted extraction	Stevioside	4.2
	Rebaudioside A	2.0
	Stevioside	5.1-9.7
	Rebaudioside A	0.4-3.7
	Stevioside	5
	Rebaudioside A	2.2

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Table 3. Classification of pressure-driven membrane processes based on the

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membrane pore size and their application in sweetener extraction.

Membrane process	Pore size range	Type of targeted components	Pressure requirement (TMP)	Applications for sweetener extraction
		Macromolecular components (Fungi, yeast, bacteria)	0.1-2 bar	Turbidity reduction of remelt syrup (Li et al., 2016).
Microfiltration (MF)	100-10,000 nm			Pre-clarification of sugarcane juice (Panigrahi et al., 2018).
		High MW compounds (Proteins, virus, polysaccharides, colloids)	0.1-7 bar	Partial removal of sugars in honey and enhancement of spray drying process (Samborska et al., 2018)
Ultrafiltration (UF)	2-100 nm			Decoloration of sugarcane molasses (Guo et al., 2018)
				Decolorization and turbidity removal of date syrup (Fathi et al., 2013)
				Separation of <i>Rebaudioside A</i> from aqueous extracts of <i>Stevia rebaudiana</i> (Díaz-Montes et al., 2020).
				Isolation of glycosides from <i>Stevia</i> (Martínez-Alvarado et al., 2017).
		Low MW compounds (Polyphenols, ions, pigments and bioactive compounds)	3- 25 bar	Concentration of Maple sap (Ali et al., 2021)
Nanofiltration (NF)	0.5-2 nm			Preclarification of sugarcane juice (Panigrahi et al., 2018).
				Sucrose purification from Molasses (Sjölin et al., 2019)

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1780 **Table 4.** Bioactive compounds contained in *Sorghum bicolor* at specific anatomic
1781 structure (de Morais Cardoso et al., 2017).

Anatomic structure	Compounds
Pericarp	Non-starch polysaccharides, phenolic compounds, carotenoids
Endosperm	Starch, proteins, B-complex vitamins, minerals
Germ	Lipids, fat-soluble vitamins, B-complex vitamins, minerals

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Table 5. Standard physicochemical composition of sorghum syrup.

Compound	Content (mg/100g)
Total sugar content	68, 570
Glucose	10,880
Fructose	5,560
Organic Acids	3,179.67
Minerals	4,607.87
Aconitic acid	2,312.87
Malic Acid	468.09
Citric acid	378.41
Succinic acid	378.41

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