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How prebiotics have been produced from agro-industrial waste: An overview of the enzymatic technologies applied and the models used to validate their health claims

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Credit authorship contribution statement

Daniela A. Gonçalves is responsible for writing chapter 2, as well as collecting the data for tables 1, 2, 3, and 4.

Abigail González is responsible for writing chapters 1 and 3, as well as collecting the data for tables 5, and 6.

Dalila Roupar is responsible for writing chapter 1, drawing figure 1, as well as collecting the data for tables 1, 2, 3, 4, 5, and 6.

José A. Teixeira is responsible for revising the manuscript, supervision, and funding acquisition.

Clarisse Nobre is responsible for revising and editing the manuscript, supervision, and funding acquisition.

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1 # How prebiotics have been produced from agro-industrial waste: an overview of the enzymatic technologies applied and the models used to validate their health claims

4

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14

15 ## Abstract

16 **Background.** The disposal of waste from the food industry represents a major
17 environmental concern. Nonetheless, agro-industrial by-products can be enzymatically
18 converted into low-cost high-value-added products, such as prebiotics, while contributing
19 to a circular economy. As a first approach for health claims validation of these novel
20 products, several gastrointestinal models have been used.

21 **Scope and Approach.** The main objective of this review is to provide a comprehensive
22 overview of the advances in the enzymatic technologies applied to the production of
23 prebiotics from agro-industrial wastes. The strategies used in the conversion of the
24 wastes, including pre-treatment processes, type of enzymes applied, and the reaction
25 conditions used are revised. Finally, from data obtained by *in vivo* trials and *in vitro*
26 gastrointestinal simulation, the functionality of the produced prebiotics and their
27 biological mechanisms of action are discussed.

28 **Key Findings and Conclusions.** Enzymatic processes have proven their efficiency for
29 the conversion of low-cost agro-industrial wastes into commercial valuable compounds,
30 such as prebiotics. Still, the potential of enzymes for the bioconversion of the vast
31 diversity of existing wastes has yet to be explored. By researching different setups of the
32 enzymatic reaction and optimization of the reaction conditions, greater yields of the
33 prebiotic extraction or synthesis may be achieved. Also, despite the many available
34 gastrointestinal models, few studies have been done on the biological function of the

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35 prebiotics obtained from agro-industrial wastes, which has been a drawback in the
36 validation of health claims associated with these novel products.
37
38 **Keywords:** agro-industrial waste; prebiotics; functional foods; enzymatic treatments; *in*
39 *vitro* digestion; prebiotic functionality.
40
41
42 1. Introduction

43 Food waste has attracted much attention in recent years due to its environmental, social,
44 and economic impact. According to data provided by the UNEP Food Waste Index Report
45 2021 it was estimated in 2019 that 931 million tons of food waste were generated,
46 representing 61 % of households, 26 % of food services, and 13 % of retail (Forbes et al.,
47 2021). In food processing industries, waste results from the separation of desired products
48 from unwanted by-products. The long-term persistence of untreated food waste in
49 landfills eventually generates a substantial amount of methane, which as a greenhouse
50 gas contributes to the global warming. In addition, this waste provides a breeding ground
51 for rot-causing microorganisms and the proliferation of pests, which in turn raises huge
52 environmental concerns.

53 The by-products generated during food processing, which are often considered waste, are
54 still packed with nutrients and bioactive compounds that can be exploited by further
55 processing to produce high-value-added products. The development of these practices at
56 an industrial level is very attractive economically since it uses a low or no-cost raw
57 material, and at the same time, it is an environmentally friendly method of waste
58 management (de la Rosa et al., 2019). A potential application for agro-wastes is the
59 production of prebiotics since food waste is often rich in carbohydrates and fibers
60 susceptible to be transformed by means of enzymatic processes. Prebiotics are
61 compounds that are not hydrolyzed by human digestive enzymes, which upon reaching
62 the colon, are fermented by the gut microorganisms, promoting the growth of beneficial
63 bacteria, which transform them into metabolites with health benefits (Nobre et al., 2022).

64 The growth of the functional food market in recent years has been remarkable,
65 specifically for foods containing prebiotics. The global prebiotics market size was over
66 U$2.9 billion in 2015 and has an expected growth of around 12.7 % by 2025, with a profit

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67 of approximately U$10.55 billion (Mano et al., 2018). Such growth may be a result of the
68 increased health-consciousness by consumers looking for food that not only meets
69 nutritional needs but can also improve overall health (Gonçalves et al., 2022). The health
70 benefits of prebiotics are related to their ability to modulate the microbiota, by increasing
71 the growth of probiotic bacteria that produce beneficial metabolites and decreasing the
72 pathogenic bacteria growth, with a subsequent decrease of intestinal permeability and
73 inflammation. Prebiotics also contribute to better intestinal absorption of minerals, such
74 as calcium and magnesium, reduce the risk of cancer and allergies, and improve the
75 immune system (Al-Sheraji et al., 2013).

76 Prebiotics must reach the gut almost intact for further fermentation by the probiotic
77 microorganisms. They must be resistant to the gastric acidic pH, and not be hydrolyzed
78 by human digestive enzymes or absorbed along the gastrointestinal tract (Davani-Davari
79 et al., 2019). These features should be evaluated by *in vivo* or *in vitro* assays that
80 reproduce or simulate human digestive physiology. Animal assays and clinical trials are
81 nowadays used for *in vivo* studies; however, their use is limited due to economic and
82 ethical issues. The *in vitro* methods used to evaluate whether a compound can be
83 considered prebiotic or not include digestibility and microbial fermentation assays, for
84 which physiologically relevant models are currently being developed and improved
85 (Roupar et al., 2021).

86 This work discusses the advances in the production of prebiotics from agro-industrial
87 wastes using enzymatic technologies, and the methodologies applied to evaluate their
88 biological functionality. A bibliometric analysis was conducted between August and
89 December 2022 using the Scopus database for the collection of the data within this
90 review. The search included the following combination of terms in the title, abstract, or
91 keywords: (agro-industrial AND waste AND prebiotics), (waste AND valorization AND
92 prebiotics OR oligosaccharides), (waste AND enzymatic AND prebiotics OR
93 oligosaccharides), (prebiotic AND digestibility AND waste), (prebiotic, *in vivo* OR *in*
94 *vitro* AND fermentation AND waste). In total, 496 publications were identified. All type
95 of publications was considered in the search except for those that were published before
96 2005 (n = 15). The selection was further narrowed down manually to exclude articles that
97 did not fulfill the inclusion criteria. Among the total, 62 articles were chosen and included
98 in this review.

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99 ## 2. Prebiotics from agro-industrial wastes

100 The agro-industrial sector is known for producing a huge amount of diverse wastes
101 **(Fig. 1)**. Residues derived from agricultural activities include materials such as straw,
102 stem, stalk, leaves, husk, shell, molasse, peel, lint, seed/stones, pulp or stubble from fruits,
103 legumes, or cereals, bagasse generated from sugarcane or sweet sorghum milling, spent
104 coffee grounds, brewer's spent grains, and many others (Sadh et al., 2018). A
105 considerable portion of the agro-industrial wastes is mainly composed of cellulose,
106 hemicellulose, and lignin, known as "lignocellulosic materials". Usually, cellulose is the
107 dominant fraction in the plant cell wall (35–50 %), followed by hemicellulose (20–35 %)
108 and lignin (10–25 %) (Mussatto et al., 2012). As a result, most oligosaccharides generated
109 from this type of residue are xylo- and cello-oligomers. Nonetheless, depending on the
110 treatment applied, mannan- and galacto-oligomers can also be extracted (Bhatia et al.,
111 2019).
112
113 *Please insert here Figure 1.*
114
115 The volume of agro-industrial wastes yearly produced is immense, representing a massive
116 prospect for economic valorization. For instance, corncobs represent a major unexplored
117 resource, as the majority of the annually produced 144 million tons are either discarded
118 or burnt (Córdoba et al., 2013), both representing a major environmental burden and an
119 opportunity for value creation. Global production of molasses accounts for around 50
120 million tons per year (Nikodinovic-Runic et al., 2013). Despite its commercial value, its
121 storage and transport raise difficulties. As a result, only about 15 % of the total molasses
122 produced is internationally traded. Peels are another major agro-industrial waste. Peels
123 represent approximately 20−30 % of 270 million metric tons (Mt) of cassava produced
124 worldwide (Ajala et al., 2020). Orange peel accounts for up to 20 % of total orange
125 production volume, resulting in an annual yield of over 15 Mt of orange peel. The
126 percentage of potatoes discarded in the peeling process is even higher, ranging between
127 10−40 % of the product mass (Sepelev & Galoburda, 2015). Since global production of
128 potatoes exceeded 359 million metric tons in 2020, it can be considered another major
129 source of agro-industrial waste (Shahbandeh, 2022).

130 Agro-industrial wastes can be converted into high-value commercial products using
131 enzymatic bioprocesses, reducing the overall production cost, and the pollution load from

132 the environment. The enzymatic hydrolysis of the residues and by-products into new food
133 ingredients with enhanced nutritional value and functionality is a cost-effective, green,
134 and safe technology (Radenkovs et al., 2018). However, each type of residue requires an
135 appropriate enzyme (or enzyme system), and its catalytic efficiency depends on several
136 parameters, including the operational conditions.

137 In this vein, much has been investigated aiming at developing new strategies for the
138 valorization of agro-industrial residues through the application of enzymatic
139 bioprocessing. A list of prebiotics produced by enzymes using different types of agro-
140 industrial residues is further discussed. Information is also provided on the pre-treatments
141 applied to the waste used and on the enzymatic process conditions applied.

142

143 *2.1. Fructo-oligosaccharides*

144 Fructo-oligosaccharides (FOS) are polymers of fructose residues linked to a terminal
145 glucose molecule. Enzymatically, FOS are synthesized from sucrose through a
146 transfructosylating reaction catalyzed by β-fructofuranosidase (FFase) or
147 fructosyltransferase (FTase) enzymes (de la Rosa et al., 2019; Nobre et al., 2018). The
148 use of sucrose-rich industrial by-products, such as molasses, can represent a cheap and
149 profitable alternative to produce this prebiotic. These by-products often do not require a
150 *prior* treatment as the needed substrate is readily available. Immersion or diffusion in hot
151 water is in most cases enough to prepare the waste for the enzymatic catalysis (Ganaie et
152 al., 2017; Smaali et al., 2012). Nonetheless, if necessary, the agro-wastes can be treated
153 for removal of proteins, using proper hydrophilic membranes (Corzo-Martínez et al.,
154 2016), or heavy metals, with ethylenediaminetetraacetic acid (EDTA) solutions (S. Zhang
155 et al., 2019).

156 Several approaches have already yielded promising results (see **Table 1**). *Aspergillus*
157 *flavus* NFCCI 2364 fructosyltransferase was exploited to synthesize FOS from sixteen
158 different agro-wastes, including wheat bran, corn straw, sugar cane bagasse, cassava
159 peels, apple pomace, banana peels, beetroot peels, orange peels, guava peels, guava seed
160 powder, pineapple peels, papaya peels, mango peels, passion fruit peels, jabuticaba peels
161 and cashew peels (Ganaie et al., 2017). A solid-state fermentation approach was
162 employed. Among the different agro-wastes, sugar cane bagasse was the most promising
163 substrate, yielding 35.95 % (w/w) under unoptimized conditions. Higher amounts of this

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164 prebiotic were obtained after optimization of the process conditions. A total of 73.42 %
165 (w/w) of FOS were produced, where 1-kestose accounted for 46.28 %, followed by
166 nystose (21.19 %) and 1^F-fructofuranosylnystose (5.95 %).

167

168 *Please insert here Table 1.*

169

170 Aguamiel of maguey was used as a culture medium to produce FOS with FTase from
171 *Aspergillus oryzae* DIA-MF, under solid-state fermentation (Muñiz-Márquez et al.,
172 2016). A yield of 0.30 (w/w) of FOS was achieved. From the 35–40 g/L of sucrose present
173 in the aguamiel, 43.8 % was converted into FOS, with a productivity of 0.097 g/(L·min).

174 FOS production from coffee silverskin reached higher values than that achieved when
175 using synthetic fiber as solid support in solid-state fermentation. Mussatto & Teixeira
176 (2010) found that this waste could provide enough nutrients for microbial development,
177 reproducing the results achieved with a nutrient-supplemented media. In the process, high
178 levels of FFase activity were attained, yielding 0.70 ± 0.04 (w/w) FOS with a productivity
179 of 8.05 ± 0.49 g/(L·h) (Mussatto & Teixeira, 2010). From an industrial point of view, it
180 is important to maximize both the formation of FOS and the enzyme transfructosylation
181 activity to achieve improved processes at reduced operational costs. After process
182 optimization, the same authors increased FOS production from 128.7 g/L to 206 g/L,
183 emphasizing the importance of process optimization (Mussatto et al., 2013). According
184 to the reported data, coffee silverskin can be considered a good candidate for the synthesis
185 of FOS at an industrial level.

186 FOS have been also produced from date by-products from *Deglet Nour* flesh (Smaali et
187 al., 2012). During the production and commercialization of date products, a considerable
188 amount of fruit waste is generated and often discarded. Dates hold a high content of
189 sucrose in their composition, 50 g of sucrose was quantified per 100 g of flesh. Aqueous
190 extracts containing sucrose from date by-products were prepared by diffusion in hot
191 water. An aqueous extract containing 231.94 g/L sucrose was used as a substrate source
192 for enzymatic FOS synthesis. Crude extract from *A. awamori* was used without enzyme
193 purification. The FFase in the crude extract was immobilized into chitosan. The
194 immobilized FFase converted 84.14 % of sucrose into 123 g/L of FOS, with a yield of
195 53.26 % and a productivity of 18.5 g/(h·100 g). Being a cheap waste material and having

196 shown the possibility of reusing the enzyme up to 11 cycles, this type of process could be
197 easily used at an industrial scale for the low-cost production of FOS.

198 Tofu whey permeate, an industrial by-product from the soybean industry, contains
199 substantial levels of sucrose (163 g/L), and, for that reason, it was tested for the enzymatic
200 synthesis of FOS. A commercial enzyme from *Aspergillus* sp., Pectinex® Ultra SP-L, was
201 capable of transfructosylate the carbohydrates present in the tofu whey permeate.
202 Maximum production of 164.2 g/L FOS and fructosylated α-galactosides was achieved
203 after 8 h of reaction, yielding 57 % (w/w) of FOS. 1-Kestose, nystose, and
204 1^F-fructofuranosylnystose accounted for 37.0 ± 1.0, 45.6 ± 1.1, and 5.4 ± 0.1 g/L,
205 respectively, of the total produced oligosaccharides (Corzo-Martínez et al., 2016).

206 Cane molasse waste is a high source of sucrose for FOS synthesis. The whole cells of
207 *Aureobasidium melanogenum,* with glucose repression in disruptant D28 relieved, were
208 applied in cane molasses waste to produce FOS in submerged fermentation. The FFase
209 showed improved β-fructofuranosidase activity, and after only 4 h reaction, 0.58 g FOS
210 per g of molasses were produced. The final FOS mixture consisted of 38.7 % 1-kestose,
211 49.3 % nystose, and 12.0 % 1^F-fructofuranosylnystose (S. Zhang et al., 2019).

212

213 ### *2.2. Galacto-oligosaccharides, Lactosucrose, and Lactulose*

214 Galacto-oligosaccharides (GalOS), galactose oligomers, are mainly synthesized by a
215 transgalactosylation reaction catalyzed by β-galactosidase enzymes, as well as the di- and
216 trisaccharide lactulose and lactosucrose (Gonçalves et al., 2022). Lactosucrose can also
217 be synthesized through a transfructosylation reaction catalyzed by levansucrases (C. Wu
218 et al., 2015), and lactulose by lactose isomerization using cellobiose 2-epimerase (CEs)
219 enzymes (Kuschel et al., 2016). Lactose is one of the substrates needed for the production
220 of these prebiotics, acting as an acceptor and/or donor of galactosyl moieties. For GalOS
221 synthesis, only lactose is required. Nevertheless, the synthesis of lactosucrose and
222 lactulose only occurs when sucrose or fructose acts as galactosyl moiety acceptors,
223 respectively (Gonçalves et al., 2022).

224 Whey, a by-product of the dairy industry, contains approximately 4.5−6.0 % lactose
225 (Kaur et al., 2020). Due to the abundant concentration of lactose in this by-product, its
226 valorization has been evaluated over the past years (see **Table 2**). Besides lactose, whey
227 contains a substantial amount of proteins. To prevent their precipitation during the

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228 prebiotic synthesis, the whey proteins may be previously digested by proteases (T. S.
229 Song et al., 2013; Bolognesi et al., 2021).

230

231 *Please insert here Table 2.*

232

233 Up to 80 % of whey-lactose was converted into GalOS using a crude recombinant β-
234 galactosidase from *Streptococcus thermophilus* DSM 20259. The reaction yielded 34.2 %
235 (w/w) galactose-oligomers with β-(1,3) and β-(1,4) linkages. When using concentrated
236 whey, GalOS yield increased up to 50 % (w/w), with ~90 % lactose conversion within
237 5 h (Geiger et al., 2016). According to the author's findings, it is possible to synthesize
238 approximately 1 kg of GalOS from 3 kg of whey permeate powder. Mano et al. (2019)
239 reported that shorter reaction times should be considered to obtain a better production
240 yield and productivity of GalOS. Proof of that was the lower GalOS production yield
241 achieved using Lactozyme™ 2600 L (25 % (w/w)). The commercial enzyme preparation
242 was found to be the most suitable for GalOS synthesis but ended up hydrolyzing the
243 produced oligomers at longer reaction times (Mano et al., 2019). The presence of
244 monomers in whey concentrates can also be a problem when trying to develop strategies
245 for GalOS production. A maximum yield of 14.8 ± 0.9 % (w/w) was reached after a 5 h
246 reaction using a concentrated acid whey containing 9.3 % of initial lactose. Similar values
247 (15.26 ± 0.05 % (w/w)) were attained at 2 h reaction using pure lactose solution. The
248 delayed GalOS synthesis may be related to enzyme activity inhibition (Zerva et al., 2021).
249 Apart from the poor results, the use of acid whey can be further explored for the synthesis
250 of GalOS with a degree of polymerization (DP) ≤ 4.

251 *Porungo* cheese whey contains approximately 4.3 % of lactose (Marim et al., 2021).
252 Contrary to the results obtained by Zerva et al. (2021), GalOS production using this
253 industrial by-product resulted in higher synthesis than that observed when used pure
254 lactose solution. A yield of 63.1 % (w/w) and a productivity of 13.6 g/(h·L) were
255 achieved, compared to the control lactose solution of 41.1 % (w/w) and 8.2 g/(h·L),
256 respectively. It seems that immobilization techniques may improve the galacto-oligomers
257 synthesis and somehow avoid enzyme inhibition (Bolognesi et al., 2021). This confirms
258 that when aiming for waste valorization all aspects should be considered for the
259 development of a suitable and economical process. It has been shown that immobilized

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260 β-galactosidase enzymes (onto glutaraldehyde-activated chitosan beads) can be used up
261 to 10 consecutive cycles, losing only 26 % of the relative activity. When applying this
262 methodology, a maximal GalOS concentration of 159.4 and 168.8 g/L was achieved using
263 milk whey and milk whey permeate, respectively, with an initial lactose concentration of
264 40 % (w/v) (Hackenhaar et al., 2021).

265 More studies have been focused on the conversion of lactose-whey into GalOS.
266 *Cryptococcus laurentii* whole-cells producing β-galactosidase produced GalOS from
267 Greek yogurt whey and concentrated whey in a yield of 34.6 ± 0.4 % and 36.1 ± 0.6
268 % (w/w), respectively. In both cases, ~50 % of the initial lactose was converted, and a
269 specific productivity of 2.2 and 2.3 mg/(U·h) was attained (Fischer & Kleinschmidt,
270 2021). An advantage of the proposed strategy is that the enzyme is able to use the
271 galactose present in the whey (up to 42.52 ± 0.52 g/L) as an acceptor substrate. Even so,
272 GalOS synthesis can be limited by the lower lactose concentrations found in the whey-
273 based medium. Because of that, only 19.41 % (w/v) of GalOS were synthesized by crude
274 β-galactosidase enzyme from *Lactobacillus paracasei* YSM0308 using a sweet whey
275 medium (T. S. Song et al., 2013). However, 32 % (w/v) of GalOS could be obtained if
276 the initial lactose concentration was increased from 300 to 500 g/L. Lastly, 38 % (w/w)
277 of GalOS were synthesized after 12 h in a reaction catalyzed by a recombinant β-
278 galactosidase (Yañez-Ñeco et al., 2021). Unexpectedly, the same production yield and
279 lactose conversion (60 %) were obtained using either whey-lactose or pure lactose
280 solutions. The main difference found was that cations present in whey favored the
281 transgalactosylation reaction, encouraging the reuse of these wastes.

282 The use of stachyose and raffinose as fructose donors for the synthesis of lactosucrose
283 has been proposed. In addition to sucrose (21 %), tofu whey has been evaluated as a
284 source of fructose. Tofu whey contains substantial amounts of stachyose (16.3 %) and
285 smaller amounts of raffinose (3.7 %). A levansucrase from *Bacillus subtilis* CECT 39 was
286 capable of producing 74 g/L of lactosucrose within a 120 min reaction time using pure
287 lactose and tofu whey as substrates. A corresponding yield of 64.9 % (w/w) was achieved
288 with a productivity of 37.0 g/(L·h). The combination of tofu whey and cheese whey
289 permeate as an alternative to pure lactose has been also evaluated, since cheese whey is
290 composed of 89.9 % lactose. Applying this strategy, a maximum yield and productivity
291 of 60.8 % and 35.8 g/(L·h) were obtained with a production of 71.5 g/L lactosucrose
292 (Corzo-Martinez et al., 2015). Both alternatives yielded good lactosucrose synthesis.

293 However, as pure lactose is expensive, the last represents the most attractive solution for
294 waste valorization as it combines the use of two industrial by-products.

295 Cheese whey powder has been tested as a possible substrate for the synthesis of lactulose
296 by enzymatic isomerization processes due to its richness in lactose. The cellobiose 2-
297 epimerase (CsCE) enzyme from *Caldicellulosiruptor saccharolyticus* has been reported
298 as the most efficient enzyme to catalyze the isomerization of lactose into lactulose.
299 Evidence of that is the results observed by L. Wu et al. (2017) in which a maximum
300 lactulose yield of 58.5 % (w/w) was attained, after a 2 h reaction. Moreover, the proposed
301 EMR system was able to promote a steady lactose conversion up to 10 batches, resulting
302 in a final production yield of 42.4 % and 84.5 g/L lactulose (L. Wu et al., 2017).

303 The synthesis of lactulose from lactose by β-galactosidases requires fructose as a
304 galactosyl acceptor. To overcome the need for this co-substrate, a dual-enzyme system of
305 β-galactosidase and glucose isomerase enzymes was used to convert pure lactose
306 solutions into lactulose in the absence of fructose. The use of lactose-whey instead of pure
307 lactose has been shown as a more economical alternative. Reaction conducted with the
308 dual-enzymes using milk-whey attained a lactulose concentration of 7.68 g/L and a
309 productivity of 0.32 mg/(U·h). The immobilized enzymes were reusable up to 7 times,
310 maintaining 57.1 % of their catalytic activity and synthesizing 4.31 g/L of lactulose (Y.
311 S. Song et al., 2013).

312

313 ### *2.3. Xylo-oligosaccharides*

314 Xylan, the major constituent of hemicellulose, is one of the most abundant
315 polysaccharides found in plant cell walls (Kallel et al., 2015). The hydrolysis of xylan by
316 autohydrolysis, chemical (acid or alkaline), and/or enzymatic methods results in the
317 production of xylo-oligomers, also referred to as xylo-oligosaccharides (XOS). These
318 prebiotics are mainly composed of xylose residues, but some side groups can be found.
319 Enzymatic methods are desired over the other methods since they do not produce toxic
320 substances nor require specific equipment (Jnawali et al., 2018). Also, XOS can be
321 extracted from hemicellulose-rich biomass, which is part of the majority of the agro-
322 industrial wastes and agricultural by-products (see **Table 3**). For that purpose, *endo*-
323 xylanase enzymes are usually used after xylan recovery from the raw materials (Jayapal
324 et al., 2013).

325

326 *Please insert here Table 3.*

327

328 A variety of pre-treatment technologies are available to aid the recovery of xylan from
329 agro-industrial by-products. If possible, autohydrolysis should be preferably applied to
330 break hemicellulose backbone owing to its high efficiency and relatively low cost (Singh
331 et al., 2019). Steam explosion also results in high sugar recoveries and low generation of
332 noxious compounds. For instance, hemicellulose from wheat straw and corncobs has been
333 extracted using this pre-treatment method (Álvarez et al., 2017; Liu et al., 2018). Yet,
334 chemical processes, including alkali and acid-based processes, have been the most
335 exploited. Normally, extraction of xylan is accomplished using a sodium hydroxide
336 (NaOH) alkaline solution (Kallel et al., 2015; Aachary & Prapulla, 2009). To improve the
337 recovery of xylan, alkali hydrolysis coupled with steam treatment has been performed,
338 for instance in coconut husk, rice husk and sugarcane bagasse residues (Jnawali et al.,
339 2018; Khat-udomkiri et al., 2018; Jayapal et al., 2013). To help delignify and increase the
340 efficiency of the enzymatic hydrolysis process of the extracted xylans, the holocellulose
341 fraction has been removed with sodium chlorite ($NaClO_2$) under acidic conditions (Ávila
342 et al., 2020). The xylan rich fractions are further enzymatically hydrolyzed to increase the
343 content of XOS.

344 Liquors obtained after biomass pre-treatments already contain lower amounts of XOS,
345 due to partial hydrolysis of the xylan polymer. Enzymatic treatments with *endo*-xylanase
346 enzymes can be used to further increase XOS concentration in the hydrolysate mixture.
347 A XOS concentration of 3.3 ± 0.1 (w/w), previously produced by autohydrolysis, was
348 augmented to a final value of 8.2 ± 0.1 % (w/w) after enzyme treatment. Xylobiose
349 accounted for 5.3 ± 0.1 % (w/w) of the produced low-DP XOS, whilst xylotriose
350 represented 3.0 ± 0.1 % (w/w) (R. D. Singh et al., 2019). Similarly, hydrolysis of a
351 banana's pseudostem pulp pre-hydrolysate by an *endo*-xylanase increased XOS content
352 from 19.7 % to 76.7 %. This coupled process resulted in the production of 6.6 g of XOS
353 per 100 g of biomass with a high proportion of xylobiose and xylotriose (Q. Wang et al.,
354 2022). The combination of alkaline pre-treatment and enzymatic hydrolysis has been also
355 evaluated to maximize the yield of XOS from rice husk. Up to 54.49 ± 0.61 % of xylan
356 were recovered, and after enzymatic treatment, a maximum XOS production of

357 17.35 ± 0.31 mg/mL was attained (Khat-udomkiri et al., 2018). Feruloylated xylo-
358 oligosaccharides (FXOS) were recovered after the digestion of the precipitated material
359 obtained from alkali-pretreated pearl millet bran byproduct. The xylanase from
360 *Trichoderma viride* could recover 1.4 % g_{FXOS} per gram of starting material, and 7.2 %
361 of other XOS (A. Singh & Eligar, 2021).

362 A combined extraction with sodium hydroxide and steam lead to high xylan extraction
363 (85 %) from sugarcane bagasse. The consequent enzymatic treatment of the xylan
364 hydrolysate, by an *endo*-xylanase from *Trichoderma viridae*, generated low-DP XOS.
365 The maximum concentration of xylobiose and xylotriose was found to be
366 1.15 ± 0.13 mg/mL, and 0.57 ± 0.21 mg/mL, respectively (Jayapal et al., 2013). A similar
367 process was used to recover xylan from coconut husk. After that, the hydrolysis was
368 catalyzed by crude xylanase yielding 1.69 mg/mL of xylobiose (Jnawali et al., 2018).
369 Furthermore, steam explosion using acidic electrolyzed water was efficiently used to
370 extract 74.8 % of xylan from corncobs. The following enzymatic hydrolysis of the xylan-
371 rich solution yielded mainly XOS with a DP 2–5, with xylobiose and xylotriose
372 accounting up to 90 %.

373 XOS (81.0 ± 3.9 %) have been also enzymatically extracted from alkali pre-treated
374 corncobs. A final oligosaccharide concentration of 10.31 ± 0.42 mg/mL was attained in
375 the final hydrolysate. According to the reported results, XOS with a DP 2−7 were
376 detected, however, xylobiose accounted for 73.5 % of the produced oligosaccharides
377 (Aachary & Prapulla, 2009). Similarly, a xylan-rich mixture obtained from garlic straw
378 was subject to hydrolysis by xylanase secreted by *Bacillus mojavensis* UEB-FK. The
379 process resulted in the production of XOS with DP 2−6. A maximum yield of 29 ± 1.74
380 % (w/w) was attained after 8 h hydrolysis, which was kept almost unchanged up to 24 h
381 reaction (Kallel et al., 2015). XOS with a DP 2−4 were the mainly produced, while longer
382 oligomers accounted for smaller amounts. A similar XOS profile was observed after
383 enzymatic hydrolysis of sugarcane straw and coffee husk. A cocktail of enzymes was able
384 to produce 10.23 and 8.45 g/L of XOS from the indicated sources (Ávila et al., 2020).
385 Furthermore, xylotetraose and xylopentaose were the main identified products from the
386 hydrolysis of coffee peel xylan. According to the authors, the treated sample contained
387 3.26 mg/mL of xylopentaose (Ratnadewi et al., 2020).

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388 The bioconversion of xylan from agricultural residues into XOS without prior pre-
389 treatment has shown to be a potentially cost-effective alternative for industrial
390 application. Crude xylanase from *Aspergillus fumigatus* R1 was able to yield 1.08 %
391 (w/w) of XOS from untreated wheat husk xylan. XOS with a DP up to 5 were detected in
392 the final hydrolysate, being xylobiose the most predominant oligosaccharide throughout
393 the entire reaction time (Jagtap et al., 2017). This type of enzymatic procedure also avoids
394 the generation of undesirable compounds commonly linked to conventional chemical
395 extraction alternatives (Jnawali et al., 2018). Brazilian Syrah grape pomace was found to
396 be a good source of xylan (91.6 % (w/w)), and the extraction of XOS either by enzymatic
397 or chemical pathways was analyzed. The authors found out that the enzymatic extraction
398 of XOS was as efficient as chemical extraction. The alkaline process allowed the recovery
399 of up to 96.3 % of XOS. On the other hand, using xylanase produced by *Aspergillus niger*
400 3T5B8, it was attained a final oligosaccharide concentration of 88.68 ± 0.13 %, whilst
401 84.09 ± 2.40 % was obtained using the Viscozyme® L cocktail. XOS with a DP ≤ 4 were
402 detected, and a minimum amount of xylose monomers were generated (Costa et al., 2019).

403 The use of enzyme systems is another suitable alternative to increase XOS yield and
404 reduce the catalysis time whilst using lignocellulosic wastes as a xylan source. While
405 using a β-glucosidase and an *endo*-xylanase enzyme, approximately 8.9 % (w/w) of XOS
406 (DP ≤ 6) were obtained from wheat straw xylan after 5 h, accounting for 90.6 % of the
407 products in the final mixture. A similar amount of XOS (87.4 %) was obtained using
408 *endo*-xylanase as a solo catalyst, however, the time required was much longer (8 h)
409 (Álvarez et al., 2017). In both cases, xylobiose, and xylotriose were the main
410 oligosaccharides produced.

411

412 ### *2.4. Other prebiotics*

413 Apart from the previously mentioned, many other prebiotic compounds can be extracted
414 or produced from several waste sources (see **Table 4**). In further sections, the strategies
415 used in the conversion of the wastes to manno-oligossacharides, isomalto-
416 oligosaccharides, and pectin oligosaccharides will be discussed.

417

418 *Please insert here Table 4.*

419 ### *2.4.1. Manno-oligosaccharides*

420 Like other prebiotics, manno-oligosaccharides (MOS) can be produced by enzymatic
421 hydrolysis. MOS are generated by the degradation of mannans using *endo*-β-(1,4)-
422 mannanase enzymes (Jana & Kango, 2020). Mannan is abundantly found in softwoods
423 and plant structures like seeds (Bangoria et al., 2021). Hence, wastes from those sources
424 can be used as substrates for the production of MOS (see **Table 4**). Chemical pre-
425 treatments are the most used techniques in the extraction of mannans from industrial
426 wastes. However, alkali treatment with NaOH appears to be more favorable than acidic
427 methods, as they are less corrosive and polluting (Zhang et al., 2021; Wongsiridetchai et
428 al., 2018).

429 Mannans from different mannan-rich agro-wastes were treated with β-mannanase
430 (ManAo) purified from *Aspergillus oryzae* MTCC 1846. From guar gum, a higher amount
431 of MOS (11.24 mg/mL) was released, followed by copra meal (7.14 mg/mL). In this
432 process, mannobiose and mannotriose were the major oligosaccharides produced (Jana &
433 Kango, 2020). Five agricultural wastes, including potato peel, soybean meal, coffee
434 residue, sugarcane bagasse, and copra meal, were also evaluated for their potential as
435 substrates for the production of MOS. The crude *Penicillium oxalicum* KUB-SN2-1
436 mannanase was able to produce MOS with a DP $\leq$ 6. Even so, the main generated products
437 were mannotetraose, mannotriose, and mannobiose (Chantorn et al., 2018). Mannan from
438 locust bean gum and palm kernel cake were converted to MOS with up to 6 mannose
439 units. Two different mannanases, Man5HJ14 and ManAJB13, were employed. Each
440 yields a different type and concentration of MOS (R. Zhang et al., 2021). These results
441 proved that the choice of catalyst represents a crucial step when aiming at the production
442 of prebiotics.

443 Similarly, mannobiose and mannotriose were obtained by enzymatic hydrolysis of spent
444 coffee grounds using a crude β-mannanase from *Bacillus* sp. GA2(1) (Wongsiridetchai et
445 al., 2018). The same substrate has been used to produce 58.22 ± 2.04 mg_{MOS}/100 mg by
446 a mannanase from *Aureobasidium pullulans* NRRL 58524 (Ibrahim et al., 2022).
447 Mannobiose accounted for 16.27 ± 0.84 mg and mannotriose for 2.85 ± 0.20 mg of the
448 total MOS content (DP > 2). *Aspergillus niger* ATCC 10864 *endo*-mannanase was able
449 to produce MOS with a DP $\leq$ 6 using guar gum and locust bean gum as raw materials.
450 From the mammans found in both substrates, 5.11 and 4.45 mg/mL of MOS were
451 attained, respectively (Magengelele et al., 2021). Locust bean gum hydrolysis led to the

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452 release of galactosyl substituted MOS (with DP > 6). In both cases, mannohexaose was
453 generated in higher concentrations. These results were similar to those obtained by
454 Pangestu et al. (2019). Manno-oligomers with the same polymerization were obtained
455 from sugar palm fruit hydrolysis by *Kitasatospora* sp. KY57667 β-mannanase. High
456 production yields were expected as treated sugar palm fruit contained about 42 %
457 galactomannan per dried weight biomass. Once more, mannohexaose was the main
458 product obtained after enzymatic hydrolysis (Pangestu et al., 2019).

459 It has been found that crude β-mannanase from *Penicillium aculeatum* APS1 can
460 hydrolyze galactomannan from locust bean gum and guar gum, and glucomannan from
461 konjac gum. The enzyme generates low molecular weight (Mw) MOS (DP ≤ 4). Even so,
462 mannotetraose was not detected in guar gum hydrolysate. From the substrates evaluated,
463 konjac gum yielded the highest MOS concentration (4.626 mg/mL), followed by guar
464 gum (2.958 mg/mL) and locust bean gum (1.364 mg/mL) (Bangoria et al., 2021). A
465 mannanase enzyme from *Streptomyces cyaenus* hydrolyzed palm cake kernel yielding
466 distinct oligo-mannans (DP ≤ 7). Mannobiose and mannotriose were detected throughout
467 all reaction time (up to 8 h), decreasing over time. A similar pattern was observed for
468 mannotetraose and mannopentaose. Mannohexaose production increased over time,
469 reaching its maximum at 8 h. Mannoheptulose could only be detected between 2 and 4 h
470 reaction (Purnawan et al., 2017). Hence, depending on the desired application, the process
471 can be further optimized for a suitable production of specific MOS.

472

473 ***2.4.2. Isomalto-oligosaccharides***

474 Isomalto-oligosaccharides (IMOs) are non-digestible oligomers constituted of glucose
475 residues. IMOs are synthesized using enzymes with transglucosidase activity such as
476 transglucosidases or α-glucosidases (J. A. Gómez et al., 2021). Even so, the IMOs
477 production involves the hydrolysis of starch using α-amylase, pullulanase, and β-amylase
478 in a first stage (Chockchaisawasdee & Poosaran, 2013).

479 A wide range of starches can be used for IMOs synthesis, including those from agro-
480 residues (see **Table 4**). Most of the wastes used in the production of IMOs do not require
481 a complex pre-treatment process. The residues mainly need to be dried and sieved to a
482 desirable particle size in order to obtain a starch-rich powder (Chockchaisawasdee &
483 Poosaran, 2013). Yet, some waste residues may require extra treatment steps. Soybean

484 molasses for example have been treated with sulfuric acid (H_2SO_4) and calcium hydroxide
485 ($Ca(OH)_2$) for the removal of viscous substances and heavy metal ions (Zhi-Peng et al.,
486 2019). To prevent browning of pulp mixtures of rejected plantain fruits, an aqueous
487 solution of ascorbic acid has been added (J. A. Gómez et al., 2021). In these cases, the
488 obtained hydrolysates have been used as substrate for the synthesis of IMOs.

489 A maximum isomaltulose concentration and yield of 96.7 g/L and 0.96 (w/w) were
490 achieved using pre-treated cane molasses. The high sucrose concentration present in cane
491 molasses makes it a perfect substrate for isomaltulose synthesis. Corn steep liquor was
492 employed as a yeast extract alternative to improve isomaltulose production and purity
493 while lowering operational costs. As a result, most sucrose in pre-treated cane molasses
494 was converted, improving the prebiotic production by 5.5 %. The organic nitrogen found
495 in corn steep liquor boosted biomass formation, enhancing sucrose isomerase production
496 and, consequently, isomaltulose synthesis. After performing a fed-batch fermentation, a
497 maximum concentration of 161.2 g/L was achieved, with a purity of 97.4 % (Z. P. Wang
498 et al., 2019).

499 Due to overproduction, many fruits at all stages of maturity go to waste. Unripe fruits
500 have a high starch content (700–800 g/kg), and therefore are suitable sources for IMOs
501 production (Chockchaisawasdee & Poosaran, 2013). For instance, the potential use of the
502 flour of rejected unripe plantain fruits for IMOs production has been evaluated.
503 Approximately 0.16 moles of maltose were obtained from 100 g of plantain fruits. The
504 process design showed that 24.48 g of IMOs (DP $\leq$ 5) could theoretically be obtained.
505 The proposed process may be a good alternative for the valorization of agro-industrial
506 plantain residues for IMOs synthesis (J. A. Gómez et al., 2021). Also, after liquefaction
507 and saccharification stages, high levels of maltose were released (45 % (w/w)) from
508 banana slurries, which were further used for IMOs synthesis by transglucosylation. After
509 12 h, a maximum amount of 76.67 $\pm$ 2.71 g/L IMOs was achieved, being isomaltotriose
510 and isomaltotetraose the main produced oligosaccharides. Hence, banana raw waste can
511 be used as a substrate for IMOs synthesis (Chockchaisawasdee & Poosaran, 2013).

512 The production of IMOs by simultaneous saccharification and transglucosylation
513 approach yielded 92.17 $\pm$ 3.43 g/L and 85.11 $\pm$ 4.30 g/L IMOs (DP $\geq$ 6) using broken rice
514 and potato processing waste as substrate sources. Both wastes contain high concentrations
515 of starch (up to 70 %) which makes them a suitable source for IMOs synthesis. This
516 approach allows a shorter reaction time, increasing overall productivity. Also, the

517 operational cost can be significantly reduced (Basu et al., 2016). Furthermore, the sugar
518 content of soy molasses accounts for more than 30 % (w/w), including stachyose and
519 raffinose. An α-galactosidase was used for their hydrolysis to sucrose aiming for the
520 maximization of IMOs synthesis. In the soy molasse hydrolysate, the final sucrose
521 concentration was raised 1.64-fold. After 72 h of fermentation, a maximum isomaltulose
522 concentration of 209.4 g/L was achieved, with a yield of 0.95 (w/w). By using whole-
523 cells of an engineered *Yarrowia lipolytica* strain, the developed bioprocess presented an
524 interesting alternative for low-cost and efficient isomaltulose synthesis from soybean
525 molasses (Zhi-Peng et al., 2019).

526

527 *2.4.3. Pectic oligosaccharides*

528 Pectic oligosaccharides (POS) are oligosaccharides that can be obtained by the
529 depolymerization of pectin-rich feedstocks (see **Table 4**). These include oligo-
530 galacturonides (OGalA), arabinogalacto-oligosaccharides (AraGalOS), arabinoxylo-
531 oligosaccharides (AraXOS), arabino-oligosaccharides (AraOS), rhamnogalacturon-
532 oligosaccharides (RhaGalAOS), and GalOS (Babbar, Dejonghe, et al., 2016). The use of
533 agricultural residues may be an interesting path in the production of these types of
534 prebiotics, as some agricultural by-products contain significant amounts of pectin
535 (Wilkowska et al., 2019).

536 The simplest method employed for the extraction of pectin from wastes is the use of water
537 at room temperature (Sabajanes et al., 2012). Other commonly strategy used is the acid
538 or base assisted extraction. For instance, water acidified with hydrochloric acid (HCl) was
539 used to extract pectin from sugar beet wastes (Combo et al., 2013). The extraction with
540 chelating agents has also been evaluated. The release of pectin from onion skins was
541 successfully achieved using a sodium hexametaphosphate ($(NaPO_3)_6$) solution (Babbar,
542 Baldassarre, et al., 2016; Baldassarre et al., 2018). The obtained crude extracts could be
543 further converted into POS by the breakdown of pectin chains using either hydrolase
544 (hydrolysis) or lyase (β-elimination) enzymes (B. Gullón et al., 2013).

545 A crude pectin extract, obtained from onion skins, was used as a substrate to produce POS
546 in an enzyme membrane reactor. By circulating the enzyme (Viscozyme® L) and substrate
547 continuously, POS were removed from the reaction mixture by permeation and the
548 retentate was recycled back to the reactor for further hydrolysis. Under optimum

549 conditions, 4.5 g/g of POS/monosaccharide ratio was produced, with a POS productivity
550 and yield of 22.0 g/(L·h) and 57 % (w/w), respectively (Baldassarre et al., 2018). In
551 another study, an *endo*-polygalacturonase was able to catalyze the conversion of pectin
552 into POS with a DP 2−10. Depending on the enzyme concentration, different POS
553 fractions were obtained. For instance, the highest amount of oligomers with a DP equal
554 to 4 was achieved using 5.2 IU/mL of the enzyme. If aiming for longer oligomers, a lower
555 enzyme load must be chosen. Hence, enzymatic production of POS with a targeted DP
556 can be achieved using onion skins as raw material (Babbar, Baldassarre, et al., 2016).

557 Oligomers with DP 2−9, described as OGalA, were produced using dried sugar beet
558 pectin. The concentration of shorter-length POS was higher than longer-chain ones. After
559 15 min reaction using the *endo*-polygalacturonase-M2 enzyme, OGalA with DP2 and
560 DP3 accounted for 3.1 and 3.7 % (w/w) of the total reaction products, respectively
561 (Combo et al., 2013). After enzymatic treatment, 31.3 kg of POS were recovered from
562 100 kg of liquor containing water-soluble compounds extracted from orange peel wastes.
563 From those, 7.5 kg were gluco-oligosaccharides (GlcOS), 4.5 kg GalOS, 6.3 kg AraOS,
564 and 13 kg OGalA. For the enzymatic hydrolysis of the polysaccharides into
565 oligosaccharides, two enzymes were applied, namely Viscozyme® L and Celluclast® 1.5L
566 (Sabajanes et al., 2012). Both residues, sugarbeet and orange peel, proved to be suitable
567 candidates for prebiotic extraction giving added-value to the wastes. Also, different types
568 of oligosaccharides can be extracted from the different feedstock types.

569

570 **2.5. Overview of the advances in the enzymatic production of prebiotics from**
571 **agro-industrial wastes**

572 One of the main advantages of using agricultural by-products is their variety and
573 abundance. As a cheap source, agro-industrial wastes can be industrially exploited as
574 alternative sources to produce prebiotics at reduced production costs. For these reasons,
575 various by-products derived from agro-industrial processes have been tested either for
576 extraction or synthesis of prebiotics, using enzymatic approaches. The residue's
577 composition defines the prebiotic which may be synthesised. The process must be
578 optimized to overcome possible technical challenges, including the correct choice of the
579 catalyst. The enzyme to be used depends on the substrate available, as well as the prebiotic
580 intended to be produced. Some examples of the binomial "prebiotic produced – enzyme

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581 applied" found in the literature are: FOS – FTase, FFase; GOS – β-galactosidase;
582 Lactosucrose – levansucrase; Lactulose – Cellobiose 2-epimerase; XOS – *endo*-xylanase;
583 MOS – β-mannanase; IMOs – α-glucosidase; and POS – *endo*-polygalacturonas.
584 Prebiotic synthesis is also highly dependent on the applied reaction conditions. Incubation
585 temperature, incubation time, pH, and enzyme dosage are the most critical operating
586 conditions that can influence production yields (**Tables 1** to **4**). Therefore, it is critical to
587 establish optimal conditions for maximal prebiotic production. Nonetheless, each residue
588 has a unique composition, and some may contain nutrients that can influence the catalytic
589 activity of the enzyme. Thus, ideal conditions may change depending on the waste used.

590 The economic viability of the process may be compromised by the need of pre-treatment
591 steps. Prebiotic production yields may be low if there is not enough available substrate
592 for synthesis. Ideally, no extraction methods should be applied. Chemical methods are
593 problematic, often resulting in the production of contaminant chemicals and in the
594 formation of undesired noxious by-products (*e.g.*, furfural). On the other hand, the use of
595 enzymes is more environment-friendly. However, the high cost of enzymes used, either
596 related to its production costs or by the acquisition of commercial enzymes, may be
597 problematic. Therefore, the huge importance of using appropriate approaches capable of
598 maximizing the production of the prebiotics and their recovery yield and to make the
599 process economically attractive.

600

601 3. Functionality tests

602 The production of prebiotics from agro-industrial wastes has shown to be effective, and
603 the use of such procedures can be a low-cost option for producing high-value-added
604 products. However, it is vital to demonstrate that the compounds generated have the
605 intended beneficial effect. Prebiotics must be resistant to gastric pH, be unable to undergo
606 hydrolysis by human digestive enzymes, not be absorbed in the gastrointestinal tract and
607 reach the colon almost intact to be fermented by the intestinal microbiota, and selectively
608 stimulate the growth and/or activity of probiotic bacteria to improve the health of the host
609 (Davani-Davari et al., 2019).

610 For this purpose, both *in vivo* and *in vitro* evaluation strategies have been used to test the
611 functionality, safety, and efficacy of new prebiotic substances for human health and well-

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612 being. Current studies conducted *in vivo* and *in vitro* on the digestibility and
613 fermentability of prebiotic candidates enzymatically produced from agro-industrial
614 wastes are discussed in the following sections.

615

616 **3.1. *In vitro gastrointestinal digestion of prebiotics***

617 Food digestion involves a complex series of dynamic processes involving the passage
618 through the different compartments of the gastrointestinal tract and the interaction with
619 the epithelium and the immune system. These processes include pH changes along the
620 digestion, gastric emptying dynamics, intestinal motility, production and release of
621 digestive enzymes and bile, as well as other processes, such as degradation by brush
622 border enzymes in the membrane of the intestinal epithelia. *In vitro* models must be
623 flexible, accurate, and reproducible although, it is not easy to simulate the complex
624 conditions of the digestive system.

625 A wide variety of gastrointestinal models have been designed to simulate the process of
626 food digestion, from simple static models to complex computer-controlled dynamic
627 multi-compartment models (Shani-Levi et al., 2017). However, due to their simplicity,
628 static models are still the most commonly used approach. Static models are used to
629 recreate the physicochemical and enzymatic environment of each single digestive phase.
630 The food product is incubated at body temperature (37 °C) for a certain time depending
631 on the digestion stage, and enzymatic solutions that simulate digestive fluids are added
632 (Ferreira-Lazarte et al., 2021). A large number of protocols have been applied to simulate
633 food digestion, which differs on many experimental conditions such as pH, duration of
634 each phase, amount of food and enzymes, source of digestive enzymes, among many
635 other parameters, which makes it difficult to compare results between studies. To
636 standardize the results, the INFOGEST network of scientists developed a harmonized and
637 consensus-based *in vitro* static digestion protocol, which aims to aid the production of
638 more consistent and comparable data (Brodkorb et al., 2019). **Table 5** shows several
639 studies in which the *in vitro* digestibility of various prebiotics obtained enzymatically
640 from agro-industrial wastes was assessed using static models.

641

642 *Please insert here Table 5.*

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644 Zidan et al. (2021) evaluated the digestibility of XOS from sugarcane pith and rind in the
645 oral and gastric phases. Sugarcane pith and rind XOS were hydrolyzed at 5.21 and 12.66
646 % after oral digestion, and 2.03 and 3.22 % after gastric digestion. Sugarcane pith XOS
647 showed higher resistance to oral/gastric digestion. Although both XOS have shown low
648 digestibility, inulin (used as a control) showed even lower digestibility values (2.58 at the
649 oral phase and 1.19 % at the gastric phase). In another study, the gastric and intestinal
650 digestibility of oligosaccharides obtained from sugarcane molasses was assessed. The
651 oligosaccharides were poorly hydrolyzed in the stomach and small intestine (10–15 %
652 and 8–10 %, respectively), which was assigned to the α-(1,6) bonds between its glucose
653 molecules. As they show resistance to low pH, they may be suitable for use in acidic
654 foods (Sharma et al., 2016). The methodologies used in both studies are, although,
655 limited, including only two stages of digestion without physiological residence time, and
656 the digestibility was not evaluated sequentially but as independent tests.

657 An increasing number of published research assessing the digestibility of food have been
658 conducted using the harmonized INFOGEST protocol. Some of these studies are
659 described below. XOS produced enzymatically from sugarcane straws and coffee husks
660 were tested for their resistance to the gastrointestinal digestion. XOS from both sources,
661 with DP 2–6, did not undergo degradation during the oral phase. After intestinal digestion,
662 XOS were hydrolyzed 4.55 % (straw) and 5.62 % (coffee husks), with fractions of DP2
663 and DP3 being the most resistant (Ávila et al., 2020). J. A. Gómez et al. (2021) evaluated
664 the digestive behavior of raw and cooked flour from discarded unripe plantain fruits. It
665 was observed a faster hydrolysis of cooked plantain flour, as compared to the raw one, in
666 the oral and intestinal phases. At the end of the digestion, 71.7 % and 52.6 % of the starch
667 of raw and cooked plantain flour, respectively, were resistant to hydrolysis, meaning that
668 a significant amount of undigested starch may reach the colon. The slower and limited
669 digestibility of the starch from the raw flour resulted also in a lower predicted glycemic
670 index, showing that the raw flour could be more suitable for people with insulin resistance
671 and diabetes than the cooked flour (J. A. Gómez et al., 2021).

672 The simulation of the upper gastrointestinal digestion of potential prebiotic carbohydrates
673 is therefore of huge importance not only to understand the resistance of the food to the
674 harsh conditions of the digestion but also to evaluate chemical and/or structural
675 modifications along the gastrointestinal tract, such as the release of glucose from
676 polysaccharides inducing a glycemic response in the consumer, allowing to assess the

677 impact of these compounds in the colonic microbiota, since even small alterations may
678 affect substantially their properties (Ferreira-Lazarte et al., 2021).

679

680 **3.2.** ***Gut microbial fermentation***

681 The conversion of prebiotics into smaller molecules by microbial activity involves
682 biochemical pathways mediated by the enzymatic activities of microorganisms. The type
683 of prebiotic and its physicochemical characteristics will affect the rate of fermentation,
684 selectivity of microorganisms to multiply, and the type and concentration of metabolites
685 released (Ashaolu et al., 2021). To verify the selectivity of a prebiotic and to follow its
686 chemical transformation, changes in the fecal microbiota, as well as the consumption and
687 production of compounds during its fermentation should be accurately monitored both *in*
688 *vitro* and *in vivo* (Al-Sheraji et al., 2013).

689 Animal models have long been used to contribute to the understanding and resolution of
690 biomedical and biotechnological challenges. *In vivo* prebiotic intestinal fermentation
691 studies are generally based on the oral administration of a certain compound over a
692 defined period. Before, throughout and/or after the study, stool samples are collected and
693 the changes in microbial composition, as well as the metabolites produced as a result of
694 the fermentation, are usually analyzed. **Table 6** presents two studies using rodents to
695 evaluate the fermentation of prebiotics obtained from agro-industrial wastes in the
696 intestinal ecosystem.

697 The beneficial effects of prebiotics on the intestinal microbiota are usually assessed by
698 the growth of probiotic bacteria, although the decrease of pathogenic microorganisms can
699 also be considered. The oral administration of 0.5 and 1.0 g/kg/day of XOS (obtained
700 from xylan of cassava dregs hydrolyzed by *endo*-β-(1,4)-xylanase) to Balb/c mice
701 resulted in significant colonic growth of the probiotic species *Lactobacillus* spp. and
702 *Bifidobacterium* spp., as well as the inhibition of the proliferation of *Escherichia coli*, a
703 potentially pathogenic bacterium (Hafidah et al., 2018). The main metabolites produced
704 by the bacteria during XOS fermentation were the short-chain fatty acids (SCFA) acetate
705 and butyrate. Acetate plays an important role in metabolism by modulating mitochondrial
706 function, and fatty acid oxidation (Sahuri-Arisoylu et al., 2016), while butyrate is an
707 important energy source for intestinal epithelial cells, as well, it has been reported to have

708 anti-inflammatory and anti-carcinogenic properties (Hamer et al., 2008). The effect of
709 dietary fiber obtained from apple by-products was also tested in rats fed a high-fat diet,
710 in which a remarkable bifidogenic, butyrogenic, and lipid-lowering effect was observed
711 compared to the control group (Mateos-Aparicio et al., 2020).

712 Animal studies provide quite valuable information, but they often involve ethical
713 concerns. It is also important to consider the physiological variations that the animal
714 model may have with the target population. On the other hand, *in vitro* methods have
715 much fewer bioethical constraints (Shani-Levi et al., 2017) and can be designed
716 considering the characteristics of the study subject. They have also the advantage of being
717 faster, less laborious, and therefore cheaper. *In vitro* fermentation models are a promising
718 tool to study the impact of prebiotics on the intestinal microbiota under strictly controlled
719 conditions (Roupar et al., 2021). These systems enable the study of the fermentation
720 profile of prebiotics, and its effect on gut microbial composition (Pham & Mohajeri,
721 2018).

722 Although pure cultures of representative beneficial bacteria (mainly *Lactobacillus* and
723 *Bifidobacterium*) have been used to determine the functionality of potential prebiotics,
724 inoculation with fecal material is a more accurate method (Bajury et al., 2018) as the
725 human intestinal microbiota is a highly complex ecosystem composed of a great variety
726 of microorganisms living in symbiosis with each other (Cockburn & Koropatkin, 2016).
727 Therefore, the *in vivo* effects of prebiotic intake may be significantly different from those
728 determined in selected culture based experiments (Ashaolu et al., 2021). **Table 6** shows
729 several studies in which the effect of various prebiotics obtained enzymatically from agro-
730 industrial wastes were evaluated *in vitro*, using either fermentation with selected bacteria
731 or with human fecal microbiota.

732

733 *Please insert here Table 6.*

734

735 The prebiotic potential is generally determined by the growth of bacteria known to be
736 beneficial to human health, being lactobacilli and bifidobacteria the most popular. Jana
737 and Kango (2020) tested the growth of *Lactobacillus delbrueckii* and *Lactobacillus*
738 *acidophilus* in media with MOS (1 mg/mL) produced from palm kernel cake, guar gum,
739 and copra meal. MOS from copra meal promoted the best growth, while MOS from locust
740 bean gum obtained the lowest growth rate. R. Zhang et al. (2021) also studied the

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741 prebiotic activity of MOS (0.5 % (w/v)) from locust bean gum and palm kernel cake in
742 the growth of *Lactobacillus plantarum*. Results showed that this bacterium could utilize
743 mannose, mannobiose, and mannotriose, in MOS from both sources, but not MOS with
744 high DP, such as mannotetroses.

745 Sugar cane by-products represent an important waste in the food industry. In fact, for
746 every 100 tons of sugar cane processed, 30 to 40 tons of residues are generated (Jayapal
747 et al., 2013). Zidan et al. (2021) obtained XOS from sugarcane pith (inner layer) and rind
748 (outer layer) and evaluated its gastrointestinal digestibility and its fermentability with
749 *Lactobacillus casei Shirota* and *Bifidobacterium animalis*. Sugarcane rind XOS promoted
750 a higher probiotic growth, which might be related to their lower DP end products (X_2 and
751 X_3) as compared to sugarcane pith, which had more content of X_4. The results also showed
752 that *L. casei Shirota* was able to consume more sugarcane pith and rind XOS than the *B.*
753 *animalis*. Bifidobacteria have been reported for their limited growth on substituted
754 branched structures, as well as their lack of β-xylosidase activity (Aachary & Prapulla,
755 2011), which may explain their limited growth in XOS observed in this study. Acetic acid
756 was the predominant SCFA produced by bacterial fermentation of both XOS, followed
757 by propionic and lactic acid. As already mentioned, acetic acid modulates mitochondrial
758 function, and fatty acid oxidation (Sahuri-Arisoylu et al., 2016) while propionic acid is a
759 precursor for the synthesis of glucose in the liver and has anti-inflammatory properties
760 (Havenaar, 2011). On the other hand, the low production of lactic acid, when compared
761 with the glucose control, indicates a slow fermentation, which is advantageous as it may
762 minimize the undesirable effects of intestinal carbohydrate fermentation such as bloating
763 and flatulence (Zidan et al., 2021).

764 Ávila et al. (2020) also used sugarcane by-products to produce XOS, in this case,
765 sugarcane straw and coffee husks. Its fermentability by two *Bifidobacterium* species (*B.*
766 *longum* and *B. lactis*) and two species of *Lactobacillus* (*L. acidophilus* and *L. paracasei*)
767 was assessed. All the probiotic cultures were able to utilize XOS produced from
768 agricultural wastes as the only carbon source, showing remarkable growth, except for *B.*
769 *lactis*, which lack β-xylosidase. As mentioned above, the lack of this enzyme is common
770 for some bifidobacteria strains.

771 Another coffee-related waste that has been used for the production of xylo-oligomers is
772 its peel, which is rich in xylan. Ratnadewi et al. (2020) tested the fermentability of XOS

773 obtained from coffee peel enzymatic hydrolysate by the probiotic *L. casei*. Acetate and
774 butyrate were the main fermentation products. Reductions in media sugar levels were
775 observed over time showing that *L. casei* utilized XOS for cell metabolism.

776 Cereal husks are another agro-industrial waste with great potential for XOS production.
777 Jagtap et al. (2017) obtained XOS from wheat husks, using the enzymatic complex of
778 *Aspergillus fumigatus*. Their prebiotic potential was evaluated using 10 probiotic strains,
779 of which only 6 were able to use the waste derived-XOS as the only carbon source (*i.e*.,
780 *Bifidobacterium bifidum* and *Bifidobacterium adolescentis, Lactobacillus rhamnosus,*
781 *Lactobacillus fermentum, Lactobacillus paraplantum* and *L. plantarum*). Results
782 indicated that the successful growth of the six tested probiotics was related to their β-
783 xylosidase activity since the highest amount of enzyme was identified for the *L.*
784 *rhamnosus* strain which coincided with the highest optical density values obtained. A
785 synergistic effect of different enzymes including β-xylosidase, α-glucuronidase, β-L-
786 arabinofuranosidase, and acetyl xylan esterase along with xylanase is required for an
787 efficient and complete degradation of XOS. Therefore, the capacity of a microorganism
788 to consume XOS is linked to its xylanolytic enzyme system efficacy. β-xylosidase and a
789 few other enzymes have been reported in some strains of *Bifidobacterium* and
790 *Lactobacillus* (Jagtap et al., 2017). Another study evaluated the prebiotic potential of
791 XOS produced from spent barley grains and husks (with different purity and Mw) by *in*
792 *vitro* fermentation with fecal microbiota. Results showed that samples with shorter Mw
793 have faster fermentation kinetics. Succinate, lactate, formate, acetate, propionate, and
794 butyrate were produced within the first 8 h of fermentation, using all tested samples,
795 demonstrating the prebiotic potential of the XOS (P. Gullón et al., 2011). Still, changes
796 in the fecal microbiota composition were not assessed in this study.

797 In a recent study, XOS were produced by the enzymatic fermentation of banana
798 pseudostems by *Aspergillus versicolor endo*-xylanase. The prebiotic effect of the XOS
799 produced was tested in two probiotic bacteria, namely *L. plantarum* and *L. fermentum*.
800 The growth of the potentially pathogenic bacteria *E. coli* was also tested. Both lactobacilli
801 were able to utilize XOS for their growth and remained in the exponential phase after 48
802 h in media containing XOS, as compared to the control media (containing glucose). *E.*
803 *coli* also consumed XOS as a carbon source, however, it reached the stationary phase after
804 24 h (de Freitas et al., 2021). Although both probiotic and pathogenic bacteria grew in the
805 presence of XOS, a competition by the substrate is established as they coexist in the gut.

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Also, as lactobacilli and other probiotic bacterial species possess antimicrobial activity and produce organic acids, the proliferation of disease-causing bacteria is inhibited (Shokryazdan et al., 2014).

Pectic oligosaccharides have been identified as potential prebiotic candidates and have been emerging as novel prebiotics (Scott et al., 2020). Citric peels are a great source of pectin, and therefore may be exploited for POS production. B. Gómez et al. (2014) obtained POS from orange peel and tested its fermentability *in vitro* using fecal samples from three healthy adult volunteers. The POS mixture contained several types of oligosaccharides, which were fermented in the following order: GlcOS > GalOS > AraOS > OGalA. Since each species of microorganism has a preference for specific substrates, the fermentation rate is highly influenced by the oligosaccharides' chemical structure (*i.e.*, degree of polymerization and esterification, type of linkages, and molecular composition) (Cockburn & Koropatkin, 2016). Bacterial growth and production of SCFA, lactate, gas, and other organic acids were observed. Higher microbial growth was observed, particularly of lactobacilli species. Due to POS fermentation, there was an increase from 0.17 to 0.27 in the ratio between the cell counts of lactobacilli and bifidobacteria and the overall cell number. The prebiotic potential of POS from lemon peel and beet pulp has been also investigated by the same research group (B. Gómez et al., 2016). Results showed that sugar-beet POS were fermented slower than lemon-peel POS, suggesting that sugar-beet POS could reach more distal parts of the colon. Both substrates promoted bifidobacteria and lactobacilli growth. Sugar-beet POS showed the strongest bifidogenic effect (from 11.8 % up to 23.4 % of total counts) while lemon-peel POS especially boosted lactobacilli population (from 6.8 % up to 14.4 % of total counts).

Besides POS, whose length range from 3 to 10 units, a growing interest in water-soluble pectin derivatives, particularly the lower Mw modified pectins, have been emerging in the past few years. Ferreira-Lazarte et al. (2018) produced modified pectins from artichoke and sunflower by-products and tested its fermentation properties and prebiotic activity by *in vitro* batch fecal fermentation. Among all assayed samples, artichoke-modified pectins was the substrate that promoted the most significant growth in bifidobacteria. These findings could be related to the high arabinose and galactose content found in artichoke-modified pectins. For all the tested substrates, acetate was the most abundant SCFA produced, followed by propionate and butyrate. The presence of high levels of acetate is consistent with the existing data found in literature since acetate is one

839 of the most abundant SCFA in the human gut. Its production has been linked to enteric
840 bacteria, namely *Bifidobacterium* and *Lactobacillus* (Cook & Sellin, 1998), which often
841 results in an increase of acetate levels and the population of these bacterial groups.
842 Therefore, the experimental results are consistent with these prior observations.
843 Furthermore, these end-products may be used as a substrate for other colonic bacteria and
844 converted into other SCFA, which can be explained by cross-feeding interactions
845 (Cockburn & Koropatkin, 2016).

846 Other prebiotic oligosaccharides have been obtained from various agro-industrial wastes,
847 such as tea leaves, soybean Okara, or artichoke by-products, and its prebiotic activity has
848 been evaluated by *in vitro* batch fermentations. Oligosaccharides obtained from spent tea
849 leaves were able to support the growth *L. acidophilus* and simultaneously inhibit the
850 growth of *E. coli* (Chimtong et al., 2016). The growth of beneficial bacteria, including
851 bifidobacteria and lactobacilli, was promoted by enzymatically treated Okara, whilst
852 pathogenic bacteria, such as clostridia and *Bacteroides,* were inhibited (Pérez-López et
853 al., 2016). The fermentation of artichoke by-product by fecal bacteria promoted the
854 growth of beneficial lactic acid bacteria and bifidobacteria, while the growth of potential
855 pathogens such as coliforms and clostridia remained lower. Moreover, the fecal
856 microorganisms were able to consume 54.6 % of the substrate and their main
857 fermentation products were the beneficial SCFA, acetic, propionic, and butyric acid,
858 while the production of lactic acid was lower than in the controls (inulin and sucrose)
859 indicating a slow fermentation (Holgado et al., 2022).

860 The aforementioned studies used batch fermentation systems (either with fecal inoculum
861 or isolated bacteria) for the evaluation of the prebiotic potential of the various
862 oligosaccharides obtained from agro-industrial wastes. These systems are the simplest
863 and most frequently used, although they have disadvantages. When the substrate is
864 limited and quickly consumed there is usually an excessive accumulation of microbial
865 metabolites that acidify the pH and hinder subsequent microbial activity, so that the
866 experiments are short-lived (Bajury et al., 2018; Roupar et al., 2021). On the other hand,
867 continuous cultures allow long-term fermentation and adjustment of parameters to
868 simulate *in vivo* conditions (Pham & Mohajeri, 2018). Continuous fermentation models
869 representing one or more regions of the human colon are currently available and under
870 development, in which parameters such as temperature, flow rate, pH, retention time, and
871 anaerobiosis of the medium are carefully controlled to accurately represent each region

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872 (Bajury et al., 2018; Roupar et al., 2021). However, the literature on studies evaluating
873 the prebiotic potential of novel products obtained from agro-industrial wastes using these
874 models is still quite limited. The appropriate dose to promote beneficial health effects
875 depends on several factors such as the nature and the purity of the produced prebiotic, as
876 well as on the consumer individual tolerability, since an excessive dose of the prebiotic
877 can result in uncomfortable side effects such as bloating, flatulence and diarrhea.
878 Therefore, a continuous intake at moderate amounts would be advisable.

879

880 4. Conclusion and future outlook

881 The reuse of agro-industrial by-products has been proved to be a feasible alternative for
882 the production of prebiotic ingredients. The combination of enzymatic processes and the
883 use of waste materials can generate high production yields. The raw material and the
884 catalyst used will determine the type of oligomers produced, which will have different
885 physicochemical and biological properties. It is important to optimize the processing
886 conditions to maximize the production of prebiotics by enzymatic mechanisms. The use
887 of immobilization approaches and enzyme systems can be helpful to increase the yield
888 and reduce the catalysis time, making it a cost-effective process for industrial
889 applications.

890 The evaluation of the digestibility of the produced prebiotics confirms that they reach the
891 colon without being hydrolyzed, while fermentability assays allow verifying their
892 selectivity to promote the growth of beneficial microorganisms and to follow its
893 conversion into bioactive metabolites, such as SCFA, which play very important roles in
894 human physiology and metabolism. Each species and strain of gut microorganisms prefer
895 specific substrates and have different enzymatic complexes, influencing the type and
896 proportion of metabolite produced. Also, the chemical structure and Mw of the prebiotic
897 will have a significant impact on its utilization by the microbiota.

898 Overall, agro-industrial wastes are raw materials of low or no cost with great potential for
899 obtaining compounds of high commercial value, namely prebiotics, and enzymatic
900 processes have demonstrated their efficiency for this conversion. Still, it is important to
901 point out that the biological activity of potential prebiotics must be evaluated before
902 commercialization and before making any health claim on these products. A step that is
903 missing for most emerging prebiotics being produced from agro-industrial wastes.

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904 **Declaration of competing interest**

905 The authors declare no competing interests.

906

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936 # References

937 Aachary, A. A., & Prapulla, S. G. (2009). Value addition to corncob: Production and characterization of
938 xylooligosaccharides from alkali pretreated lignin-saccharide complex using *Aspergillus oryzae*
939 MTCC 5154. *Bioresource Technology*, *100*(2), 991–995.
940 https://doi.org/10.1016/j.biortech.2008.06.050

941 Aachary, A. A., & Prapulla, S. G. (2011). Xylooligosaccharides (XOS) as an Emerging Prebiotic: Microbial
942 Synthesis, Utilization, Structural Characterization, Bioactive Properties, and Applications.
943 *Comprehensive Reviews in Food Science and Food Safety*, *10*(1), 2–16.

944 Ajala, E. O., Ajala, M. A., Tijani, I. A., Adebisi, A. A., & Suru, I. (2020). Kinetics modelling of acid
945 hydrolysis of cassava (Manihot esculanta Cranz) peel and its hydrolysate chemical characterisation.
946 *Journal of King Saud University - Science*, *32*(4), 2284–2292.
947 https://doi.org/10.1016/j.jksus.2020.03.003

948 Al-Sheraji, S. H., Ismail, A., Manap, M. Y., Mustafa, S., Yusof, R. M., & Hassan, F. A. (2013). Prebiotics
949 as functional foods: A review. *Journal of Functional Foods*, *5*(4), 1542–1553.
950 https://doi.org/10.1016/j.jff.2013.08.009

951 Álvarez, C., González, A., Negro, M. J., Ballesteros, I., Oliva, J. M., & Sáez, F. (2017). Optimized use of
952 hemicellulose within a biorefinery for processing high value-added xylooligosaccharides. *Industrial*
953 *Crops and Products*, *99*, 41–48. https://doi.org/10.1016/j.indcrop.2017.01.034

954 Ashaolu, T. J., Ashaolu, J. O., & Adeyeye, S. A. O. (2021). Fermentation of prebiotics by human colonic
955 microbiota *in vitro* and short-chain fatty acids production: a critical review. *Journal of Applied*
956 *Microbiology*, *130*(3), 677–687. https://doi.org/10.1111/jam.14843

957 Ávila, P. F., Martins, M., de Almeida Costa, F. A., & Goldbeck, R. (2020). Xylooligosaccharides
958 production by commercial enzyme mixture from agricultural wastes and their prebiotic and
959 antioxidant potential. *Bioactive Carbohydrates and Dietary Fibre*, *24*, 100234.
960 https://doi.org/10.1016/j.bcdf.2020.100234

961 Babbar, N., Baldassarre, S., Maesen, M., Prandi, B., Dejonghe, W., Sforza, S., & Elst, K. (2016). Enzymatic
962 production of pectic oligosaccharides from onion skins. *Carbohydrate Polymers*, *146*, 245–252.
963 https://doi.org/10.1016/j.carbpol.2016.03.011

964 Babbar, N., Dejonghe, W., Gatti, M., Sforza, S., & Elst, K. (2016). Pectic oligosaccharides from agricultural
965 by-products: production, characterization and health benefits. *Critical Reviews in Biotechnology*,
966 *36*(4), 594–606. https://doi.org/10.3109/07388551.2014.996732

967 Bajury, D. M., Nashri, S. M., King Jie Hung, P., & Sarbini, S. R. (2018). Evaluation of potential prebiotics:
968 a review. *Food Reviews International*, *34*(7), 639–664.
969 https://doi.org/10.1080/87559129.2017.1373287

970 Baldassarre, S., Babbar, N., Van Roy, S., Dejonghe, W., Maesen, M., Sforza, S., & Elst, K. (2018).
971 Continuous production of pectic oligosaccharides from onion skins with an enzyme membrane
972 reactor. *Food Chemistry*, *267*, 101–110. https://doi.org/10.1016/j.foodchem.2017.10.055

973 Bangoria, P., Divecha, J., & Shah, A. R. (2021). Production of mannooligosaccharides producing β-
974 Mannanase by newly isolated *Penicillium aculeatum* APS1 using oil seed residues under solid state
975 fermentation. *Biocatalysis and Agricultural Biotechnology*, *34*, 102023.
976 https://doi.org/10.1016/j.bcab.2021.102023

977 Basu, A., Mutturi, S., & Prapulla, S. G. (2016). Production of isomaltooligosaccharides (IMO) using
978 simultaneous saccharification and transglucosylation from starch and sustainable sources. *Process*
979 *Biochemistry*, *51*(10), 1464–1471. https://doi.org/10.1016/j.procbio.2016.06.008

Journal Pre-proof

Journal Pre-proof

980 Bhatia, L., Sharma, A., Bachheti, R. K., & Chandel, A. K. (2019). Lignocellulose derived functional
981 oligosaccharides: production, properties, and health benefits. *Preparative Biochemistry and*
982 *Biotechnology*, *49*(8), 744–758. https://doi.org/10.1080/10826068.2019.1608446

983 Bolognesi, L. S., Gabardo, S., Dall Cortivo, P. R., & Ayub, M. A. Z. (2021). Biotechnological production
984 of galactooligosaccharides (GOS) using porungo cheese whey. *Food Science and Technology*.
985 https://doi.org/10.1590/fst.64520

986 Brodkorb, A., Egger, L., Alminger, M., Alvito, P., Assunção, R., Ballance, S., Bohn, T., Bourlieu-Lacanal,
987 C., Boutrou, R., Carrière, F., Clemente, A., Corredig, M., Dupont, D., Dufour, C., Edwards, C.,
988 Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., … Recio, I. (2019). INFOGEST static in
989 vitro simulation of gastrointestinal food digestion. *Nature Protocols*, *14*(4), 991–1014.
990 https://doi.org/10.1038/S41596-018-0119-1

991 Chantorn, S., Piyapittayanun, C., & Dangpram, P. (2018). Bioconversion of Agricultural Wastes to
992 Mannooligosaccharides and Their Prebiotic Potential. *Chiang Mai Journal of Science*, *45*(1), 60–67.

993 Chimtong, S., Saenphoom, P., Karageat, N., & Somtua, S. (2016). Oligosaccharide Production from
994 Agricultural Residues by Non-starch Polysaccharide Degrading Enzymes and Their Prebiotic
995 Properties. *Agriculture and Agricultural Science Procedia*, *11*, 131–136.
996 https://doi.org/10.1016/j.aaspro.2016.12.022

997 Chockchaisawasdee, S., & Poosaran, N. (2013). Production of isomaltooligosaccharides from banana flour.
998 *Journal of the Science of Food and Agriculture*, *93*(1), 180–186. https://doi.org/10.1002/jsfa.5747

999 Cockburn, D. W., & Koropatkin, N. M. (2016). Polysaccharide Degradation by the Intestinal Microbiota
1000 and Its Influence on Human Health and Disease. *Journal of Molecular Biology*, *428*(16), 3230–3252.
1001 https://doi.org/10.1016/j.jmb.2016.06.021

1002 Combo, A. M. M., Aguedo, M., Quiévy, N., Danthine, S., Goffin, D., Jacquet, N., Blecker, C., Devaux, J.,
1003 & Paquot, M. (2013). Characterization of sugar beet pectic-derived oligosaccharides obtained by
1004 enzymatic hydrolysis. *International Journal of Biological Macromolecules*, *52*(1), 148–156.
1005 https://doi.org/10.1016/j.ijbiomac.2012.09.006

1006 Cook, S. I., & Sellin, J. H. (1998). Review article: Short chain fatty acids in health and disease. *Alimentary*
1007 *Pharmacology and Therapeutics*, *12*(6), 499–507. https://doi.org/10.1046/j.1365-2036.1998.00337.x

1008 Córdoba, J. A., Salcedo, E., Rodríguez, R., Zamora, J. F., Manríquez, R., Contreras, H., Robledo, J., &
1009 Delgado, E. (2013). Caracterización y valoración química del olote: Degradación hidrotérmica bajo
1010 condiciones subcríticas. *Revista Latinoamericana de Quimica*, *41*(3), 171–184.

1011 Corzo-Martínez, M., García-Campos, G., Montilla, A., & Moreno, F. J. (2016). Tofu whey permeate is an
1012 efficient source to enzymatically produce prebiotic fructooligosaccharides and novel fructosylated α-
1013 galactosides. *Journal of Agricultural and Food Chemistry*, *64*(21), 4346–4352.
1014 https://doi.org/10.1021/acs.jafc.6b00779

1015 Corzo-Martinez, M., Luscher, A., De Las Rivas, B., Muñoz, R., & Moreno, F. J. (2015). Valorization of
1016 cheese and tofu whey through enzymatic synthesis of lactosucrose. *PLoS ONE*, *10*(9).
1017 https://doi.org/10.1371/journal.pone.0139035

1018 Costa, J. R., Tonon, R. V., Gottschalk, L. M. F., Santiago, M. C. de A., Mellinger-Silva, C., Pastrana, L.,
1019 Pintado, M. M., & Cabral, L. M. C. (2019). Enzymatic production of xylooligosaccharides from
1020 Brazilian Syrah grape pomace flour: a green alternative to conventional methods for adding value to
1021 agricultural by-products. *Journal of the Science of Food and Agriculture*, *99*(3), 1250–1257.
1022 https://doi.org/10.1002/jsfa.9297

1023 Davani-Davari, D., Negahdaripour, M., Karimzadeh, I., Seifan, M., Mohkam, M., Masoumi, S. J.,
1024 Berenjian, A., & Ghasemi, Y. (2019). Prebiotics: Definition, types, sources, mechanisms, and clinical
1025 applications. *Foods*, *8*(3), 92. https://doi.org/10.3390/foods8030092

Journal Pre-proof

Journal Pre-proof

1026 de Freitas, C., Terrone, C. C., Masarin, F., Carmona, E. C., & Brienzo, M. (2021). *In vitro* study of the
1027 effect of xylooligosaccharides obtained from banana pseudostem xylan by enzymatic hydrolysis on
1028 probiotic bacteria. *Biocatalysis and Agricultural Biotechnology*, *33*, 101973.
1029 https://doi.org/10.1016/j.bcab.2021.101973

1030 de la Rosa, O., Flores-Gallegos, A. C., Muñíz-Marquez, D., Nobre, C., Contreras-Esquivel, J. C., & Aguilar,
1031 C. N. (2019). Fructooligosaccharides production from agro-wastes as alternative low-cost source.
1032 *Trends in Food Science and Technology*, *91*, 139–146. https://doi.org/10.1016/j.tifs.2019.06.013

1033 Ferreira-Lazarte, A., Kachrimanidou, V., Villamiel, M., Rastall, R. A., & Moreno, F. J. (2018). *In vitro*
1034 fermentation properties of pectins and enzymatic-modified pectins obtained from different renewable
1035 bioresources. *Carbohydrate Polymers*, *199*, 482–491. https://doi.org/10.1016/j.carbpol.2018.07.041

1036 Ferreira-Lazarte, A., Moreno, F. J., & Villamiel, M. (2021). Bringing the digestibility of prebiotics into
1037 focus: update of carbohydrate digestion models. *Critical Reviews in Food Science and Nutrition*,
1038 *61*(19), 3267–3278. https://doi.org/10.1080/10408398.2020.1798344

1039 Fischer, C., & Kleinschmidt, T. (2021). Synthesis of galactooligosaccharides by *Cryptococcus laurentii*
1040 and *Aspergillus oryzae* using different kinds of acid whey. *International Dairy Journal*, *112*, 104867.
1041 https://doi.org/10.1016/j.idairyj.2020.104867

1042 Forbes, H., Quested, T., & O'Connor, C. (2021). *Food Waste Index Report 2021*.

1043 Ganaie, M. A., Soni, H., Naikoo, G. A., Santos Oliveira, L. T., Rawat, H. K., Mehta, P. K., & Narain, N.
1044 (2017). Screening of low cost agricultural wastes to maximize the fructosyltransferase production
1045 and its applicability in generation of fructooligosaccharides by solid state fermentation. *International*
1046 *Biodeterioration and Biodegradation*, *118*, 19–26. https://doi.org/10.1016/j.ibiod.2017.01.006

1047 Geiger, B., Nguyen, H. M., Wenig, S., Nguyen, H. A., Lorenz, C., Kittl, R., Mathiesen, G., Eijsink, V. G.
1048 H., Haltrich, D., & Nguyen, T. H. (2016). From by-product to valuable components: Efficient
1049 enzymatic conversion of lactose in whey using β-galactosidase from *Streptococcus thermophilus*.
1050 *Biochemical Engineering Journal*, *116*, 45–53. https://doi.org/10.1016/j.bej.2016.04.003

1051 Gómez, B., Gullón, B., Remoroza, C., Schols, H. A., Parajó, J. C., & Alonso, J. L. (2014). Purification,
1052 characterization, and prebiotic properties of pectic oligosaccharides from orange peel wastes. *Journal*
1053 *of Agricultural and Food Chemistry*, *62*(40), 9769–9782. https://doi.org/10.1021/jf503475b

1054 Gómez, B., Gullón, B., Yáñez, R., Schols, H., & Alonso, J. L. (2016). Prebiotic potential of pectins and
1055 pectic oligosaccharides derived from lemon peel wastes and sugar beet pulp: A comparative
1056 evaluation. *Journal of Functional Foods*, *20*, 108–121. https://doi.org/10.1016/j.jff.2015.10.029

1057 Gómez, J. A., Pino-Hernández, E., Abrunhosa, L., Matallana, L. G., Sánchez, Ó. J., Teixeira, J. A., &
1058 Nobre, C. (2021). Valorisation of rejected unripe plantain fruits of *Musa* AAB Simmonds: From
1059 nutritional characterisation to the conceptual process design for prebiotic production. *Food and*
1060 *Function*, *12*(7), 3009–3021. https://doi.org/10.1039/d0fo03379k

1061 Gonçalves, D. A., Teixeira, J. A., & Nobre, C. (2022). *In situ* enzymatic synthesis of prebiotics to improve
1062 food functionality. In *Value-Addition in Food Products and Processing Through Enzyme Technology*
1063 (pp. 253–267). Academic Press. https://doi.org/10.1016/b978-0-323-89929-1.00026-3

1064 Gullón, B., Gómez, B., Martínez-Sabajanes, M., Yáñez, R., Parajó, J. C., & Alonso, J. L. (2013). Pectic
1065 oligosaccharides: Manufacture and functional properties. *Trends in Food Science & Technology*,
1066 *30*(2), 153–161. https://doi.org/10.1016/J.TIFS.2013.01.006

1067 Gullón, P., González-Muñoz, M. J., & Parajó, J. C. (2011). Manufacture and prebiotic potential of
1068 oligosaccharides derived from industrial solid wastes. *Bioresource Technology*, *102*(10), 6112–6119.
1069 https://doi.org/10.1016/j.biortech.2011.02.059

1070 Hackenhaar, C. R., Spolidoro, L. S., Flores, E. E. E., Klein, M. P., & Hertz, P. F. (2021). Batch synthesis
1071 of galactooligosaccharides from co-products of milk processing using immobilized β-galactosidase

1072 from *Bacillus circulans*. *Biocatalysis and Agricultural Biotechnology*, *36*, 102136.
1073 https://doi.org/10.1016/j.bcab.2021.102136

1074 Hafidah, A. H., Sulistyaningsih, E., Handayani, W., & Ratnadewi, A. (2018). Prebiotic Potential of
1075 Xylooligosaccharides Derived from Cassava Dregs in Balb / c Mice Colon. *Pertanika Journal of*
1076 *Tropical Agricultural Science*, *41*(3), 1021–1031.

1077 Hamer, H. M., Jonkers, D., Venema, K., Vanhoutvin, S., Troost, F. J., & Brummer, R. J. (2008). The role
1078 of butyrate on colonic function. *Alimentary Pharmacology and Therapeutics*, *27*(2), 104–119.
1079 https://doi.org/10.1111/j.1365-2036.2007.03562.x

1080 Havenaar, R. (2011). Intestinal health functions of colonic microbial metabolites: A review. *Beneficial*
1081 *Microbes*, *2*(2), 103–114. https://doi.org/10.3920/BM2011.0003

1082 Holgado, F., Campos-Monfort, G., de las Heras, C., & Rupérez, P. (2022). Assessment of the prebiotic
1083 potential of globe artichoke by-product through *in vitro* fermentation by human faecal microbiota.
1084 *Bioactive Carbohydrates and Dietary Fibre*, *28*, 100328. https://doi.org/10.1016/j.bcdf.2022.100328

1085 Ibrahim, S. N. M. M., Bankeeree, W., Prasongsuk, S., Punnapayak, H., & Lotrakul, P. (2022). Production
1086 and characterization of thermostable acidophilic β-mannanase from *Aureobasidium pullulans* NRRL
1087 58524 and its potential in mannooligosaccharide production from spent coffee ground
1088 galactomannan. *3 Biotech*, *12*(9), 237. https://doi.org/10.1007/s13205-022-03301-4

1089 Jagtap, S., Deshmukh, R. A., Menon, S., & Das, S. (2017). Xylooligosaccharides production by crude
1090 microbial enzymes from agricultural waste without prior treatment and their potential application as
1091 nutraceuticals. *Bioresource Technology*, *245*, 283–288.
1092 https://doi.org/10.1016/j.biortech.2017.08.174

1093 Jana, U. K., & Kango, N. (2020). Characteristics and bioactive properties of mannooligosaccharides derived
1094 from agro-waste mannans. *International Journal of Biological Macromolecules*, *149*, 931–940.
1095 https://doi.org/10.1016/j.ijbiomac.2020.01.304

1096 Jayapal, N., Samanta, A. K., Kolte, A. P., Senani, S., Sridhar, M., Suresh, K. P., & Sampath, K. T. (2013).
1097 Value addition to sugarcane bagasse: Xylan extraction and its process optimization for
1098 xylooligosaccharides production. *Industrial Crops and Products*, *42*(1), 14–24.
1099 https://doi.org/10.1016/j.indcrop.2012.05.019

1100 Jnawali, P., Kumar, V., Tanwar, B., Hirdyani, H., & Gupta, P. (2018). Enzymatic Production of
1101 Xylooligosaccharides from Brown Coconut Husk Treated with Sodium Hydroxide. *Waste and*
1102 *Biomass Valorization*, *9*(10), 1757–1766. https://doi.org/10.1007/s12649-017-9963-4

1103 Kallel, F., Driss, D., Bouaziz, F., Neifer, M., Ghorbel, R., & Ellouz Chaabouni, S. (2015). Production of
1104 xylooligosaccharides from garlic straw xylan by purified xylanase from *Bacillus mojavensis* UEB-
1105 FK and their *in vitro* evaluation as prebiotics. *Food and Bioproducts Processing*, *94*, 536–546.
1106 https://doi.org/10.1016/j.fbp.2014.07.012

1107 Kaur, R., Panwar, D., & Panesar, P. S. (2020). Biotechnological approach for valorization of whey for
1108 value-added products. In *Food Industry Wastes* (pp. 275–302). Academic Press.
1109 https://doi.org/10.1016/b978-0-12-817121-9.00013-9

1110 Khat-udomkiri, N., Sivamaruthi, B. S., Sirilun, S., Lailerd, N., Peerajan, S., & Chaiyasut, C. (2018).
1111 Optimization of alkaline pretreatment and enzymatic hydrolysis for the extraction of
1112 xylooligosaccharide from rice husk. *AMB Express*, *8*(1), 1–10. https://doi.org/10.1186/s13568-018-
1113 0645-9

1114 Kuschel, B., Claaßen, W., Mu, W., Jiang, B., Stressler, T., & Fischer, L. (2016). Reaction investigation of
1115 lactulose-producing cellobiose 2-epimerases under operational relevant conditions. *Journal of*
1116 *Molecular Catalysis B: Enzymatic*, *133*, S80–S87. https://doi.org/10.1016/j.molcatb.2016.11.022

1117 Liu, X., Liu, Y., Jiang, Z., Liu, H., Yang, S., & Yan, Q. (2018). Biochemical characterization of a novel
1118 xylanase from *Paenibacillus barengoltzii* and its application in xylooligosaccharides production from
1119 corncobs. *Food Chemistry*, *264*, 310–318. https://doi.org/10.1016/j.foodchem.2018.05.023

1120 Magengelele, M., Hlalukana, N., Malgas, S., Rose, S. H., van Zyl, W. H., & Pletschke, B. I. (2021).
1121 Production and *in vitro* evaluation of prebiotic manno-oligosaccharides prepared with a recombinant
1122 *Aspergillus niger* endo-mannanase, Man26A. *Enzyme and Microbial Technology*, *150*, 109893.
1123 https://doi.org/10.1016/j.enzmictec.2021.109893

1124 Mano, M. C. R., Neri-Numa, I. A., da Silva, J. B., Paulino, B. N., Pessoa, M. G., & Pastore, G. M. (2018).
1125 Oligosaccharide biotechnology: an approach of prebiotic revolution on the industry. *Applied*
1126 *Microbiology and Biotechnology*, *102*(1), 17–37. https://doi.org/10.1007/s00253-017-8564-2

1127 Mano, M. C. R., Paulino, B. N., & Pastore, G. M. (2019). Whey permeate as the raw material in galacto-
1128 oligosaccharide synthesis using commercial enzymes. *Food Research International*, *124*, 78–85.
1129 https://doi.org/10.1016/j.foodres.2018.09.019

1130 Marim, A. V. C., Gabardo, S., & Ayub, M. A. Z. (2021). Porungo cheese whey: β-galactosidase production,
1131 characterization and lactose hydrolysis. *Brazilian Journal of Food Technology*, *24*.
1132 https://doi.org/10.1590/1981-6723.03821

1133 Mateos-Aparicio, I., De la Peña Armada, R., Pérez-Cózar, M. L., Rupérez, P., Redondo-Cuenca, A., &
1134 Villanueva-Suárez, M. J. (2020). Apple by-product dietary fibre exhibits potential prebiotic and
1135 hypolipidemic effectsin high-fat fed Wistar rats. *Bioactive Carbohydrates and Dietary Fibre*, *23*,
1136 100219. https://doi.org/10.1016/j.bcdf.2020.100219

1137 Muñiz-Márquez, D. B., Contreras, J. C., Rodríguez, R., Mussatto, S. I., Teixeira, J. A., & Aguilar, C. N.
1138 (2016). Enhancement of fructosyltransferase and fructooligosaccharides production by *A. oryzae*
1139 DIA-MF in Solid-State Fermentation using aguamiel as culture medium. *Bioresource Technology*,
1140 *213*, 276–282. https://doi.org/10.1016/j.biortech.2016.03.022

1141 Mussatto, S. I., Ballesteros, L. F., Martins, S., Maltos, D. A. F., Aguilar, C. N., & Teixeira, J. A. (2013).
1142 Maximization of Fructooligosaccharides and β-Fructofuranosidase Production by *Aspergillus*
1143 *japonicus* under Solid-State Fermentation Conditions. *Food and Bioprocess Technology*, *6*(8), 2128–
1144 2134. https://doi.org/10.1007/s11947-012-0873-y

1145 Mussatto, S. I., Ballesteros, L. F., Martins, S., & Teixeira, J. A. (2012). Use of Agro-Industrial Wastes in
1146 Solid-State Fermentation Processes. In Kuan-Yeow Show (Ed.), *Industrial Waste* (pp. 120–140).
1147 InTech. https://doi.org/10.5772/36310

1148 Mussatto, S. I., & Teixeira, J. A. (2010). Increase in the fructooligosaccharides yield and productivity by
1149 solid-state fermentation with *Aspergillus japonicus* using agro-industrial residues as support and
1150 nutrient source. *Biochemical Engineering Journal*, *53*(1), 154–157.
1151 https://doi.org/10.1016/j.bej.2010.09.012

1152 Nikodinovic-Runic, J., Guzik, M., Kenny, S. T., Babu, R., Werker, A., & O'Connor, K. E. (2013). Carbon-
1153 rich wastes as feedstocks for biodegradable polymer (polyhydroxyalkanoate) production using
1154 bacteria. In *Advances in Applied Microbiology* (Vol. 84, pp. 139–200). Academic Press.
1155 https://doi.org/10.1016/B978-0-12-407673-0.00004-7

1156 Nobre, C., Gonçalves, D. A., Teixeira, J. A., & Rodrigues, L. R. (2018). One-step co-culture fermentation
1157 strategy to produce high-content fructo-oligosaccharides. *Carbohydrate Polymers*, *201*, 31–38.
1158 https://doi.org/10.1016/j.carbpol.2018.08.051

1159 Nobre, C., Simões, L. S., Gonçalves, D. A., Berni, P., & Teixeira, J. A. (2022). Fructooligosaccharides
1160 production and the health benefits of prebiotics. In *Current Developments in Biotechnology and*
1161 *Bioengineering* (pp. 109–138). Elsevier. https://doi.org/10.1016/b978-0-12-823506-5.00002-3

1162 Pangestu, R., Rahmani, N., Palar, R., Lisdiyanti, P., & Yopi. (2019). The effect of biomass particle size and
1163 chemical structure on the enzymatic hydrolysis reaction of galactomannan from sugar palm fruit by

Journal Pre-proof

1164 β-mannanase from *Kitasatospora* sp. KY576672. *IOP Conference Series: Earth and Environmental*
1165 *Science*, *251*(1). https://doi.org/10.1088/1755-1315/251/1/012008

1166 Pérez-López, E., Cela, D., Costabile, A., Mateos-Aparicio, I., & Rupérez, P. (2016). *In vitro* fermentability
1167 and prebiotic potential of soyabean Okara by human faecal microbiota. *British Journal of Nutrition*,
1168 *116*(6), 1116–1124. https://doi.org/10.1017/S0007114516002816

1169 Pham, V. T., & Mohajeri, M. H. (2018). The application of *in vitro* human intestinal models on the
1170 screening and development of pre- and probiotics. *Beneficial Microbes*, *9*(5), 725–742.
1171 https://doi.org/10.3920/BM2017.0164

1172 Purnawan, A., Yopi, Y., & Irawadi, T. T. (2017). Production of Manooligomannan from Palm Kernel Cake
1173 by Mannanase Produced from *Streptomyces Cyaenus*. *Biosaintifika: Journal of Biology & Biology*
1174 *Education*, *9*(1), 73. https://doi.org/10.15294/biosaintifika.v9i1.9201

1175 Radenkovs, V., Juhnevica-Radenkova, K., Górnaś, P., & Seglina, D. (2018). Non-waste technology through
1176 the enzymatic hydrolysis of agro-industrial by-products. *Trends in Food Science and Technology*, *77*,
1177 64–76. https://doi.org/10.1016/j.tifs.2018.05.013

1178 Ratnadewi, A. A. I., Amaliyah Zain, M. H., Nara Kusuma, A. A. N., Handayani, W., Nugraha, A. S., &
1179 Siswoyo, T. A. (2020). *Lactobacillus casei* fermentation towards xylooligosaccharide (XOS)
1180 obtained from coffee peel enzymatic hydrolysate. *Biocatalysis and Agricultural Biotechnology*, *23*,
1181 101446. https://doi.org/10.1016/j.bcab.2019.101446

1182 Roupar, D., Berni, P., Martins, J. T., Caetano, A. C., Teixeira, J. A., & Nobre, C. (2021). Bioengineering
1183 approaches to simulate human colon microbiome ecosystem. *Trends in Food Science and*
1184 *Technology*, *112*, 808–822. https://doi.org/10.1016/j.tifs.2021.04.035

1185 Sabajanes, M. M., Yáñez, R., Alonso, J. L., & Parajó, J. C. (2012). Pectic oligosaccharides production from
1186 orange peel waste by enzymatic hydrolysis. *International Journal of Food Science and Technology*,
1187 *47*(4), 747–754. https://doi.org/10.1111/j.1365-2621.2011.02903.x

1188 Sadh, P. K., Duhan, S., & Duhan, J. S. (2018). Agro-industrial wastes and their utilization using solid state
1189 fermentation: a review. *Bioresources and Bioprocessing*, *5*(1), 1–15. https://doi.org/10.1186/s40643-
1190 017-0187-z

1191 Sahuri-Arisoylu, M., Brody, L. P., Parkinson, J. R., Parkes, H., Navaratnam, N., Miller, A. D., Thomas, E.
1192 L., Frost, G., & Bell, J. D. (2016). Reprogramming of hepatic fat accumulation and “browning” of
1193 adipose tissue by the short-chain fatty acid acetate. *International Journal of Obesity*, *40*(6), 955–963.
1194 https://doi.org/10.1038/ijo.2016.23

1195 Scott, K. P., Grimaldi, R., Cunningham, M., Sarbini, S. R., Wijeyesekera, A., Tang, M. L. K., Lee, J. C. Y.,
1196 Yau, Y. F., Ansell, J., Theis, S., Yang, K., Menon, R., Arfsten, J., Manurung, S., Gourineni, V., &
1197 Gibson, G. R. (2020). Developments in understanding and applying prebiotics in research and
1198 practice—an ISAPP conference paper. *Journal of Applied Microbiology*, *128*(4), 934–949.
1199 https://doi.org/10.1111/jam.14424

1200 Sepelev, I., & Galoburda, R. (2015). Industrial potato peel waste application in food production: A Review.
1201 *Research for Rural Development*, *1*, 130–136.

1202 Shahbandeh, M. (2022). *Potato industry - statistics & facts*. https://www.statista.com/topics/2379/potato-
1203 industry/#dossierKeyfigures

1204 Shani-Levi, C., Alvito, P., Andrés, A., Assunção, R., Barberá, R., Blanquet-Diot, S., Bourlieu, C.,
1205 Brodkorb, A., Cilla, A., Deglaire, A., Denis, S., Dupont, D., Heredia, A., Karakaya, S., Giosafatto,
1206 C. V. L., Mariniello, L., Martins, C., Ménard, O., El, S. N., … Lesmes, U. (2017). Extending in vitro
1207 digestion models to specific human populations: Perspectives, practical tools and bio-relevant
1208 information. *Trends in Food Science and Technology*, *60*, 52–63.
1209 https://doi.org/10.1016/j.tifs.2016.10.017

Journal Pre-proof

1210 Sharma, M., Patel, S. N., Lata, K., Singh, U., Krishania, M., Sangwan, R. S., & Singh, S. P. (2016). A novel
1211 approach of integrated bioprocessing of cane molasses for production of prebiotic and functional
1212 bioproducts. *Bioresource Technology*, *219*, 311–318. https://doi.org/10.1016/j.biortech.2016.07.131

1213 Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Faseleh Jahromi, M., & Ho, Y.
1214 W. (2014). Probiotic Potential of *Lactobacillus* Strains with Antimicrobial Activity against Some
1215 Human Pathogenic Strains. *BioMed Research International*, *2014*, 1–16.
1216 https://doi.org/10.1155/2014/927268

1217 Singh, A., & Eligar, S. M. (2021). Bioactive feruloylated xylooligosaccharides derived from Pearl millet
1218 (*Pennisetum glaucum*) bran with antiglycation and antioxidant properties. *Journal of Food*
1219 *Measurement and Characterization*, *15*(6), 5695–5706. https://doi.org/10.1007/s11694-021-01139-7

1220 Singh, R. D., Nadar, C. G., Muir, J., & Arora, A. (2019). Green and clean process to obtain low degree of
1221 polymerisation xylooligosaccharides from almond shell. *Journal of Cleaner Production*, *241*,
1222 118237. https://doi.org/10.1016/j.jclepro.2019.118237

1223 Smaali, I., Jazzar, S., Soussi, A., Muzard, M., Aubry, N., & Nejib Marzouki, M. (2012). Enzymatic
1224 synthesis of fructooligosaccharides from date by-products using an immobilized crude enzyme
1225 preparation of β-dfructofuranosidase from *Aspergillus awamori* NBRC 4033. *Biotechnology and*
1226 *Bioprocess Engineering*, *17*(2), 385–392. https://doi.org/10.1007/s12257-011-0388-9

1227 Song, T. S., Lee, K. S., Kang, S. B., Yoo, S. H., Lee, J. I., & Yoon, S. S. (2013). Synthesis of
1228 galactooligosaccharides in the cheese whey-based medium by a lactase from *Lactobacillus paracasei*
1229 YSM0308. *Korean Journal for Food Science of Animal Resources*, *33*(5), 565–571.
1230 https://doi.org/10.5851/kosfa.2013.33.5.565

1231 Song, Y. S., Lee, H. U., Park, C., & Kim, S. W. (2013). Optimization of lactulose synthesis from whey
1232 lactose by immobilized β-galactosidase and glucose isomerase. *Carbohydrate Research*, *369*, 1–5.
1233 https://doi.org/10.1016/j.carres.2013.01.002

1234 Wang, Q., Su, Y., Gu, Y., Lai, C., Ling, Z., & Yong, Q. (2022). Valorization of bamboo shoot shell waste
1235 for the coproduction of fermentable sugars and xylooligosaccharides. *Frontiers in Bioengineering*
1236 *and Biotechnology*, *10*, 1651. https://doi.org/10.3389/fbioe.2022.1006925

1237 Wang, Z. P., Wang, Q. Q., Liu, S., Liu, X. F., Yu, X. J., & Jiang, Y. L. (2019). Efficient Conversion of
1238 Cane Molasses Towards High-Purity Isomaltulose and Cellular Lipid Using an Engineered *Yarrowia*
1239 *lipolytica* Strain in Fed-Batch Fermentation. *Molecules*, *24*(7).
1240 https://doi.org/10.3390/molecules24071228

1241 Wilkowska, A., Nowak, A., Antczak-Chrobot, A., Motyl, I., Czy, A., & Paliwoda, A. (2019). Structurally
1242 Different Pectic Oligosaccharides Produced from Apple Pomace and Their Biological Activity *In*
1243 *Vitro*. *Foods*, *8*(9). https://doi.org/10.3390/foods8090365

1244 Wongsiridetchai, C., Chiangkham, W., Khlaihiran, N., Sawangwan, T., Wongwathanarat, P., Charoenrat,
1245 T., & Chantorn, S. (2018). Alkaline pretreatment of spent coffee grounds for oligosaccharides
1246 production by mannanase from *Bacillus* sp. GA2(1). *Agriculture and Natural Resources*, *52*(3), 222–
1247 227. https://doi.org/10.1016/j.anres.2018.09.012

1248 Wu, C., Zhang, T., Mu, W., Miao, M., & Jiang, B. (2015). Biosynthesis of lactosylfructoside by an
1249 intracellular levansucrase from *Bacillus methylotrophicus* SK 21.002. *Carbohydrate Research*, *401*,
1250 122–126. https://doi.org/10.1016/j.carres.2014.11.001

1251 Wu, L., Xu, C., Li, S., Liang, J., Xu, H., & Xu, Z. (2017). Efficient production of lactulose from whey
1252 powder by cellobiose 2-epimerase in an enzymatic membrane reactor. *Bioresource Technology*, *233*,
1253 305–312. https://doi.org/10.1016/j.biortech.2017.02.089

1254 Yañez-Ñeco, C. V., Cervantes, F. V., Amaya-Delgado, L., Ballesteros, A. O., Plou, F. J., & Arrizon, J.
1255 (2021). Synthesis of β(1→3) and β(1→6) galactooligosaccharides from lactose and whey using a
1256 recombinant β-galactosidase from *Pantoea anthophila*. *Electronic Journal of Biotechnology*, *49*, 14–
1257 21. https://doi.org/10.1016/j.ejbt.2020.10.004

1258 Zerva, A., Limnaios, A., Kritikou, A. S., Thomaidis, N. S., Taoukis, P., & Topakas, E. (2021). A novel
1259 thermophile β-galactosidase from *Thermothielavioides terrestris* producing galactooligosaccharides
1260 from acid whey. *New Biotechnology*, *63*, 45–53. https://doi.org/10.1016/j.nbt.2021.03.002

1261 Zhang, R., Li, X. Y., Cen, X. L., Gao, Q. H., Zhang, M., Li, K. Y., Wu, Q., Mu, Y. L., Tang, X. H., Zhou,
1262 J. P., & Huang, Z. X. (2021). Enzymatic preparation of manno-oligosaccharides from locust bean
1263 gum and palm kernel cake, and investigations into its prebiotic activity. *Electronic Journal of*
1264 *Biotechnology*, *49*, 64–71. https://doi.org/10.1016/j.ejbt.2020.11.001

1265 Zhang, S., Jiang, H., Xue, S., Ge, N., Sun, Y., Chi, Z., Liu, G., & Chi, Z. (2019). Efficient Conversion of
1266 Cane Molasses into Fructooligosaccharides by a Glucose Derepression Mutant of *Aureobasidium*
1267 *melanogenum* with High β-Fructofuranosidase Activity. *Journal of Agricultural and Food Chemistry*,
1268 *67*(49), 13665–13672. https://doi.org/10.1021/acs.jafc.9b05826

1269 Zhi-Peng, W., Lin-Lin, Z., Liu, S., Xiao-Yan, L., & Xin-Jun, Y. (2019). Whole conversion of soybean
1270 molasses into isomaltulose and ethanol by combining enzymatic hydrolysis and successive selective
1271 fermentations. *Biomolecules*, *9*(8). https://doi.org/10.3390/biom9080353

1272 Zidan, D., Sabran, M. R., Ramli, N. S., Shafie, S. R., & Fikry, M. (2021). Prebiotic properties of
1273 xylooligosaccharide extracted from sugarcane wastes (pith and rind): a comparative study.
1274 *International Journal of Food Science and Technology*, *56*(5), 2175–2181.
1275 https://doi.org/10.1111/ijfs.14956

1276

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1 # Tables

2

3 **Table 1.** Examples of enzymatic production of fructo-oligosaccharides from agro-industrial wastes.

Source(s)	Microorganism(s)	Enzyme(s)	Pre-treatment	Reaction Type and Conditions	Main Results	Reference
Sugar cane bagasse (SCB)	*Aspergillus flavus* NFCCI 2364	Fructosyltransferase	(i) SCB was washed with distilled water; (ii) Blenched by immersing in hot water (70−80 °C, for 10 min); (iii) Material was dried overnight and further grinded	SSF – Two stages: (i) 5 g substrate and 0.1 g/gds yeast extract were added to 5 mL water, and 1 × 10^7 spores were inoculated, for 72 h; (ii) 50 mL water was added to the fermented medium, for 24 h at 150 rpm; 28 °C, and pH 4.8	73.42 % (w/w)	(Ganaie et al., 2017)
Aguamiel	*Aspergillus oryzae* DIA-MF	Fructosyltransferase	Aguamiel was sterilized at 121 °C for 15 min	SSF: Polyurethane foam was used as inert support; aguamiel with 35–40 g/L sucrose content was inoculated with 2 × 10^7 spores/g of support; 30 °C, pH 5.0, for 120 min	0.30 (w/w); 0.097 g/(L·min)	(Muñiz-Márquez et al., 2016)
Coffee silverskin (CS)	*Aspergillus japonicus* ATCC 20236	β-fructofuranosidase	CS was sterilized at 121 °C for 20 min	SSF: 3 g of CS (moistened with a 200 g/L sucrose solution to attain 70 % moisture content), and of 2 × 10^6 spores/g of inoculum; 28 °C	128.7 g/L; 0.70 ± 0.04 (w/w); 8.05 ± 0.49 g/(L·h)	(Mussatto & Teixeira, 2010)
Coffee silverskin (CS)	*Aspergillus japonicus* ATCC 20236	β-fructofuranosidase	CS was sterilized at 121 °C for 20 min	SSF: 2.5 g of CS (moistened with a 240 g/L sucrose solution to attain 60 % moisture content), and 1.7 × 10^7 spores/g of inoculum; 28 °C	206 g/L; 10.44 g/(L·h)	(Mussatto et al., 2013)
Date by-products	*Aspergillus awamori* NBRC4033	β-fructofuranosidase	(i) Fruit flesh was collected and cut into small pieces; (ii) Aqueous extracts were obtained by diffusion in hot water	SmF: 1 g of enzyme per 10 mL of substrate (containing 403.5 g/L sucrose); 50 °C, pH 5.0, for 1 h	123 g/L; 53.26 % (w/w); 18.5 g/(h·100 g)	(Smaali et al., 2012)
Tofu whey permeate (TWP)	*Aspergillus aculeatus*	Pectinex® Ultra SP-L	(i) TWP was centrifuged at 4,000×g for 90 min; (ii) Supernatant was ultrafiltered through hydrophilic membranes (removal of proteins)	SmF: 60 % (w/v) TWP, and 9 U/mL of enzyme; 60 °C, pH 5.5, 1350 rpm, for 8 h	GF_2: 37.0 ± 1.0 g/L; GF_3: 45.6 ± 1.1 g/L; and GF_4: 5.4 ± 0.1 g/L.	(Corzo-Martínez et al., 2016)
Cane molasses (CM)	*Aureobasidium melanogenum* D28	β-fructofuranosidase	(i) 1 mM of EDTA solution was added to the CM solution to bind heavy metals; (ii) Treated CM was centrifuged at 12,000×g for 0.5 h; (iii) Color for the supernatant was removed by adding activated charcoal	SmF: 375 U/g of enzyme and 35 % (w/v) molasses sugar; 50 °C, pH 4.5, and 100 rpm	0.58 (w/w)	(S. Zhang et al., 2019)

4 GF_2 – 1-Kestose; GF_3 − Nystose; GF_4 −1^F-fructofuranosylnystose; SSF − Solid-state fermentation; SmF − Submerged fermentation.

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5 **Table 2.** Examples of enzymatic production of lactose-derivated prebiotics from agro-industrial wastes.

Prebiotic Produced	Source(s)	Microorganism(s)	Enzyme(s)	Pre-treatment	Reaction Type and Conditions	Results	Reference
GalOS	Whey permeate (WP)	*Streptococcus thermophilus* DSM 20259	β-Galactosidase (expressed in *Lactobacillus plantarum* WCFS1)	WP powder (containing 65 % (w/w) lactose) was dissolved in 50 mM sodium phosphate buffer with 10 mM $MgCl_2$	SmF: WP solution (equivalent to 50 g/L lactose) and 1.35 U/mL of enzyme; 37 °C and pH 6.5	34.2 % (w/w); β-(1,6) and β-(1,3)-linked	(Geiger et al., 2016)
	Whey permeate (WP)	*Kluyveromyces lactis*	Lactozyme™ 2600 L	5.4 g whey powder was dissolved in 12.6 g of 200 mM potassium phosphate	SmF: WP with 30 % (w/w) lactose concentration and 2 g of enzyme solution (50 U/g); 35 °C, pH 7.0, 200 rpm, for 12 h	25 % (w/w); 51 g/(g_{enzyme}·h)	(Mano et al., 2019)
	Acid whey	*Thermothielavioides terrestris*	β-galactosidase (expressed in *Pichia pastoris*)	An evaporator was used for the concentration of whey lactose (52 °C and 120 rpm)	SmF: 9.3 % (w/v) lactose (concentrated whey), and 0.5 U/mL of enzyme; 50 °C, for 5 h	14.9 ± 0.08 g/L; 1.48 ± 0.08 % (w/v); 0.17 ± 0.04 (w/(v·h))	(Zerva et al., 2021)
	Acid whey	*Cryptococcus laurentii* DSM 27153	β-galactosidase	—	SmF: enzyme/substrate ratio of 0.94 U_{oNPG}/g; 55 °C, and no pH adjustment	NCW: 34.6 ± 0.4 % (w/w) and 2.3 mg/(U·h); CW: 36.1 ± 0.6 % (w/w) and 2.3 mg/(U·h)	(Fischer & Kleinschmidt, 2021)
	Sweet whey	*Lactobacillus paracasei* YSM0308	β-galactosidase	(i) Whey powder was reconstituted at 10 % (w/v); (ii) Proteins from the whey were partially digested by protease	SmF: 30 % (w/v) sweet whey and crude enzyme; 30 °C, pH 6.5−7.0, for 4 h	19.41 % (w/v)	(T. S. Song et al., 2013)
	Sweet whey	*Pantoea anthophila*	β-galactosidase (expressed in *Escherichia coli*)	Whey powder was dissolved in a 50 mM sodium phosphate	SmF: 15 U/mL of enzyme, and 300 g/L lactose (whey concentrate equivalent); 50 °C, pH 7.0, and 150 rpm	38 % (w/w)	(Yañez-Ñeco et al., 2021)
	Porungo cheese whey (PCW)	*Kluyveromyces lactis*	Maxilact LGi 5000	(i) PCW was diluted in 0.1 M sodium phosphate buffer; (ii) Proteins from the whey were partially digested by a protease	SmF: 8 mL of immobilized enzyme and 20 mL of PCW; 37 °C, pH 7.0, 150 rpm, for 180 min	63.1 % (w/w); 13.6 g/(h·L)	(Bolognesi et al., 2021)
	Milk whey (MW) and Milk whey permeate (MWP)	*Bacillus circulans*	β-galactosidase	—	SmF: 5 mL of substrate (with a lactose concentration equivalent to 40 % (w/v)), and 100 beads of immobilized enzyme; 50 °C, and pH 7	159.4 g/L for MW; 168.8 g/L for MWP	(Hackenhaar et al., 2021)

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Lactosucrose	Tofu whey (TW)	*Bacillus subtilis* CECT 39	Levansucrase	—	SmF: 0.5 U/mL enzyme; TW:Lac ratio of 47.3 %:12.5 %; 37 °C, pH 6.0, for 120 min	74 g/L; 64.9 % (w/w); 37.0 g/(L·h)	(Corzo-Martinez et al., 2015)
	Tofu whey (TWP) and Cheese whey permeate (CWP)	*Bacillus subtilis* CECT 39	Levansucrase	—	SmF: 0.5 U/mL enzyme; TW:CWP ratio of 47.3 %:13.9 %; 37 °C, pH 6.0, for 120 min	71.5 g/L; 60.8 % (w/w); 35.8 g/(L·h)	(Corzo-Martinez et al., 2015)
Lactulose	Cheese whey powder	*Caldicellulosiruptor saccharolyticus*	Cellobiose 2-epimerase (expressed in *Bacillus subtilis* WB800)	(i) Spray-dried powder was added to phosphate buffer (pH 7.0) and mixed; (ii) Mixture was centrifuged at 8,000×g for 20 min	SmF: 3 L of whey powder solution and 7.5 U/mL of enzyme; 70 °C, pH 7.0, for 2 h	58.5 % (w/w) after 2 h; 42.4 % (w/w) after 10 batches (EMR)	(L. Wu et al., 2017)
	Whey powder	*Kluyveromyces lactis* and *Streptomyces rubiginosus*	β-galactosidase and Glucose isomerase	(i) Whey powder was added to sodium phosphate buffer (pH 7.5) and was mixed; (ii) Mixture was centrifuged at 13,000×g for 20 min, at 25 °C	SmF: 20 % (w/v) of whey preparation, 12 U/mL of immobilized β-galactosidase, and 60 U/mL of immobilized glucose isomerase; 53.5 °C, and pH 7.5	7.68 g/L; 0.32 mg/(U·h)	(Y. S. Song et al., 2013)

6 GalOS – Galacto-oligosaccharides; SSF – Solid-state fermentation; SmF – Submerged fermentation.

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8 **Table 3.** Examples of enzymatic production of xylo-oligosaccharides from agro-industrial wastes.

Source(s)	Microorganism(s)	Enzyme(s)	Pre-treatment	Reaction Type and Conditions	Results	Reference
Garlic straw (GS)	*Bacillus mojavensis* UEB-FK	Xylanase	(i) Xylan from GS was recovered using alkali extraction (15 % NaOH 1M, 90 °C, 90 min); (ii) Xylan was precipitated in ethanol and separated by filtration. The precipitate was dissolved in distilled water, dialyzed, and lyophilized. (iii) The purified mixture was dissolved in 50 mM sodium acetate buffer (pH 4.0)	SmF: 2 % (w/v) of the xylan mixture and 12 U/g of enzyme; 50 °C, pH 4.0, 100 rpm, for 8 h	DP ≤ 6: 29 ± 1.74 % (w/w)	(Kallel et al., 2015)
Pearl millet bran (PMB)	*Bacillus licheniformis, Bacillus licheniformis, Aspergillus niger* and *Trichoderma viride*	α-amylase (Termamyl® 120L), protease, amyloglucosidase and xylanase	(i) The defatted PMB was autoclaved for 45 min at 121 °C; (ii) Material was de-starched and deproteinized; (iii) The precipitated material was recovered for further digestion	SmF: Precipitated residue of PMB, and 10 U/g xylanase, at 30 °C, pH 4.5, 200 rpm, for 24 h; Reaction terminated by adding three volumes of EtOH 95 %; Samples were centrifuged at 8000 rpm (4 °C), for 20 min	1.4 % g_{FXOS} and 7.2 % g_{XOS} per g of starting material	(A. Singh & Eligar, 2021)
Bamboo shoot shell (BSS)	—	*Endo*-xylanase	(i) BSS autohydrolysis (100 mL water and 10.0 g of dry BSS) at 170 °C, 150 rpm, for 50 min in an oil bath; (iii) The reactor was soaked in a cold-water bath for 4 h; (iii) The pre-hydrolysate was separated by filtration and stored at 4 °C	SmF: 15 % (v/v) endo-xylanase (3 IU/mL), at 50 °C, 150 rpm, for 12 h; Enzymes were heat inactivated (100 °C, 5 min) and the aliquots were kept at 4 °C	6.6 g_{XOS} per 100 g_{BSS}: 76.7 % of X_2 and X_3	(Q. Wang et al., 2022)
Sugarcane straw (SS)	*Aspergillus niger* and *Clostridium thermocellum*	*Endo*-xylanase (GH11), α-L-arabinofuranosidase (GH51), and Feruloyl Esterase (CE1)	(i) SS was pre-incubated (70 °C, 16 h) in deionized water, and further drained; (ii) Holocellulose was extracted with sodium chlorite under acidic conditions; (iii) The mixture was filtered and precipitated. The precipitated material was centrifuged at 4,000×g for 15 min, washed and dried	SmF: 0.63 mg/g of CE1, 5 mg/g of GH11, 2.70 mg/g of GH5, and 50 mg of substrate; 50 °C, pH 5.0, and 1000 rpm	DP ≤ 6: 10.23 ± 0.56 g/L; 205 mg/g; 4.27 mg/(g·h)	(Ávila et al., 2020)
Coffee husk (CH)	*Aspergillus niger* and *Clostridium thermocellum*	*Endo*-xylanase (GH11), α-L-arabinofuranosidase (GH51), and Feruloyl Esterase (CE1)	(i) CH was pre-incubated (70 °C, 16 h) in deionized water, and further drained; (ii) Holocellulose was extracted with sodium chlorite under acidic conditions; (iii) The mixture was filtered and precipitated. The precipitated material was centrifuged at 4,000×g for 15 min, washed and dried	SmF: 0.63 mg/g of CE1, 6 mg/g of GH11, 0.80 mg/g of GH51, and 50 mg of substrate; 50 °C, pH 5.0, and 1000 rpm	DP ≤ 6: 8.45 ± 0.65 g/L; 169 mg/g; 3.52 mg/(g·h)	(Ávila et al., 2020)
Grape pomace (GP)	*Aspergillus niger* 3T5B8	Xylanase	GP was dried (45 °C for 24) and milled	SmF: 100 mg of substrate with a substrate:water ratio of 1:18, and 10 IU/g of enzyme; 40 °C, pH 5.0, 200 rpm, for 6 h	88.68 ± 0.12 %	(Costa et al., 2019)

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Grape pomace (GP)	*Aspergillus aculeatus*	Viscozyme® L	GP was dried (45 °C for 24) and milled	SmF: 100 mg of the substrate with a substrate:water ratio of 1:18, and 10 IU/g of enzyme; 40 °C, pH 5.0, 200 rpm, for 4 h	84.09 ± 2.40 %	(Costa et al., 2019)
Coffee peel (CP)	*Bacillus subtilis*	*Endo*-xylanase	(i) CP was grounded and sieved; (ii) The powder was extracted and stored	SmF: 0.8 % (w/v) substrate solution, 4.53 U/mg of enzyme, with an enzyme:substrate ratio of 1:1; 40 °C, for 24 h	3.26 mg/mL X_5	(Ratnadewi et al., 2020)
Coconut husk (CH)	—	Crude xylanase preparation	(i) CH was shredded dried; (ii) The dried husk was ground into powder; (iii) Alkali treatment (20 % NaOH) coupled with steam treatment for 60 min	SmF: 2 % of solubilized xylan in 10 mL of sodium citrate buffer, and 4.50 % of crude xylanase; 55 °C, pH 5.0, for 18 h	1.69 mg/mL X_2	(Jnawali et al., 2018)
Almond shell (AS)	*Thermomyces lanuginosus*	*Endo*-xylanase (expressed in *Aspergillus oryzae*)	(i) Autohydrolysis pretreatment of AS powder (200 °C, 5 min); (ii) The mixture was filtered; (iii) The autohydrolysate was centrifuged at 5,000 rpm for 10 min. The supernatant was stored at 4 ºC	SmF: Material was loaded into the reactor at a 1:10 (w/v) ratio, with 10 U of enzyme; 50 °C, pH 5.5, for 36 h	5.3 ± 0.1 % (w/w) X_2; 3.0 ± 0.1 % (w/w) X_3	(Singh et al., 2019)
Rice husk (RH)	*Thermomyces lanuginosus*	Pentopan™ MonoBG	(i) Material was dried, powdered, and sieved; (ii) Alkaline treatment coupled with steam; (iii) The treated solution was centrifuged at 5,000 rpm for 20 min. The supernatant was acidified (pH 5.0); (iv) Xylan fraction was recovered after precipitation with ethanol and centrifuged at 4,480×g for 10 min. The xylan precipitate was collected and dried	SmF: 5 % of xylan solution, and 6.25 mg of enzyme per g of xylan; 50 °C, pH 6.0, for 9 h	17.35 ± 0.31 mg/mL	(Khat-udomkiri et al., 2018)
Wheat husk (WH)	*Aspergillus fumigatus* R1	Xylanase	Dried WH was dissolved in 50 mM phosphate buffer (pH 7.0)	SmF: 2 % (w/v) wheat husk xylan, and 42 IU/g of enzyme; 37 °C, pH 7.0, 100 rpm, for 6 h	1.08 g/(100 g)	(Jagtap et al., 2017)
Wheat straw (WS)	—	*Endo*-xylanase	(i) WS was milled; (ii) Hydrothermal pre-treatment (Steam explosion, 200 °C) was applied; (iii) Slurry was filtrated and the liquid fraction was recovered	SmF: 7.2 U of enzyme per mL of liquid fraction; 50 °C, pH 4.8, and 150 rpm	87.4 % (w/w): 59.6 % X_2, 30.7 % X_3, 6.8 % X_4, 1.9 % X_5 and 1.0 % X_6	(Álvarez et al., 2017)
Wheat straw (WS)	—	*Endo*-xylanase and β-glucosidase	(i) WS was milled; (ii) Hydrothermal pre-treatment (Steam explosion, 200 °C) was applied; (iii) Slurry was filtrated and the liquid fraction was recovered	SmF: 7.2 U of endo -xylanase and 1.2 U of β-glucosidase enzyme per mL of liquid fraction; 50 °C, pH 4.8, and 150 rpm	90.6 % (w/w): 44.4 % X_2, 32.0 % X_3, 10.7 %) X_4, 2.4 % X_5 and 1.2 % X_6; 8.9 g/(100 g)	(Álvarez et al., 2017)

Sugarcane bagasse (SB)	*Trichoderma viridae*	*Endo*-xylanase	(i) Xylan from SB was alkali extracted (12 % NaOH) combined with steam; (ii) The soluble xylan was recovered and dried	SmF: 2.65 U of enzyme, and 2 % (w/v) substrate concentration; 40 °C, pH 4.0, for 8 h	1.153 ± 0.13 mg/mL X_2; 0.565 ± 0.21 mg/mL X_3	(Jayapal et al., 2013)
Corncob (CC)	*Aspergillus oryzae* MTCC 5154	*Endo*-xylanase	(i) CC was grounded and sieved; (ii) CC powder was pretreated by alkali treatment; (iii) Treated CC powder was dried	SmF: 14 U/mL of endo-xylanase, and 6 % corncob powder; 50 °C, pH 5.4, for 14 h.	10.2 ± 0.14 mg/mL; 81.0 ± 3.9 % (w/v)	(Aachary & Prapulla, 2009)
Corncob (CC)	*Paenibacillus barengoltzii* CAU904	Xylanase (expressed in *Escherichia coli*)	(i) Dry CC was powdered and soaked in water; (ii) The mixture was pretreated by a steam explosion at 165 °C for 35 min	SmF: 50 U/mL of enzyme was added into 25 mL of treated CC mixture; 60 °C, pH 6.5, 150 rpm, for 4 h	75 % (w/w); 90 % DP 2−4	(Liu et al., 2018)

9 X_2 – Xylobiose; X_3 – Xylotriose; X_4 – Xylotetraose; X_5 – Xylopentose; X_6 – Xylohexose; FXOS – Feruloylated xylo-oligosaccharides; SSF − Solid-state fermentation; SmF −
10 Submerged fermentation.

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13 **Table 4.** Examples of enzymatic production of manno-oligosaccharides, isomalto-oligosaccharides, and pectic oligosaccharides from agro-industrial wastes.

Prebiotic Produced	Source(s)	Microorganism(s)	Enzyme(s)	Pre-treatment	Reaction Type and Conditions	Results	Reference
MOS	Guar gum (GG)	*Aspergillus oryzae* MTCC 1846	β-mannanase (ManAo)	—	SmF: 100 mL of 2 % (w/v) waste, and 0.5 U/mL enzyme; 50 °C, pH 5.0, 150 rpm, for 12 h	DP 2−4; 11.24 mg/mL	(Jana & Kango, 2020)
	Copra meal (CM)	*Aspergillus oryzae* MTCC 1846	β-mannanase (ManAo)	—	SmF: 100 mL of 2 % (w/v) waste, and 0.5 U/mL of enzyme; 50 °C, pH 5.0, 150 rpm, for 12 h	DP 2−4; 7.14 mg/mL	(Jana & Kango, 2020)
	Copra meal (CM), and Coffee residue (CR)	*Penicillium oxalicum* KUB-SN2-1	β-mannanase	Wastes were dried (60 °C, for 48 h), blended, milled, and sieved	SmF: 80 mL of crude enzyme (36.01 ± 0.13 U/mL), and 80 mL of waste solution (10 mM citrate-phosphate buffer); 60 °C, pH 4.0, for 48 h	DP 2−4	(Chantorn et al., 2018)
	Locust bean gum (LBG) and palm kernel cake (PKC)	*Bacillus* sp. HJ14, and *Sphingomonas* sp. JB13	β-mannanase (Man5HJ14 and ManAJB13)	(i) PKC (50 g) was pre-treated with 1 % (w/v) NaOH solution (121 °C,15 lbs pressure, for 20 min); (ii) Biomass was washed with dilute HCl, harvested and dried	SmF: 0.5 % (w/v) LBG or 1:5 PKC solution, and 10 U/mL of enzyme; 60 °C (Man5HJ14) or 37 °C (ManAJB13), pH 6.5, for 8 h	DP 2−6 from LBG; DP 2−4 from PKC	(R. Zhang et al., 2021)
	Spent coffee grounds (SCGs)	*Bacillus* sp. GA2(1)	β-mannanase	(i) The SCGs were dried (60 °C, for 24 h) and sieved; (ii) Biomass was subject to an alkaline pretreatment with 0.5 N NaOH (50 °C, for 6 h)	SmF: 5 mL of the crude enzyme, 0.05 g treated SCGs, and 5 mL of 50 mM citrate-phosphate buffer; 50 °C, pH 6.5, for 5 h	DP 2−3	(Wongsiridetchai et al., 2018)
	Spent coffee grounds (SCGs)	*Aureobasidium pullulans* NRRL 58524	β-mannanase	(i) SCG was washed, dried at 60 °C, sieved (60-mesh size), and stored in vacuum desiccator; (ii) Alkali pretreatment was performed by incubation of SCG with 4 M sodium hydroxide and 0.02 M sodium borohydride (liquid:solid ratio of 10:1) at room temperature, overnight; (iv) The mixture was centrifuged at 4,000×g, for 15 min, filtered and acidified to pH 5.0 (1 M acetic acid). The filtrate was concentrated by ultrafiltration, and freeze-dried at -60 °C, for 6 h; (v) Extracted mannan was dissolved in 50 mM citrate buffer (pH 4.0) to obtain the final concentration of 1% (w/v)	SmF: 84.87 U/g of enzyme, at 55 °C, pH 4.0, 150 rpm, for 41 h and 34 min; Reaction was terminated by boiling the mixture for 15 min	58.22 ± 2.04 mgMOS/(100 mg): 16.27 ± 0.84 M2 and 2.85 ± 0.20 mg M3	(Ibrahim et al., 2022)

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	Guar gum (GG), and Locust bean gum (LBG)	*Aspergillus niger* ATCC 10864	*Endo*-mannanase (Man26A)	—	SmF: 2 % (w/v) mannans (LBG, and GG) were suspended in 10 mL of 50 mM citrate buffer, and the enzyme was loaded at a concentration of 0.625 mg/g; 50 °C, pH 5.0, 70 rpm, for 24 h	DP 2−6; 5.11 mg/mL from LBG, and 4.45 mg/mL from GG	(Magengelele et al., 2021)
	Sugar palm fruit (SPF)	*Kitasatospora* sp. KY576672	β-mannanase	(i) SPF was sliced, dried (40−50 °C), and crushed; (ii) The powder was sieved; (iii) The fraction was diluted with 50 mM phosphate buffer	SmF: 200 mL SPF solution, and 200 mL crude enzyme; 40 °C, pH 6.0, 190 rpm	DP 2−6	(Pangestu et al., 2019)
	Locust bean gum (LBG), konjac glucomannan (KG), and Guar gum (GG)	*Penicillium aculeatum* APS1	β-mannanase	—	SmF: 1 % substrate and 100 U/g crude enzyme in 50 mM sodium citrate buffer; 50 °C, pH 5.3, for 3 h	DP 2−4: 1.364 mg/mL from LBG, and 4.626 mg/mL from KG; DP 2−3: 2.958 mg/mL from GG	(Bangoria et al., 2021)
	Palm cake kernel (PKC)	*Streptomyces cyaenus*	β-mannanase	—	SmF: Enzyme (1.706 U/mL) solution was reacted with 0.5 % PKC substrate (1:1); 37 °C, pH 6.0, 150 rpm, for 8 h	DP ≤ 7	(Purnawan et al., 2017)
IMOs	Cane molasses (PCM), and Corn steep liquor (CSL)	*Yarrowia lipolytica* S47	Sucrose Isomerase (SIase)	—	SmF: 3.6 U/mL enzyme, 350 g/L PCM, 1.0 g/L CSL; 30 °C, pH 6.0	102.6 g/L isomaltulose, 97.4 % purity, 0.96 (w/w)	(Z. P. Wang et al., 2019)
	Pulp of plantain fruits (PPF)	—	β-amylase, pullulanase and α-glucosidase	(i) Rejected plantain fruits were disinfected and peeled; (ii) The pulp was cut into slices and immersed in a 5 % (w/v) ascorbic acid solution for 5 min; (iii) The slices were dehydrated (45 °C for 48 h), grounded and sieved. The plantain flour (PF) was stored under vacuum in laminated multilayer bags	SmF – Three stages: (i) 0.15 % (w/w) α-amylase and 30 % (w/v) substrate, for 3 h, (ii) 0.1 % (w/w) β-amylase and 0.05 % (w/w) pullulanase, for 24 h, and (iii) 0.1 % (w/w) α-glucosidase, for 24 h; pH and temperature are adjusted to the optimal conditions of the enzymes in each stage; 150 rpm	24.48 g: 7.49 g IM_2, 6.20 g IM_3, 5.58 g IM_4 and 5.21 g IM_5	(J. A. Gómez et al., 2021)
	Soybean molasses (SM)	*Rhizomucor miehei*	α-galactosidase (expressed in *Yarrowia lipolytica* S47)	Viscous substances were removed from soy molasses using H_2SO_4 and $Ca(OH)_2$	SmF – Three stages: (i) 700 g/L soy molasses hydrolysate (SMH), 200 mL strain culture, for 32 h, (ii) 700 g/L SMH added, for 16 h, and (iii) 300 g/L SMH added, for 24 h; 30 °C, pH	209.4 g/L isomaltulose, 0.95 (w/w)	(Zhi-Peng et al., 2019)

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					6.5, 300 rpm, aeration rate of 5 L/min		
	Broken rice (BR)	*Aspergillus niger* PFS08	Fungamyl® 800L and α-glucosidase	(i) Rice grains were milled and sieved; (ii) Powder was stored in an airtight plastic container; (iii) Samples were mixed with 0.7 M HCl and neutralized with 5 M NaOH (pH 7.0)	SmF: 0.06 U of Fungamyl® 800L and 1.05 U of α-glucosidase were added together to a total reaction volume of 5 mL; 55 °C, pH 5.0, 12 h	92.17 ± 3.43 g/L	(Basu et al., 2016)
	Potato processing waste (PPW)	*Aspergillus niger* PFS08	Fungamyl® 800L and α-glucosidase	(i) The solid in the waste was allowed to settle, and the sediments were collected and washed; (ii) Sediments were dried and sieved. The starch powder was stored in an airtight plastic container; (iii) Samples were mixed with 0.7 M HCl and neutralized with 5 M NaOH (pH 7.0)	SmF: 0.06 U of Fungamyl® 800L and 1.05 U of α-glucosidase were added together to a total reaction volume of 5 mL; 55 °C, pH 5.0, 12 h	85.11 ± 4.30 g/L	(Basu et al., 2016)
	Banana slurries (BS)	—	Termamyl® SC, Fungamyl® 800 L and Transglucosidase L	(i) Banana was dried and sieved; (ii) Banana slurry with a concentration of 250 g/kg was prepared	SmF – Three stages: (i) 0.15 mL Termamyl® SC and 500 g banana slurry, 93–95 °C, pH 5.5−6.0, (ii) 0.3 mL Fungamyl® 800 L, 50 °C, pH 5.5−6.0, for 24 h, and (iii) 0.3 mL Transglucosidase L, 60 °C, pH 5.5, for 12 h	76.67 ± 2.71 g/L: 57.66 ± 2.60 IM_3; 19.11 ± 1.24 IM_4	(Chockchaisawasdee & Poosaran, 2013)
Pectic Oligosaccharides	Onion skins (OS)	*Aspergillus aculeatus*	Viscozyme® L	(i) OS were grinded; (ii) Pectin was extracted with 2 % sodium hexametaphosphate solution (95 °C, 0.5 h); (iii) The biomass was centrifuged at 4,500×g for 10 min. The crude pectin was collected from the supernatant	SmF: 50 g/L substrate and 60 mL of enzyme (41.4 U/mL); 45 °C, pH 4.5, 200 rpm, for 15−30 min	22.0 g/(L·h); 57 % (w/w)	(Baldassarre et al., 2018)
	Onion skins (OS)	*Aspergillus aculeatus*	*Endo*-polygalacturonase M2	(i) Dried OS were milled and sieved; (ii) Powder was pretreated with 2 % sodium hexametaphosphate (95 °C, 0.5 h); (iii) Biomass was centrifuged at 5,000×g for 10 min.The crude pectin was collected from the supernatant	SmF: 10 % (v/v) of the diluted enzyme/pectic solution (accounting to 5.2 U/mL enzyme); 45 °C, 150 rpm, for 2 h	2.5–3.0 % (w/w) DP2; 5.5–5.6 % (w/w) DP3; and 5.2 to 5.5 % (w/w) DP4	(Babbar, Baldassarre, et al., 2016)
	Sugar beet (SB)	—	*Endo*-polygalacturonase M2 or Rapidase Smart®	(i) SB was suspended in a solution with HCl (ratio of 1:29), heated, and stirred; (ii) The macerate was rapidly cooled and filtered; (iii) pH was adjusted with 0.2 M KOH; (iv) The extract was dispersed into ethanol; (v) Pectin gel was washed, hand-squeezed in nylon cloth, dried and finely grounded	SmF: 0.5 % (w/v) pectin, and 20 µL of each enzyme; 50 °C, pH 5.0, for 15 min	3.1 % (w/w) DP2; and 3.7 % (w/w) DP3	(Combo et al., 2013)

	Orange peel waste (OPW)	*Trichoderma reesei* and *Aspergillus aculeatus*	Celluclast® 1.5L and Viscozyme® L	(i) OPWs were milled; (ii) Samples were mixed with water and stirred; (iii) Solids were recovered by centrifugation and extracted with water two additional times	SmF: 12 (w/w) liquor to solid ratio, 45 U/g of Viscozyme® L, and 5 FPU/g of Celluclast® 1.5L; 37 °C, pH 5, 150 rpm, for 20 h	31.3 kg/(100 kg): 7.5 kg GlcOS, 4.5 kg GalOS, 6.3 kg AraOS and 13 kg OGalA	(Sabajanes et al., 2012)

14 MOS – Manno-oligosaccharides; M_2 – Mannobiose; M_3 – Mannotriose; IMOs – Isomalto-oligosaccharides; IM_2 − Isomaltose; IM_3 − Isomaltotriose; IM_4 − Isomaltotetraose;
15 IM_5 – Isomaltopentaose; GlcOS – Gluco-oligosaccharides; OGalA – Oligo-galacturonides; AraOS – Arabino-oligosaccharides; GalOS – Galacto-oligosaccharides; SSF − Solid-
16 state fermentation; SmF − Submerged fermentation.

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18 **Table 5.** *In vitro* evaluation of gastrointestinal digestibility of several prebiotic ingredients enzymatically produced from agro-industrial wastes.

Waste used as raw material	Prebiotic produced	Main findings	Reference
Cane molasses	Prebiotic oligosaccharides	Hydrolysis of 8−10 % by α-amylase and 10−15 % by gastric juice (3,6 and 24 h incubation in each enzyme solution).	(Sharma et al., 2016)
Sugarcane pith and rind	XOS	Hydrolysis of 5.21 % of pith XOS and 12.66 % of rind XOS by α-amylase (6 h incubation). Hydrolysis of 2.03 % of pith XOS and 3.22 % of rind XOS under gastric simulation (6 h incubation).	(Zidan et al., 2021)
Sugarcane straw and coffee husks	XOS	Hydrolysis of 4.55 % for sugarcane XOS and 5.62 % for coffee husk XOS at the end of the intestinal phase.	(Ávila et al., 2020)
Rejected unripe plantain fruits	IMOs	The resistant starch content after small intestinal digestion of raw plantain flour was higher (71.7 %) than in the cooked flour (52.6 %).	(J. A. Gómez et al., 2021)

19 IMOs − Isomalto-oligosaccharides; XOS − Xylo-oligosaccharides.
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21 **Table 6.** *In vivo* and *in vitro* gut fermentation of several prebiotics enzymatically produced from agro-industrial wastes.

Type of test	Source of prebiotic	Prebiotic produced	Dose tested	Main findings	Reference
Animal studies					
27 mice divided into 9 groups: 3 control groups, 3 low-dose groups and 3 high-dose groups for 14, 21 and 28 days	Cassava dregs	XOS	Low-dose groups: 0.5 g/kg of BW High-dose groups: 1.0 g/kg of BW	Feeding with XOS at 0.5 and 1.0 g/kg of BW increased *Bifidobacterium* and *Lactobacillus* and decreased *Escherichia.coli*. SCFA such as acetic and butyric acid were produced. A high acidic pH was determined in the treatment group.	(Hafidah et al., 2018)
24 rats divided in two groups: 1 experimental and 1 control group	Apple by-products	Dietary fibre	20 % of dietary fiber	The ingestion of a diet supplemented with dietary fiber for 5 weeks showed a potential bifidogenic, butyrogenic, and hypolipidemic effect.	(Mateos-Aparicio et al., 2020)
***In vitro* studies**					
Fermentation with selected gut bacteria	Palm kernel cake, locust bean gum, and copra meal	MOS	1 mg/mL of medium	Copra meal hydrolysate obtained the highest growth of *Lactobacillus delbrueckii* and *L. acidophilus* among all the mannans.	(Jana & Kango, 2020)
	Locust bean gum and palm kernel cake	MOS	0.5 % (w/v)	*Lactobacillus plantarum* could utilize M_1, M_2, and M_3, but not M_4 and above.	(R. Zhang et al., 2021)
	Spent tea leaves	Prebiotic oligosaccharides	150, 300, 400, 600, and 700 µg/mL	Oligosaccharides obtained from spent tea leaves inhibited the growth of pathogens and support the growth of beneficial bacteria. At higher concentrations, the effect was enhanced.	(Chimtong et al., 2016)
	Sugarcane straw and coffee husks	XOS	0.35 % (w/v)	Three of four probiotic cultures of *Lactobacillus* and *Bifidobacterium* tested were able to utilize the XOS and showed remarkable growth consuming preferentially the X_2 and X_3 fractions as the sole carbon source.	(Ávila et al., 2020)
	Wheat husk	XOS	0.35 % (w/v)	Six of ten probiotic cultures tested were able to utilize the XOS with a remarkable growth in the media containing XOS as only carbon source.	(Jagtap et al., 2017)
	Coffee peel	XOS	0, 10, 20 and 30 % (v/v) of XOS solution	Among the four concentrations tested (0, 10, 20 and 30 %) 20 % XOS stimulated the highest growth (8.75 log CFU/mL) of *Lactobacillus casei* after 12 h fermentation.	(Ratnadewi et al., 2020)
	Sugarcane pith and rind	XOS	1 % (w/v)	*Lactobacillus casei Shirota* (LcS) and *Bifidobacterium animalis* subsp. *Lactis* significantly grew in both XOS sources after 48 h of incubation. XOS obtained from rind promoted a higher growth. Both XOS were found to be more fermentable by LcS. Acetic acid was the main fermentation end-product.	(Zidan et al., 2021)

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	Banana pseudostem	XOS	N.D.	The medium enriched with XOS stimulated the growth of the prebiotics *Lactobacillus plantarum* and *Lactobacillus fermentum*. Probiotic growth continued increasing after 48 h, while in the control (medium with glucose) it remained stationary by that time.	(de Freitas et al., 2021)
Fermentation with human stool	Lemon peel wastes and sugar beet pulp	Pectins and POS	N.D.	Populations of bifidobacteria and lactobacilli increased from 19 % up to 29 % and 34 % in cultures with oligosaccharides from lemon peel and sugar beet pulp, respectively. *Faecalibacterium* and *Roseburia* also grew with both substrates.	(B. Gómez et al., 2016)
	Orange peel wastes	POS	N.D.	POS increased bifidobacteria and lactobacilli counts, so their ratio among total bacteria increased from 17 % in the inocula to 27 % upon fermentation.	(B. Gómez et al., 2014)
	Artichoke and sunflower by-products	POS	1 % (w/v)	Reduction of the Mw of artichoke pectin resulted in greater stimulation of the growth of *Bifidobacterium*, *Lactobacillus*, and *Bacteroides/Prevotella*, whilst this effect was observed only in *Bacteroides/Prevotella* for sunflower samples.	(Ferreira-Lazarte et al., 2018)
	Artichoke by-product	Prebiotic oligosaccharides	1 % (w/v)	Growth of beneficial lactic acid bacteria and bifidobacteria. Lower counts of potentially pathogenic bacteria (up to 2 log UFC/mL). Higher production of acetic, propionic and butyric acids, and lower production of lactic acid than on controls. Consumption 54.6 % of the substrate by fecal bacteria.	(Holgado et al., 2022)
	Soyabean Okara	Prebiotic oligosaccharides	0.5 g per 45 mL	Treated Okara promoted higher growth of beneficial bacteria and inhibited potentially harmful bacterial groups.	(Pérez-López et al., 2016)
	Spent barley grains and husks	XOS	5 and 10 g/L	Samples with shorter Mw showed faster fermentation kinetics. Succinate, lactate, formate, acetate, propionate, and butyrate were produced during fermentation.	(P. Gullón et al., 2011)

22 BW – Body weight; MOS − Manno-oligosaccharides; M_1 − Mannose; M_2 − Mannobiose M_3 − Mannotriose; M_4 − Mannotetrose; N.D. – Not described; POS − Pectic-oligosaccharides; XOS −
23 Xylo-oligosaccharides; X_2 − Xylobiose; X_3 − Xylotriose; SCFA − Short-chain fatty acids; Mw − Molecular weight.
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Figure Captions

Fig. 1. Classification of agro-industrial wastes. Adapted from Sadh *et al.* (2018).

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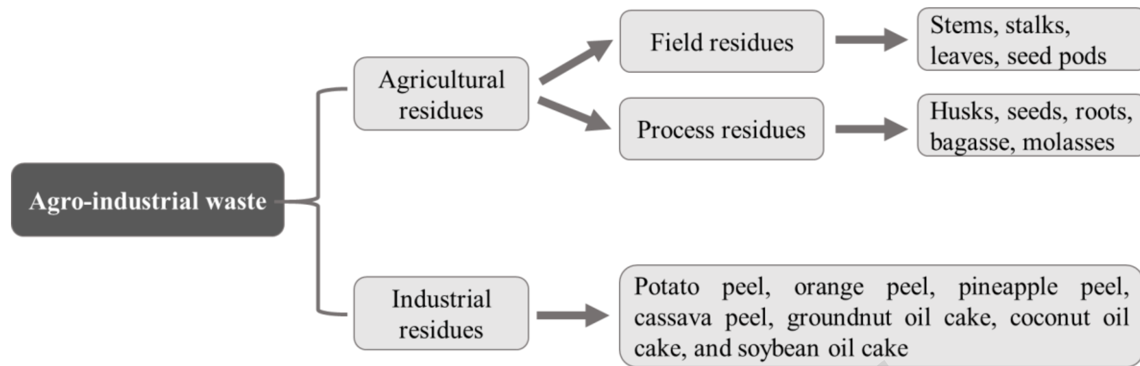
Figure 1.



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How prebiotics have been produced from agro-industrial waste: an overview of the enzymatic technologies applied and the models used to validate their health claims

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Article Highlights

- Agro-industrial wastes can be exploited to produce high-value prebiotic compounds.
- Enzymatic approaches are suitable to produce prebiotics from agro-industrial waste.
- Research efforts are still required to increase enzyme catalytic efficiency.
- Prebiotics functionality have been assessed by *in vitro* digestion.
- Waste-derived prebiotic functionality is poorly explored.

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