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1 **Sweet as Sugar - Efficient conversion of lactose into sweet sugars using a novel whole-cell-**

2 **catalyst**

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20 **Abstract**

21 Lactose, the sugar contained in milk, has a low sweetness. We have constructed an efficient whole-
22 cell catalyst (WCC), that can be grown on dairy waste and which is able to convert lactose into a
23 mixture of sugars as sweet as sucrose. The WCC is based on *Corynebacterium glutamicum*
24 ATCC13032, which has been engineered to metabolize lactose, to express xylose and arabinose
25 isomerase and to eliminate byproduct formation. When introduced in concentrated cheese whey
26 permeate, its content of 98 g/L lactose was completely hydrolyzed and the liberated sugars partially
27 isomerized into 23.5 g/L fructose and 20.4 g/L tagatose, which corresponds to a 49% conversion of
28 the glucose and a 44% conversion of galactose. The latter is similar to what can be obtained using
29 purified enzymes. The new technology enables better resource utilization and allows for dairy waste
30 to be converted into a valuable food sweetener with many potential uses.

31 **Keywords:** whole-cell catalyst, *Corynebacterium glutamicum*, lactose hydrolysis, sugar
32 isomerization, sweetener

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40 **Introduction**

41 Lactose, a disaccharide consisting of one glucose molecule joined to a galactose molecule by a ß-1,
42 4-glycosidic linkage, is the primary sugar found in the milk of mammals. Lactose first emerged as a
43 commercial commodity during the 18th century [1], and with the later expansion of the dairy industry
44 increasing volumes of various lactose-containing (waste) streams followed. Whey and whey
45 permeate are examples of such streams that are generated from manufacture of cheese and other
46 fermented dairy products. The amount of lactose anually produced in the form of dried whey is
47 estimated to be more than 1,400,000 tons [2].

48 Whey permeate, or deproteinized whey, has several applications within the food and confectionery
49 industry, where it promotes sweetness and adds flavor to the product [1]. Beyond food applications,
50 lactose is also used by the pharmaceutical industry as a bulking agent [3]. With the explosive rise in
51 cheese production since the latter half of the 20th century, an urgent need for new alternative ways
52 to utilize whey has arisen. Two types of use have gained most attention, where the first is microbial
53 conversion of lactose into different value added compounds [4,5], and the second involves hydrolysis
54 of lactose into glucose and galactose that can be used for sweetening purposes .

55 Lactose has a low sweetness (16% of the Perceived Sweetness of Sucrose, PSS) compared to its
56 monosaccharide moieties, glucose (74% PSS) and galactose (32% PSS) [6]. Hydrolysis of lactose into
57 the glucose/galactose syrup extends the use of whey permeate as a sweetening agent, not only
58 because the syrup is three times sweeter than lactose [7], but also because 70% of the world's
59 population suffer from lactose intolerance, due to an inability to metabolize lactose [8].

60 Chemical hydrolysis of lactose requires harsh conditions such as very high temperatures (up to
61 150°C) and extremely acidic conditions (pH<1.5), which result in formation of undesirable
62 byproducts [9] and hydrolysis using enzymes is thus the preferred choice. Enzymatic hydrolysis of

63 lactose is a quite common procedure, and is typically carried out at temperatures between 30°C and
64 50°C [10]. The purified beta-galactosidase are normally derived from various microbes, either natural
65 or recombinant [11].

66 Although hydrolysis of lactose increases sweetness, the liberated glucose and galactose are not
67 excessively sweet, and, in particular, galactose has a low sweetness. It is possible to isomerize
68 glucose into fructose and galactose into tagatose by using enzymes, and thereby not only increase
69 the overall sweetness but also reduce the glycemic index, which is desirable for food applications [12].
70 As for lactose hydrolysis, isomerization using purified microbial enzymes is preferred before the
71 use of chemical catalysts, as the conditions are milder and the process less sophisticated [13,14]. Since
72 the 1970s, high-fructose corn sirup (HFCS) has been used as a sweetener in foods and soft drinks
73 and the market for HFCS is immense [15]. Tagatose came on the market later, however, is also
74 considered a promising sugar substitute due to its high-sweetness (92% to sucrose) and low-calorie
75 features [16].

76 Whole-cell catalysis is not a novel approach, and in the past few decades, WCC have been used to
77 produce a wide variety of products in various industries [17,18]. Whole-cell catalysis potentially has
78 several advantages over the use of purified enzymes [18]. First, there is no need for a costly enzyme
79 purification step [18]. Second, the protected intracellular environment stabilizes enzymes under harsh
80 conditions. Third, for re-use, whole cells can easily be recovered by simple filtration or the cells can
81 be immobilized [19].

82 In this study, we modify *C. glutamicum*, a Generally Recognized As Safe (GRAS) Gram-positive
83 bacterium to become a host for expressing the relevant enzymes needed for lactose hydrolysis and
84 subsequent isomerization. The strain applied is able to ferment lactose, and lacks lactate
85 dehydrogenase. We demonstrate that this engineered *C. glutamicum* strain efficiently can convert

86 whey permeate into a sweet syrup in a single-step process.

87 Methods

88 Growth medium and conditions.

89 *Escherichia coli* (*E. coli*) strains were grown aerobically in Luria-Bertani broth (LB) [20] at 37°C,
90 and *C. glutamicum* strains were cultivated in Brain Heart Infusion broth (BHI) or CGXII minimal
91 medium at 30°C with 200-rpm shaking [21]. When appropriate, kanamycin was added to a
92 concentration of 50 µg/mL for *E. coli* and 25 µg/mL for *C. glutamicum*, and chloramphenicol was
93 used at a concentration of 25 µg/mL for *E. coli* and 8 µg/mL for *C. glutamicum*. When needed, one
94 mM of IPTG (isopropyl β-D-1-thiogalactopyranoside) was added to induce gene expression.

95 Deletion of the *ldhA* gene in *C. glutamicum*.

96 Preparation of electrocompetent *C. glutamicum* cells as well as transformation were carried out as
97 described previously [22]. The deletion of the *ldhA* gene in JS95 [23] was conducted via a two-step
98 homologous recombination procedure using the vector pK18*mobsacB* [24]. Briefly, two 800-bp
99 downstream and upstream gBlock fragments of *ldhA* were ordered from Integrated DNA
100 Technologies (Coralville, IA, U.S.) and cloned into the multiple cloning site of pK18*mobsacB* with
101 the Gibson cloning kit (SGI-DNA, La Jolla, CA, U.S.). The resulting plasmids were transformed
102 into the electrocompetent JS95, and clones with the plasmid successfully integrated were selected
103 on BHI plates with kanamycin. Counter-selection was performed on BHI with 10% sucrose.
104 Successful *ldhA* knockouts were confirmed by Sanger sequencing, and strain JS99 is one of these.

105 Plasmid-based expression of xylose isomerase and arabinose isomerase (construct A).

106 The *xylA* gene from *Arthrobacter* sp. NRRL B3728 [25] encoding xylose isomerase and the *araA* gene
107 from *Bacillus coagulans* NL01 [26] encoding arabinose isomerase were chosen for the purpose of

108 isomerizing glucose into fructose and galactose into tagatose respectively. Two gBlock gene
109 fragments were ordered from Integrated DNA Technologies (Coralville, IA, U.S.) and assembled
110 with the *Xba*I digested vector pEC-XC99E [27]. The ligation mix was first electroporated into the *E.*
111 *coli* TOP10 cells. Colonies with correct construct (construct A, Figure S1) were verified by colony
112 PCR and followed by Sanger sequencing. The plasmids were subsequently isolated from *E. coli*
113 using the Zyppy Plasmid Miniprep Kit (Irvine, CA, U.S.) and electroporated into the *C. glutamicum*
114 competent cells.

115 **Plasmid-based expression of xylose isomerase and arabinose isomerase using tandem**
116 **repetitive promoters (construct B)**

117 The shuttle plasmid pEC-XC99E for *C. glutamicum* was used as the backbone for the construction
118 of the new expression vector. First, two gBlock gene fragments (Integrated DNA Technologies,
119 Coralville, IA, U.S.), each of which contained a *trc* promoter sequence, were assembled into the
120 *BamH*I site of pEC-XC99E using the Gibson cloning kit (SGI-DNA, La Jolla, CA, U.S.) resulting
121 in the plasmid pJS150. Afterwards, two additional *trc* promoter sequences were introduced into the
122 *Sal*I site of pJS150 with the same cloning strategy and generated a new *Sal*I site at the 3´ of the
123 promoter sequence. The final vector, pJS151, contained four additional *trc* promoters downstream
124 of the original promoter in pEC-XC99E. The *xylA* and *araA* genes were cloned into the new *Sal*I
125 site of pJS151 to generate the construct B.

126 **Construction of the expression plasmid with *lacZ* (construct C).**

127 The *lacZ* gene was PCR amplified from the genomic DNA of *Streptococcus thermophilus* (*S.*
128 *thermophilus*) with the following primers p*lacZ*-F:
129 ACCTCCTGGGCTCCCGCTAATCGACCTGCAGTTATTACCTTCAAAAAGG and p*lacZ*-
130 R:CATCCGCCAAAACAGCCAAGCTTGCATGCCCTAATTTAGTGGTTCAATCA. The

131 fragment was cloned into the *pst*I site downstream the *xylA* and *araA* gene in the construct B with
132 the Gibson cloning (SGI-DNA, La Jolla, CA, U.S.) to generate construct C.

133 **Sugar hydrolysis and isomerization.**

134 One mL of overnight culture of the cell catalyst in BHI medium was inoculated in 50-ml CGXII
135 supplied with 5% lactose and 1mM IPTG. After 24 hours of cultivation, the cells were harvested by
136 centrifugation (5,000 g for 10 minutes). The pellet was washed once with 0.9 % NaCl solution. For
137 the hydrolysis of lactose and further isomerization, 15 g/L DCW (dry cell weight) cells were
138 resuspended in 40 mM MOPS solution (pH = 7.5) containing lactose, and incubated under static
139 conditions at 60°C. For the hydrolysis of the whey lactose, the pH of ultra-filtrated cheese whey
140 was adjusted to pH 7.5 prior to the use for the whole-cell catalysis. The concentration of cells and
141 other reaction settings used in whey was same as that used in lactose MOPS solution.

142 **Thermal stability test for β-galactosidase, xylose isomerase and tagatose isomerase.**

143 The thermal stability of β-galactosidase and isomerases was monitored after incubation of the WCC
144 in whey permeate under static conditions at 60°C. The WCC was sampled at periodic intervals for
145 the measurement of the residual activity in 40 mM MOPS solution (pH = 7.5) with either 50 g/L
146 lactose, glucose or galactose. The activities at 0 hour were defined as 100%.

147 **HPLC analysis of sugars and acids.**

148 The concentration of lactate was determined on an Ultimate 3000 high-performance liquid
149 chromatography system (Dionex) equipped with an Aminex HPX-87H column (Bio-Rad) and a
150 Shodex RI-101 detector (Showa Denko K.K.). The column oven temperature was set to 60°C and
151 the mobile phase consisted of 5 mM H_2SO_4. The flow rate used was 0.5 mL/min [28]. For the

152 measurement of lactose, glucose, galactose, fructose and tagatose (Sigma-Aldrich), the same system
153 and setup were used, except for the column (Agilent Hi-Plex Ca column) and mobile phase (water).

154 **Results & Discussion**

155 ***C. glutamicum* retains a low glycolytic activity at 60°C**

156 Previously we have engineered *C. glutamicum* into efficiently metabolizing lactose, by introducing
157 the lactose permease and the β-galactosidase from *Streptococcus thermophilus* and the Leloir
158 pathway from *Lactococcus lactis* on the chromosome [23]. *C. glutamicum* readily grows under aerated
159 conditions, but not when deprived of oxygen. We hypothesized that the engineered strain, JS95,
160 when incubated at elevated temperatures could serve as a whole-cell catalyst for hydrolyzing
161 lactose due to heat-induced permeabilization of its membrane, and tested this. Using 15 g/L DCW
162 cells, 114.20 g/L lactose could be completely hydrolyzed within 24 hours at 60°C, and a total of
163 108.13 g/L glucose and galactose were generated (Table 1). The sugar recovery rate reached
164 94.68%. Besides the glucose and galactose, we found that lactate had been formed in small amounts
165 (3.05 g/L). *C. glutamicum* is an aerobic bacterium that ceases to grow under anaerobic conditions,
166 however, it retains the ability to metabolize sugars into organic acids, mainly lactate, due to an
167 active glycolysis and lactate dehydrogenase [29]. We expected that the high temperature incubation
168 would inactivate central metabolism in the mesophilic *C. glutamicum*, however, lactate formation
169 indicated that this was not the case. Previously it has been reported for some mesophiles, with a
170 high abundance of glycolytic enzymes, that some residual activity persists for a period after high
171 temperature exposure [30]. Howell *et al.* demonstrated that most glycolytic enzymes in mesophilic
172 bacteria still maintained 50% activity after a half-hour heat treatment at 55°C - 70°C [31]. To
173 eliminate lactate formation, we decided to delete the *ldhA* gene, which is solely responsible for the
174 formation of lactate in *C. glutamicum* [32]. Indeed after deleting *ldhA*, lactate formation ceased, and

175 the sugar recovery rate increased to 97.21 % compared to 94.68% with the reference strain JS95
176 (Table 1). The *ldhA*-null strain JS99 appeared to be an ideal starting point for further engineering.

177 **Concurrent hydrolysis of lactose and isomerization of its constituent monosaccharides by**
178 **using recombinant *C. glutamicum*-based cell catalysts.**

179 To isomerize glucose and galactose into fructose and tagatose respectively, we decided to rely on
180 the promiscuous activities of xylose isomerase (fructose isomerase) and arabinose isomerase
181 (tagatose isomerase). First, we introduced *xylA* gene from *Arthrobacter* sp. NRRL B3728 [25] and
182 *araA* gene from *Bacillus coagulans* strain NL01 [26] in strain JS99, by using the plasmid pEC-XC99E
183 as expression vector (construct A), and obtained strain JS154. After a 72-h static incubation with 15
184 g/L DCW cells of JS154 at 60°C, 95 g/L lactose could be hydrolyzed and converted into a mixture
185 of 23.0 ± 0.5 g/L glucose, 22.8 ± 0.2 g/L fructose, 32.7 ± 0.9 g/L galactose, and 12.8 ± 0.4 g/L
186 tagatose. In this process, the high temperature exposure created a leaky cell envelope, through
187 which the substrate could enter and the products could leave. The conversion was efficient and only
188 small amounts of lactose remained (0.8 ± 0.0 g/L). The amount of glucose and galactose converted
189 into fructose and tagatose were 50% and 28% respectively. In a comparable study [26], where the
190 *araA* gene from *Bacillus coagulans* strain NL01 was expressed in *E. coli*, the amount of galactose
191 converted into tagatose reached a maximum of 40% when applying whole-cell catalysis with
192 galactose as the substrate. The lower sugar isomerization efficiency achieved in our setting
193 indicated that there was room for improvement. It is possible that the *araA* gene was expressed less
194 well in *C. glutamicum* than in *E. coli*, e.g. because of differences in transcription/translation
195 efficiency or because of differences in copy-number of the plasmid used for expressing *araA*. For *E.*
196 *coli*, high copy-number expression vectors and strong inducible promoters are widely available, but
197 this is hardly the case for *C. glutamicum* [33]. Second, the fact that we had integrated the β-
198 galactosidase in the chromosome, could have generated a bottleneck for subsequent isomerization.

199 It is possible that lactose hydrolysis was a rate-limiting step which potentially could exacerbate the
200 effect due to loss of activity of the xylose isomerase and arabinose isomerase at high temperatures
201 [26]. For all of these reasons, we attempted to improve the expression of the three genes.

202 **Optimization of isomerization efficiency by increasing gene expression.**

203 In order for whole-cell catalysts to be efficient, it is essential that the relevant enzymes are
204 expressed to a sufficiently high level [18]. Most of the expression vectors for *C. glutamicum* rely on
205 the tac/trc promoters, and although these promoters are derived from *E. coli*, they provide inducible
206 high-level expression of target genes in *C. glutamicum* [34]. However, the copy number of *C.*
207 *glutamicum* vectors is generally low (~20 copies per cell), and this could be a limiting factor for
208 overall expression. Shin *et al.* compared the influence of different media on the level of
209 heterologous protein expression in *C. glutamicum*, and found that Riesenberg broth supported the
210 highest expression levels when compared to other commonly used media such as BHI and CGXII
211 [19]. However, the use of a specific medium could restrict industrial applications, as industrial media
212 often contain many complex components. When we used construct A, the conversion of galactose
213 into tagatose was merely 12% lower than that obtained in the previous study using *E. coli*. To avoid
214 the risk of overloading cellular metabolism by using high copy number expression vectors, we
215 decided to introduce multiple *trc* promoters upstream of the genes in the vector pEC-XC99E, which
216 appeared to be a fast and controllable manner in which the expression could be increased [35].

217 Four additional *trc* promoters were introduced and in the new construct (B), the *xylA* and *araA*
218 genes were transcribed from five tandem *trc* promoters (Figure S1). The strain JS99 with construct
219 B was designated JS155. When the isomerization was performed with JS155, an increase in
220 productivity of approximately 60% and 120% for fructose and tagatose respectively was observed
221 (Figure 1& 2B), and the tagatose titer increased from 12.8 g/L to 19.4 g/L. With the new construct

222 B, 42% of the released galactose was converted into tagatose (Figure 2A), which clearly
223 demonstrates that the expression level was the limiting factor. The final fructose titer did not
224 change, however, this was not expected as a 50% conversion corresponds to the equilibrium
225 between galactose and tagatose at 60°C [12]. Therefore, with a higher *xylA* expression, only the
226 maximum productivity could be improved, and not the conversion ratio. The xylose isomerase used
227 in this study is a thermally stable enzyme [12], and the partial thermal denaturation taking place
228 during the isomerization process (72h at 60°C) apparently was not significant as equilibrium could
229 be reached. This was clearly not the case for arabinose isomerase, where complete loss of activity
230 was observed before equilibrium could be reached (Figure 2).

231 Only one copy of the *lacZ* gene was present in the chromosome of strain JS99, in contrast to the
232 *xylA* and *araA* genes, that both were expressed on plasmids from strong promoters. The relatively
233 low intracellular level of β-galactosidase probably limited the maximum productivity of fructose
234 and tagatose, due to a slow release of glucose and galactose. To overcome this potential limitation,
235 we made a new construct C, by inserting an additional *lacZ* gene downstream of the *xylA* and *araA*
236 genes in construct B (Figure S1). Strain JS99 carrying construct C was designated JS156. This final
237 catalyst was able to completely hydrolyze lactose within 12 hours, as compared to 24 hours for the
238 catalyst based on construct B (Figure 3) (the construct B). With construct C, both the maximum
239 production rates of fructose and tagatose were further improved by 45% and 50% compared to
240 construct B on lactose respectively. As expected, the increased cleavage rate of lactose did not
241 improve the conversion into fructose and tagatose, as the equilibrium between glucose/fructose and
242 galactose/tagatose at 60°C had been reached [12,26].

243 **Simultaneous whey lactose hydrolysis and isomerization of glucose and galactose**

244 Eventually, we applied the cell catalyst JS156 on concentrated cheese whey permeate. The cheese

245 whey ultra-filtrated permeates contained approximately 98 g/L lactose. With the incubation of 15
246 g/L DCW JS156 cells at 60°C for 48 hours, the final whey-based GGFT syrup was composed of
247 24.3 ± 0.0 g/L glucose, 25.7 ± 0.1 g/L galactose, 23.5 ± 0.3 g/L fructose and 20.4 ± 0.1 g/L
248 tagatose. A high thermostability is a desired property for a catalyst to be applied at elevated
249 temperatures, and we therefore examined the stability of the three enzyme activities over time at
250 60°C. It was found (Figure S2) that the activity of the two isomerases remained high and unaffected
251 by the high temperature exposure for more than 48 hours. The β-galactosidase activity, however,
252 started to reduce after 12 hours, but prior to this, the lactose had been completely hydrolyzed
253 (Figure 3).

254 In recent years, tagatose, as a GRAS food additive, has been extensively researched as a functional
255 sweetener[36]. Several researches on tagatose production from lactose or lactose-containing feedstock
256 have been reported. Xu et al. reported a single-step process for tagatose production from lactose
257 based on recombinant *E. coli* whole cells expressing an arabinose isomerase from *Lactobacillus*
258 *fermentum* CGMCC2921 and a β-galactosidase from *Thermus thermophiles* HB27, where a
259 maximum yield of 20.2% tagatose could be reached[37]. To improve the productivity and yield, cell
260 permeabilization and immobilization usually is carried out. Jayamuthunagai et al. produced tagatose
261 from lactose in whey permeate by using a combination of enzyme treatment (beta-galactosidase)
262 and alginate immobilized *Lactobacillus plantarum* cells (arabinose isomerase), and achieved a
263 conversion of 38%[38], but the research only focused on conversion of the galactose moiety of lactose
264 without considering to the possibility of generating sweetness from glucose. Rhimi et al. have
265 previously reported that it is possible to produce tagatose and fructose from a mixture of galactose
266 and glucose using an *E. coli* WCC expressing arabinose isomerase and xylose isomerase[39]. Rhimi et
267 al. used a mixture of glucose and galactose in MOPS buffer and a WCC that had been grown in LB
268 medium, a setup that is quite far from real-life applications. Even though Torres et al.[40] reported the

269 use of an immobilized tri-enzymatic system composed of β-galactosidase, xylose isomerase and
270 arabinose isomerase for bioconversion of lactose, simultaneous hydrolysis of lactose and
271 isomerization of the released sugars using a whole cell catalyst has not been reported. Torres et al.
272 found that lactose hydrolysis was incomplete in all their experiments employing tri-enzymatic
273 system because of product inhibition by galactose and glucose[40]. When we employed our whole-cell
274 catalyst in whey permeate, not only did we achieve complete lactose hydrolysis, but also efficient
275 isomerization of glucose and galactose. There was no residual lactose, a 49% conversion of the
276 glucose into fructose and a 44% conversion of the galactose into tagatose, which is comparable to
277 what can be achieve using purified enzymes.

278 In a one-step process, the sweetness of whey was increased by 440% and 160% compared to the
279 untreated whey and whey that merely had been hydrolyzed into glucose and galactose, respectively
280 (Figure 4). The conversion was associated with a decrease in glycemic index by 25%, when
281 compared to untreated whey (Figure 4). The results clearly show that microbial catalysis is an
282 efficient approach for turning cheese whey into a value-added sweetener, with potential benefits to
283 human health.

284 In conclusion, we have presented a novel approach for valorizing cheese whey permeate, a low- or
285 negative value side stream generated in enormous amounts by the dairy industry. By co-expressing
286 β-galactosidase, xylose isomerase and arabinose isomerase in *C. glutamicum*, a cell catalyst was
287 made that efficiently could convert whey lactose into a sweet GGFT syrup, with lower calorie
288 content and glycemic index. It is well-established that a high sugar consumption can cause various
289 lifestyle-diseases such as obesity and type 2 diabetes [41] and there have been several initiatives from
290 organizations (FAO/WHO) and health authorities aiming at lowering the consumption of sugar. The
291 technology developed addresses these societal challenges, while at the same time supporting a
292 better resource utilization at dairies. The generation of the *C. glutamicum* cell catalyst is also cost-

293 effective, since we previously have demonstrated that the host strain can grow on whey permeate
294 without addition of expensive nutrients. We believe that it is possible to optimize the performance
295 of the cell catalyst, e.g. by making the cells more permeable. To increase stability and to facilitate
296 reuse, immobilization approaches could be tested.

297

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309 **Notes**

310 The authors declare no competing financial interest.

311 **Supporting Information Statement**

312 The Supporting Information is available free of charge on the ACS Publications website.

313 Figure S1. The plasmid maps of different constructs used in the study. The *xylA* gene sequence. The
314 *araA* gene sequence. Two gBlocks containing trc promoter sequence.

315

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437 Figure captions

438 Figure 1. Comparison of fructose and tagatose formation from lactose using JS154 and JS155.
439 JS154: JS99 harboring construct A, where the isomerase genes (*xylA*, *araA*) are expressed from the
440 *tac* promoter in pEC-XC99E; JS155: JS99 harboring construct B, where the isomerase genes are
441 expressed from multiple tandem promoters inserted upstream the tac promoter in pEC-XC99E. The
442 schematic of the plasmids used can be seen in Figure S1.

443 Figure 2. Comparison of productivity and conversion ratio for different cell catalysts. A, fraction of
444 glucose and galactose converted into fructose and tagatose; B, the maximum productivity of
445 fructose and tagatose during the one-step lactose conversion. JS154: JS99 harboring construct A,
446 where *xylA* and *araB* are co-expressed on pEC-XC99E; JS155: JS99 harboring construct B, where
447 *xylA* and *araB* are co-expressed on pEC-XC99E from four additional *trc* promoters; JS156: JS99
448 harboring construct C, where *xylA*, *araB* and *lacZ* were co-expressed on pEC-XC99E from four
449 additional *trc* promoters (plasmid maps can be seen in Figure S1).

450 Figure 3. Comparison of lactose hydrolysis rate with JS155 and JS156. JS155: only one copy of
451 *lacZ* on the chromosome of JS99. JS156: additional *lacZ* were co-expressed on pEC-XC99E from
452 four additional *trc* promoters in JS99 (The illustration of plasmid maps can be seen in Figure S1).

453 Figure 4. Comparison of relative sweetness and glycemic index between raw whey and enzyme-
454 treated whey. Lactose-hydrolyzed whey, treated with JS99; Sugar-isomerized whey, treated with
455 JS156 which is JS99 bearing the construct C.

456

Table 1

Strain	Glu + Gal (g/L)	Lactate (g/L)	Sugar Recovery (%)
JS95	108.13 ± 0.59	3.05 ± 0.06	94.68
JS99	111.01 ± 0.07	0 ± 0	97.21

Table 1. The effect of inactivating lactate dehydrogenase in the whole-cell catalyst JS95 on sugar recovery and lactate formation. JS99: the ldh deficient derivative of JS95.

Figure 1

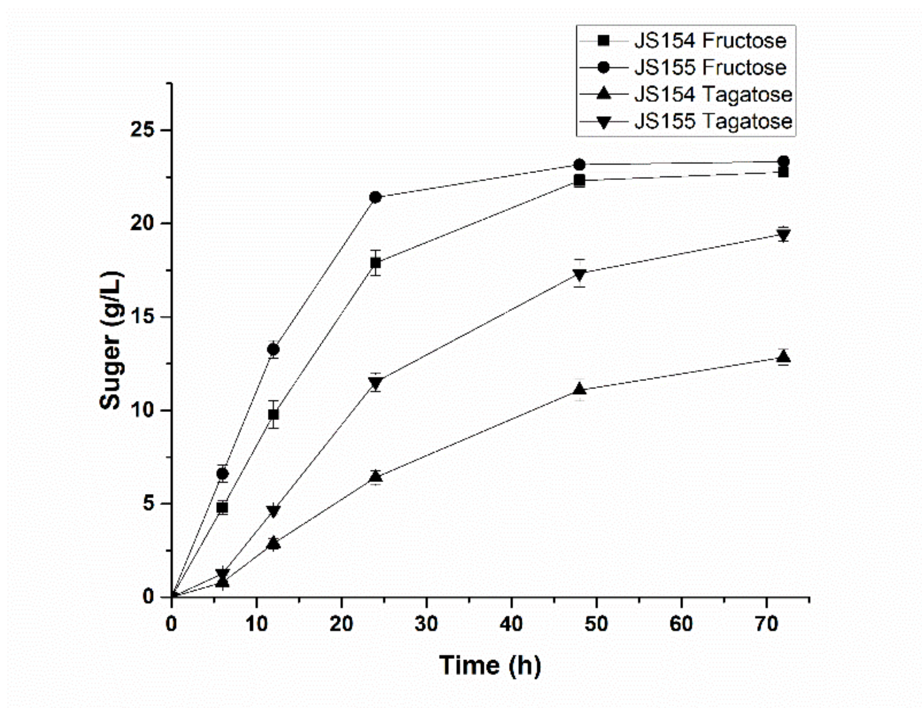


Figure 2

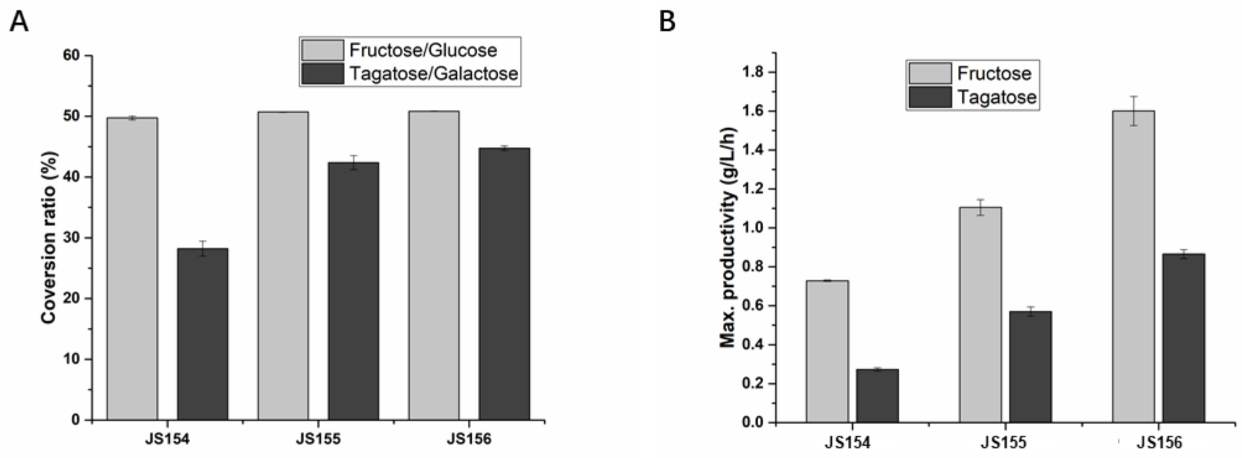


Figure 3

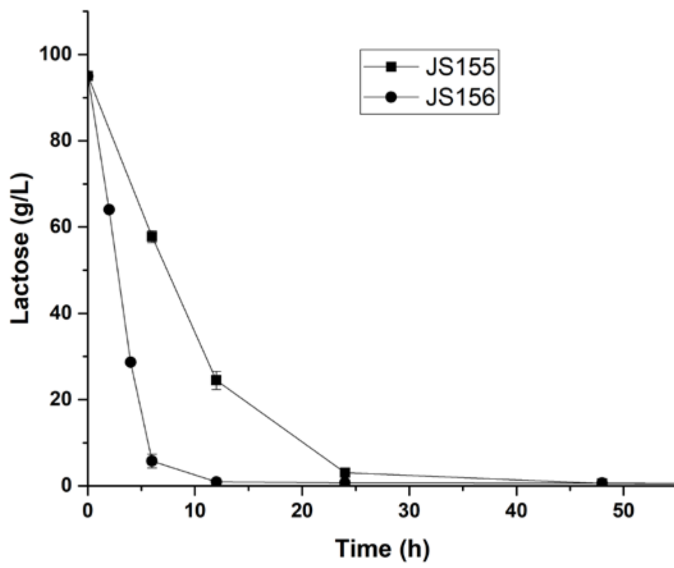
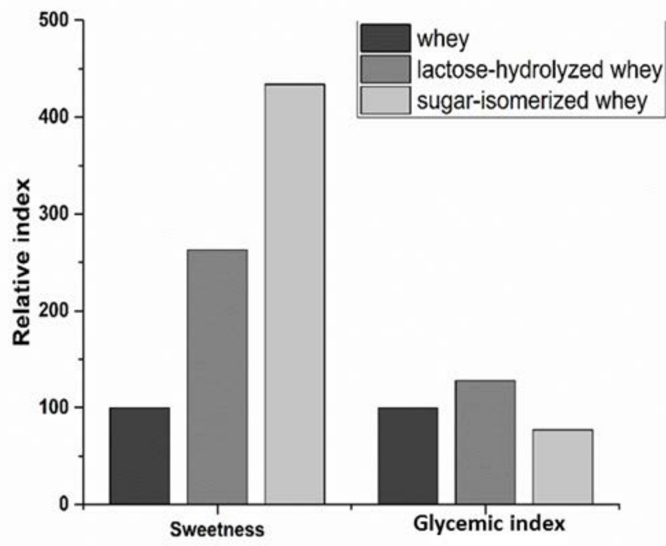


Figure 4


500
400
300
200
100
0
Relative index
whey
lactose-hydrolyzed whey
sugar-isomerized whey
Sweetness
Glycemic index