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Selective oxidation of glucose-galactose syrup to gluconic and galactonic acids†

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The global food industry, inclusive of dairy, is searching for sustainable upgrading of the large volumes of inedible waste they produce. Presented here is the selective oxidation of Greek yoghurt acid-whey derived glucose—galactose syrup (GGS) to gluconic and galactonic acids. Aldonic acids are high value biproducts with many applications. Our straightforward approach combines efficient catalytic oxidation and simple separation to upgrade a

large dairy waste product to higher value products under base free conditions, using an Au/Al_2O_3 catalyst with 73% and 55% selectivity towards gluconic and galactonic acids, respectively, under conditions of 2.5 bar O_2 for 24 h at 80 °C with a low Au: GGS ratio of 1:1000. A selective separation method was developed allowing the separation of the two epimeric acids based solely on their solubilities with high purity.

Green foundation

- 1. An inedible waste stream from the production of Greek yoghurt has been utilised to produce products listed in the top 10 high value bio-products by the Department of Energy. In addition, manipulation of the epimeric relationship of glucose and galactose is performed for the separation of mixed products gluconic and galactonic acids based on solubilities.
- 2. Exploration beyond the conventional lignocellulosic biomass for upgrading of waste to useful products has provided a route to gluconic and galactonic acids simultaneously. Selectivities of 73% and 55% to these aldonic acids using a gold catalyst with an extremely low loading ratio of 1:1000 of Au: feedstock has been achieved. Separation of gluconic acid and galactonic acid as calcium salts is purely based on their solubilities, utilising the uniquely intense hydrogen bonding in galactose due to its symmetry.
- 3. To make the work greener, a non-noble metal catalyst could be used. Further directed scale-up research is required to assess the process feasibility and economics to elevate the research.

Introduction

Exploitation of unavoidable side-streams and lessening dependence on petroleum derived chemical products are simultaneously achievable by targeted valorisation of biomass waste. In addition, reduction of environmental impact of food production is vital in ensuring sustainable maintenance of worldwide food supplies. The dairy industry aims to reduce greenhouse gas emissions, land and water use, which they hold a large share of among the food industry and the wider sectors. Greek yoghurt production has significantly

increased in the last decade, growing from a 1% market share of yogurt products to 40% and 20% in the US and Canada, respectively.³⁻⁵ This increase in demand yields large amounts of inedible Greek yoghurt acidic whey (GAW), around 2.1 million tonnes per year,^{6,7} which is unavoidable due to the yoghurt production process.⁸ There are limited feasible solutions to upgrading of GAW, which is the most significant byproduct of the dairy industry in the US;⁹ hence it requires a huge waste disposal effort. Therefore, there is an opportunity for bioproducts produced from the waste to provide an additional revenue stream, but more importantly, an environmental benefit for a circular economy.

GAW has a high chemical oxygen demand (COD), acidity and mineral content making environmental disposal challenging without further processing.¹⁰ Unlike cheese whey, it cannot be used as fertilizer or livestock feed.

Recently a highly effective, multistep process for converting GAW into high purity glucose-galactose syrup (GGS) was devel-

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oped at the University of Wisconsin-Madison⁶ as shown in Fig. 1. GGS has a carbohydrate composition and sweetness level similar to sucrose, making it a viable alternative to high-fructose corn syrup (HFCS). Based on our techno-economic analysis, selling GGS at a price comparable to HFCS would make the process economically viable. We have successfully developed a pilot-scale GGS production system at the University of Wisconsin-Madison. Our current focus is to further improve the process economics by upgrading GGS into higher-value products. We identified an opportunity to valorise the GAW-derived product, GGS, as an attractive feedstock for the production of gluconic acid (GA) and galactonic acid (GAL).

GA is an aldonic acid formed via oxidation of the aldehyde group of glucose to a carboxylic acid, and similarly GAL is the monoacid product of galactose oxidation (Fig. 1). GA has applications in a wide array of industries including food additives, 11 pharmaceuticals, 12 and metal-chelating agents 13 and has been found to be an excellent additive for cement which currently constitutes its largest demand by industry (45%). 14,15 Owing to these applications the Department of Energy (DoE) named GA in the top 10 bioproduct targets and its market value is predicted to hit \$1.9 billion in 2028.16

Fermentation is a common route used for the production of GA from glucose, 17,18 with batch processing producing high yields yet hindered by difficulties in separation and operating time and other production roadblocks.¹⁹ Thermochemical routes directly from glucose utilising Pt and Au catalysts²⁰⁻²⁵ are being developed but with applied alkaline conditions the longevity of the catalyst on various supports poses a feasibility problem. Alternative catalysts such as CuO nanoleaves have been reported for these conversions showing promising high selectivity for the oxidation of glucose or cellobiose.^{26,27}

Galactonic acid (GAL), an epimer of gluconic acid, is not as widespread in production nor application, due to the lower availability of galactose compared to glucose. Owing to its similar structure to GA it may have potential applications in similar use cases. For example, the same anti-retardation of

the cement effect is observed for GAL as for GA, 28 acting by slowing the dehydration of concrete and allowing longer handling times with suitable addition. Although the orientation of the hydroxyl group may not impact its application and value, it impacts the solubility of GAL compared to that of GA, more exaggerated when used as calcium salts.²⁹ A difference in solubility is also observed when comparing aldaric (dicarboxylic) acids derived from glucose and galactose. Glucaric acid from glucose has a high solubility in water; however galactaric acid is insoluble in water and in virtually all common organic solvents.³⁰ This is due to the tighter crystalline packing possible due to its optical symmetry, a trend extended to GA and GAL. The solubility of calcium gluconate is 3.5 g per 100 mL (ref. 31), whereas calcium galactonate has a low solubility in water, even when heat is applied. In this work the solubility is utilised for selective precipitation of the two epimers, aided simply by the low solubility of calcium galactonate.

Here we report a selective, catalytic oxidation of wastederived GGS into gluconic and galactonic acids using Au catalysts, on various supports, using hydrogen peroxide as a green, more convenient oxidant and molecular O2 for a more economical process. The oxidation does not require the addition of a base, and inexpensive additives are used. Selective precipitation utilising just the solubilities of the aldonic acid calcium salts allows two high-value product streams to be produced as secondary products from GAW.

Experimental

Catalyst preparation

Au/Al₂O₃ and Au/TiO₂ were prepared by deposition-precipitation (DP)³²⁻³⁴ using gamma-phase Al₂O₃ (Thermo Fisher) and TiO₂ (Aeroxide P25, Thermo Fisher) as supports. For the Au precursor, HAuCl₄·3H₂O (Aldrich >99.5%) was used. The selected support was dried in an oven overnight and a 100 mL solution of $HAuCl_4 \cdot 3H_2O$ at 4.2×10^{-3} M was heated to 80 °C and the pH

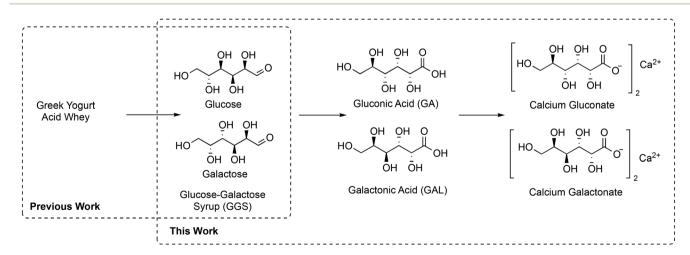


Fig. 1 Schematic overview of this work and the developed process for the oxidation of GGS to gluconic acid (GA) and galactonic acid (GAL) and the precipitation of their calcium salts.

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adjusted to 8 using a 1 M solution of NaOH. After maintaining the pH for some time, 1 g of the dried solid support was added, and the resulting slurry was readjusted to pH 8 before stirring for 2 h. After this time the solids were separated by centrifugation, washed thoroughly with H2O and dried overnight. The catalyst material was then calcined in a furnace at 300 °C with a ramp rate of 2 °C min⁻¹ and the target temperature was maintained for 4 h. Other catalysts used in this work, Pt/C (5 wt% & 10 wt%), Pt/ Al₂O₃ (5 wt%) and Pd/C (5 wt%), were sourced commercially from Sigma-Aldrich.

Catalyst characterisation

Metal loadings were determined using an Oxford INCA 350 energy-dispersive X-ray microanalysis system (EDS) connected with a Hitachi S-4800 field emission scanning electron microscope (FESEM) used for the energy-dispersive X-ray spectrometry (EDS) measurements. EDS spectra are shown in the ESI (Fig. S1 & S2†). Using the TEM images captured with a TE attachment for the EDS system, the particle size distribution (Fig. 2b) and average particle size were calculated for the prepared catalysts (Table 1). Ultraviolet-Visible (UV-Vis) spectroscopy of solid samples was performed using an Agilent Cary 5000 UV-Vis-NIR spectrophotometer equipped with a Praying Mantis diffuse reflectance accessory. The prepared catalysts were referenced against the relevant support materials. A characteristic absorbance at ca. 540 nm confirmed deposition of Au onto the surface (Fig. 3).

Product analysis

Product analysis was performed on an Agilent 1200 series HPLC with a diode array detector (DAD) and a refractive index detector (RID) using a Phenomenex Rezex ROA-Organic Acid H+ (8%) column with a 5 mM H₂SO₄ mobile phase. Product quantities of gluconic, galactonic and glucaric acids were

Table 1 Metal composition and particle diameter of the prepared catalyst

Catalyst	Metal loading/wt%	Particle diameter/nm
Au/Al ₂ O ₃	1	6.7 ± 1.1
Au/TiO_2	3	4.9 ± 1.0

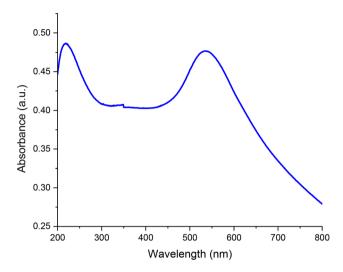
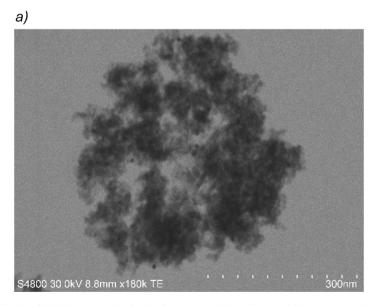


Fig. 3 UV-Vis absorbance spectra of Au/Al₂O₃ prepared by DP with Al₂O₃ as baseline correction.

measured at 210 nm in the UV range using external standard curves. Carbohydrate conversions were determined using the RID detector with additional external standard curves. The RID signal for both gluconic and galactonic acids and their respective carbohydrate precursor overlap; however as the carbohydrates are not UV active it was possible to subtract a



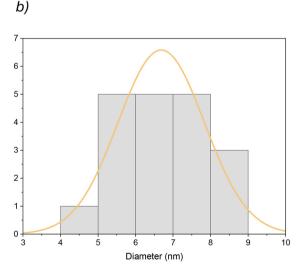


Fig. 2 (a) TEM image of Au/Al_2O_3 (scale bar = 300 nm) scale. (b) Particle size distribution of Au NPs on Au/Al_2O_3 .

calculated RID response based on the UV quantifications of gluconic and galactonic acids to quantify the remaining unreacted carbohydrates. Example chromatograms are provided in the ESI (Fig. S3 and S4 \dagger). Total organic carbon (TOC) was measured with a Shimadzu© TOC-VCPH Analyzer. Samples were diluted in MQ H₂O and degassed, and a 1000 ppm potassium hydrogen phthalate standard was used for the quantification of the organic carbons in the liquid samples. The results are shown in ESI Table S4. \dagger

Results and discussion

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Catalytic oxidation of GGS using hydrogen peroxide as the oxidant

Building on our previous research using Au NP catalysts to successfully oxidise glucose to gluconic acid under microwave irradiation in $\rm H_2O$ without adding a base, ²² we began a trial oxidation of GGS simulation feeds with various catalysts using hydrogen peroxide as the oxidant. Only stochiometric amounts of $\rm H_2O_2$ are required. Initially microwave (MW) experiments were performed to establish catalyst performance and optimal reaction temperature which can be adapted to conventional heating apparatus. Based on the initial screening it was concluded that $\rm Au/Al_2O_3$ at 80 °C provided the highest selectivity for gluconic acid production (Table 2).

Previous reports of Au, Pt and Pd catalysts for the oxidation of carbohydrates focused on the production of aldaric acids. As these di-acids are not the target in this work we restricted the amount of oxidant to stochiometric amounts to avoid overoxidation. The importance of oxidant concentration conversion and selectivity of GGS oxidation was assessed by creating a series of $\rm H_2O_2$ equivalents. Lower conversion was observed: 72% and 82% for glucose and galactose with 62% and 48% selectivities to the respective aldonic acid using 1 equivalent $\rm H_2O_2$ (Fig. 4). This improved to 98% and 94% conversion of glucose and galactose using 4 equivalents with 69% and 57% selectivity, respectively. With the addition of greater equivalents of $\rm H_2O_2$ we noticed a slight decrease in selectivity, which is a result of further oxidation to aldaric acids, and small amounts with <10% selectivity to glucaric acid were observed.

The dependence on time was assessed. Glucose and galactose conversions in a 3-hour reaction were moderate (and the respective selectivity). A 6-hour reaction was required for >90% conversion (Fig. 5). After 24 hours 98% and 93% conversions

Table 2 GGS oxidation with Au/Al_2O_2 as the catalyst: 2.2 wt% GGS solution, 4 equivalents H_2O_2 , 18 mg Au/Al_2O_3 for 25 min MW irradiation

Temp/°C	GLU conv./%	GAL conv./%	GA selectivity/%	GALA selectivity/%
40	0	0	0	0
60	0	0	0	0
80	43	62	91	16
100	61	71	85	36
120	76	81	56	29
140	95	87	56	51

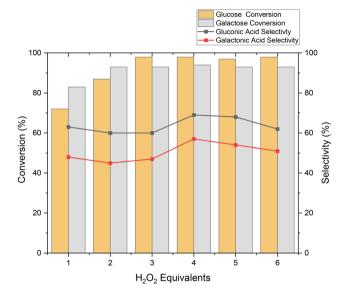


Fig. 4 Oxidation of GGS with varying H_2O_2 equivalents. Reaction conditions: 80 °C for 24 h, 10 mL of 2% solution of GGS with 18 mg Au/Al_2O_3 (1:1000, GGS: Au).

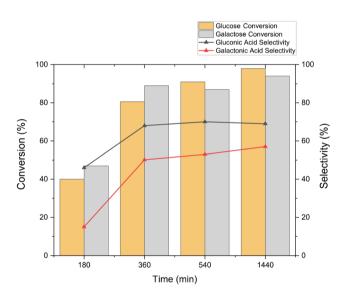


Fig. 5 GGS oxidation as a function of reaction time. Reaction conditions: 80 °C, 10 mL of 2% solution of GGS with 18 mg catalyst (1:1000, GGS:Au) and $4 equiv. H_2O_2$.

of glucose and galactose were achieved with 69% selectivity to gluconic acid and 57% to galactonic acid. Equipped with these results we replaced H_2O_2 with molecular O_2 as the oxidant for more economical conversions. As the stoichiometry of H_2O_2 can be controlled in an easier way, the insights gained from these oxidations can be applied to those with molecular O_2 .

Catalytic oxidation experimental optimisation using O_2 as the oxidant of simulation feeds

Studying O_2 as the oxidant for the reaction, we observe selectivity towards the aldonic acids. In a 24 h reaction at 7 bar O_2

Pd/C

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Glucose Conversion Glucose Conversion Gluconic Acid Selectivity Gluconic Acid Selectivity Galactose Conversion
Galactonic Acid Selectivity a) 2.5 bar Galactose Conversion b) 7 bar Galactonic Acid Selectivity 100 100 100 80 80 80 80 Conversion (%) Conversion (%) Selectivity (%) Selectivity (%) 60 60 40 40 40 20 20

Fig. 6 GGS oxidation with different supported metal catalysts and pressure using O_2 as the oxidant. Reaction conditions: 90 mg glucose, 90 mg galactose, 30 mg lactose, 18 mg catalyst, 10 mL H_2O with (a) 2.5 bar and (b) 7 bar O_2 at 80 °C for 24 h.

Au/Al2O3

Au/TiO2

pressure the Au catalysts (Au/Al₂O₃ and Au/TiO₂) outperformed Pt and Pd catalysts (Fig. 6), even with significantly lower metal loading (1–3 wt% Au and 5–10 wt% Pt and Pd) and the selectivity and conversion were comparable to those of reactions using H_2O_2 . The reactions were carried out without the addition of a base, a benefit of using Au catalysts which does not favour Pt & Pd oxidation as reported in the literature.³⁵

Pt/C

Catalyst

Pt/Alummina

Au/TiO2

Au/Al2O3

Oxidations of glucose with Pt/C are well reported, for the purpose of producing glucaric acid by the oxidation of glucose. ^{20,36,37} Comparably high conversions of glucose and galactose were observed using Pt/C under the applied conditions (Fig. 6), but the selectivity was low, 29% and 21% for gluconic and galactonic acid, respectively. This can be explained by quantifying the glucaric acid in the reaction mixture signifying overoxidation (Fig. S7†), while for galactaric acid it is insoluble and will not be detectable in solution *via* HPLC analysis. For a 24 h reaction at 5 bar O₂ pressure at 80 °C the conversion of glucose and galactose was 79% and 95%, respectively. This translated into 58% and 31% selectivities to gluconic and galactonic acids, with a selectivity to glucaric acid of 32%. This shows easier accessible oxidation of

the terminal hydroxyl group with Pt/C supported by studies on the adsorption and reactivity of aldoses on Pt surfaces, ³⁸ which for the scope of aldonic acid production is an undesired side product. Au/Al₂O₃ and Au/TiO₂ have lower selectivity towards glucaric acid, reflecting decreased activity of Au for the oxidation of terminal hydroxyl groups.

Pt/C

Catalyst

Pt/Alummina

The conversion at 7 bar (Fig. 6b) for 24 h using Au/Al $_2$ O $_3$ was 80% and 88% for glucose and galactose, respectively (52% and 33% selectivities). However, using hydrogen peroxide under the same conditions higher selectivity and conversions were observed for the oxidation products. It was noted that lower pressures favoured higher conversion and acid selectivity. The reaction at 2.5 bar O $_2$ for 24 h at 80 °C gave 98 and 95% conversions of glucose and galactose with 73% and 55% selectivities for gluconic and galactonic acids from the respective monosaccharide (Fig. 6a). This was a marked improvement and highlighted the importance of controlling the O $_2$ pressure during the reaction. The trends in Table 3 for a 6 h reaction showcase the effect of pressure on the GGS conversion and selectivity which emphasises the requirement for appropriate oxygen pressure to reduce catalytic site blocking and destruc-

Table 3 Conversions and selectivity of oxidation of GGS using varying oxygen pressures

O ₂ pressure (bar)	Time (h)	Temp. (°C)	Glucose conversion (%)	Galactose conversion (%)	Selectivity GA (%)	Selectivity GAL (%)
1.5	6	80	32%	39%	52%	45%
3	6	80	73%	75%	76%	45%
7	6	80	67%	73%	51%	33%
12	6	80	74%	79%	33%	22%

Reaction conditions: 90 mg glucose, 90 mg galactose, 30 mg lactose, 18 mg catalyst, and 10 mL $_{2}$ O

tion of reaction products. This was confirmed using liquid total organic carbon (TOC) analysis. The TOC showed that the pressure applied was positively correlated with total carbon loss (see the ESI for more details†). Catalyst loading is another important aspect to ensure high selectivity and a ratio of 1:1000. Au:GGS was found to be optimal for adequate conversions and high selectivity, and an increase in the ratio to

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 Table 4
 Solubilities of glucose and galactose and their respective sugar

 acids and calcium salts

1:2000 decreased the conversion. A decrease in the ratio to

Solute	Solubility in water mg mL ⁻¹	Ref.
Glucose	909.0	41
Galactose	475.2	42
Gluconic acid	316.0	43
Galactonic acid	159.0	44
Calcium gluconate	35.0	31
Calcium galactonate	9.7	45
Glucaric acid	912.0	46
Galactaric acid	3.3	47

1:500 and further to 1:250 showed a higher conversion but a decrease in selectivity. This was explained by a positive linear correlation between catalyst: feed and total carbon loss (monitored by TOC and detailed in Table S14†).

Selective separations of gluconic and galactonic salts

As with numerous carbohydrate conversions, the separation of products is a vital and usually a challenging aspect of the process and can account for 20–50% of the total cost of the process. Thermo-catalytic treatment of carbohydrates often leads to small organic acidic side products and humin formation. As this oxidation is active at 80 °C, humin formation and side product formation are minimal. However, if the intended applications of the gluconic and galactonic acids are pharmaceuticals and food products, these are high purity demanding applications. Therefore, it is desired to have a separation toolkit for further applications. The difference in solubilities in glucose and galactose become more pronounced when oxidised to aldonic and aldaric acids and even more so as calcium salts. The calcium salts calcium gluconate and

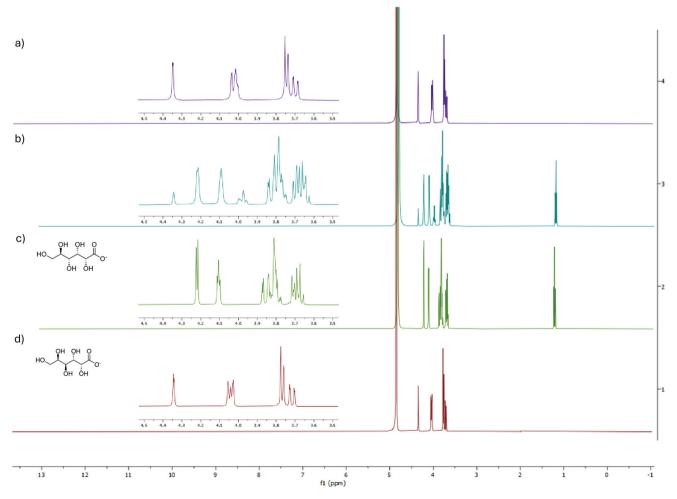


Fig. 7 1 H NMR (400 MHz D₂O). (a) Spectrum of the precipitate of the solution of calcium gluconate and galactonate in H₂O showing pure Cagalactonate, (b) spectrum of the precipitate after addition of EtOH, showing majority Ca-gluconate with Ca-galactonate impurities, (c) spectrum of the Ca-gluconate standard, and (d) spectrum of the Ca-galactonate standard.

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calcium galactonate are reported to have much lower solubility than the free acids. These are summarised in Table 4 from reported literature values.

A simulated mixture of calcium carboxylates obtained from the GGS oxidation, of 5 wt% calcium gluconate and 3 wt% calcium galactonate in $\rm H_2O$, was prepared to test crystallization as a separation approach. Upon cooling to 0 °C, a precipitate formed, and this was confirmed by $^1\rm H$ NMR spectroscopy to be calcium galactonate with high purity (>99%) (Fig. 7a). A solution of calcium gluconate in $\rm H_2O$ was left. To precipitate the calcium gluconate, EtOH was added, as the solubility of the salts in EtOH is generally poor. This successfully precipitated calcium gluconate confirmed by $^1\rm H$ NMR with a purity of 83% (Fig. 7b) with the major impurity being calcium galactonate, lending to its trace solubility in $\rm H_2O$.

Applying the successful model precipitation experiments to the obtained oxidation reaction solutions first the solid catalyst was recovered for reuse, then 5 equivalents CaCO3 were added as an inexpensive base and calcium source and the solution was stirred for 2 h. With a solution of calcium gluconate, calcium galactonate and unreacted sugars the selective precipitation method was tried. Initially this was unsuccessful in precipitating calcium galactonate in H₂O with both calcium salts co-precipitating once EtOH was added. This can be suggested to be the consequence of strong hydrogen bonding between the components in solution. To promote the calcium galactonate to precipitate out of the solution seed crystallisation of calcium galactonate was tested. A small portion of pure calcium gluconate was added, and the concentrated solution was cooled (detailed in the ESI†). This caused the calcium galactonate in solution to precipitate and the purity was monitored by ¹H NMR. After this was separated, we added EtOH to the solution to precipitate calcium gluconate and this was obtained with a purity of 86%.

Conclusions

An efficient approach for the conversion of a dairy wastederived product GGS from an inedible Greek yogurt side product is outlined. The developed two-step process provides new feedstock streams for the renewable chemical industry. The Au/Al_2O_3 catalyst prepared selectively oxidized glucose and galactose into their respective aldonic acids, using either H_2O_2 or O_2 as the oxidant with a very low catalyst loading (Au: GGS ratio of 1:1000).

Gluconic acid, galactonic acid and their respective calcium salts are desired products as they already have a plethora of industrial applications. By manipulating the solubility difference of the produced gluconic and galactonic acids as calcium salts, an effective, simple, and selective precipitation of epimeric products is presented, providing opportunities for high-purity product streams of valuable bioproducts from the mixed monosaccharide GGS. Further studies on the scale-up potential of the oxidation and separation procedures will allow realisation of the utilisation of the large volume of Greek

yogurt waste, an important step in removing some of the environmental burden from the dairy industry. To align with a wider theme of a green chemistry future, the applications of sugar derived acids are still being developed and when attention is turned to renewable feedstocks in contrast to petroleum sourced chemicals, we can provide new chemical building blocks with improved functionality as well as helping to secure a greener chemical industry.⁴⁸

Data availability

The data used for the preparation of this communication are listed in the ESI.†

Conflicts of interest

There are no conflicts to declare.

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