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ORIGINAL ARTICLE

Sustainable production of pure L-arabinose from brewer's spent grain biomass

Paola D'Arrigo[1,2] · **Letizia Anna Maria Rossato**[1] · **Stefano Serra**[2]

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Abstract
The current work presents the innovative isolation and purification of a commercially valuable monosaccharide, L-arabinose, which is largely utilized as a natural sweetener and food additive, from brewers' spent grain (BSG), one of the most abundant agri-food waste and the primary by-product of the brewing industry. The utilization of BSG for the extraction of industrially relevant compounds has recently gained significant attention due to its potential for waste reduction and natural resources optimization. Moreover, L-arabinose recovery from BSG would represent a valid green alternative to the commonly used depolymerization of gum Arabic, a high-cost raw material (35 €/kg), which requires several steps of purification and, consequently, the use of hazardous solvents, higher costs, and time. In this work, a process based on an initial water treatment followed by a selective controlled hydrolysis step is presented, with the final aim to specifically break down the glycosidic bonds between D-xylose and L-arabinose to obtain the latter one release and final recovery in high purity, leaving the remaining biomass unaffected. In order to achieve this result, the kinetic of the process has been studied and optimized, and 20% of the total L-arabinose present in BSG has been recovered. This research aims to develop a new cost-effective and environmentally friendly method for the isolation of high-purity L-arabinose from brewery residues, contributing to the advancement of circular economy practices in the brewing industry.

Keywords Lignocellulose · Hemicellulose · L-arabinose · Fractionation

1 Introduction

In the last decades, the research exploiting the use of renewable carbon sources has been greatly investigated, due to the necessity of finding alternative low-cost raw materials for the production of high valuable compounds. Among the various renewable resource options, lignocellulosic biomass is one of the major contributor, and it is usually considered as a waste or at least a substrate for thermovalorization. In particular, the carbohydrate fraction (cellulose and hemicellulose) is largely investigated for the preparation of bio-based compounds, since it can be effectively hydrolyzed to monosaccharides that could be further transformed into high added value chemicals via fermentation or chemical synthesis. In the last years, researchers have mainly focused on the valorization of different lignocellulosic biomasses for the recovery of the two main monosaccharides, D-glucose, and D-xylose [1], which are highly requested for the synthesis of a variety of products, including sugars, value-added chemicals, and biofuels [2–5]. On the other hand, L-arabinose was left apart or recovered as a contaminant in D-xylose production, even if it is the second most abundant sugar in hemicellulose moiety and its demand is rapidly increasing [6–8]. In fact, L-arabinose applications are widespread across various industrial sectors. It constitutes a valuable carbon source for the production of organic compounds and is largely employed in food industry as a sweetener and a flavor enhancer because of its low calories content, which makes it desirable for health-conscious consumers [9].

✉ Letizia Anna Maria Rossato
letiziaanna.rossato@polimi.it

Paola D'Arrigo
paola.darrigo@polimi.it

Stefano Serra
stefano.serra@cnr.it

[1] Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Politecnico Di Milano, p.zza Leonardo da Vinci 32, 20133 Milan, Italy

[2] Istituto Di Scienze E Tecnologie Chimiche "Giulio Natta" — Consiglio Nazionale delle Ricerche (SCITEC-CNR), Via Luigi Mancinelli 7, 20131 Milan, Italy

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Moreover, L-arabinose is largely studied in pharmaceutical industry for the synthesis of drugs such as nucleoside analogues with antiviral and antitumor activity [10, 11]. In addition, L-arabinose could be used as precursor for the preparation of derivatives with interesting properties, [12] has also shown to help in blood sugar reduction and has beneficial effects on human diabetes, thanks to its antioxidant activity. Finally, the fermentation of this sugar is highly important in the development of alternative energy sources because the process produces ethanol, a renewable biofuel, contributing to the setup of sustainable energy solutions [8, 13–16].

Despite its large demand, to the best of our knowledge, examples of L-arabinose isolation from lignocellulose are not reported in literature [8, 17, 18]. In fact, most of the works focus on the complete or partial hydrolysis of hemicellulose, mostly obtaining a mixture of hexoses-pentoses sugars, without any further monosaccharide isolation and purification [19–21]. Actually, the separation of L-arabinose in its pure form from the pentose mixtures resulting from hemicellulose hydrolysis is a challenging and demanding process, especially because of its low content respect to D-xylose, which has very similar physical and chemical properties. Therefore, the separation of these two monosaccharides through the most common techniques employed in chemical industry (such as crystallization, distillation, and chromatography) is particularly difficult. For all of these limitations in purifying L-arabinose from lignocellulose hydrolysates, nowadays, commercial L-arabinose is obtained by depolymerization of gum Arabic by acid hydrolysis, followed by its purification through multiple procedures such as a neutralization step, ion exchange and other chromatographic separations, or chemical modifications, such as ketal formation. Gum Arabic has been chosen because of its high L-arabinose content, 30–45% depending on its origin [22], but, however, this methodology presents some obvious limitations: the high raw material cost (35 €/kg), the requirement of complex steps of purification, and the use of hazardous solvents [23, 24]. All of these features make L-arabinose particularly expensive, especially when compared to the other monosaccharides. In fact, considering big, international dealers, L-(+)-arabinose costs in a range between 500 and 1300 €/kg, while D-(+)-xylose 60–100 €/kg depending on the provider, packaging, and purity of the compounds. This economic aspect consequently limits its use, especially as food additive where high-cost commodities are unsuitable. Therefore, there is a strong commercial interest in the development of new cost-effective and high-performance methods for obtaining high-purity grade L-arabinose [25, 26]. For all the cited reasons, in this work, brewer's spent grain (BSG) was selected as raw material for L-arabinose extraction, due to BSG high abundance worldwide [27]. In fact, it constitutes the solid residue obtained during the beer-making process, representing about 85% (w/w) of the total waste by-products generated during this process. For every kilogram of beer, about 0.12 kg of dried BSG is produced (equivalent to 0.20 kg of wet BSG), and the estimated global annual production of beer is currently around 38,600,000 metric tons with a constant growth [28, 29]. In fact, beer is the most popular alcoholic beverage worldwide and the third-most popular drink overall after water and tea [30–33]. BSG composition is strictly dependent on several factors (type of barley grains, harvesting time, brewing technology, and process parameters) [34]. However, typically, it contains 15–25% proteins (hordeins, glutelins, globulins, and albumins), 50–70% fiber (hemicellulose, cellulose, and lignin), 5–10% fats (triglycerides and fatty acids), and 2–5% ashes [35]. The main polysaccharides of BSG are cellulose and hemicellulose, which are composed of D-glucose (cellulose), and D-xylose and L-arabinose (hemicellulose), which have been widely investigated for their interesting properties and applications in several sectors, such as material science and medicine [36–38]. In particular, BSG contains 11% w/w of L-arabinose. The valorization of BSG from a circular economy perspective for L-arabinose selective recovery not only would thus contribute to saving resources, reducing land use, and decreasing the carbon footprint of the brewing process, but also would help the establishment of novel waste processing streams, thus introducing little but significant societal changes [39, 40].

Recently, the authors have proposed the valorization of BSG through a new multistep method for its fractionation recovering the different main components of the biomass [41]. Accordingly, a first pretreatment with hot water in autoclave allowed the separation of an aqueous medium containing ~ 25% of the starting biomass. This fraction, mainly composed of soluble polysaccharides, proteins, and other minor components, was exploited in different microbial fermentations for the production of high-value branched-chain fatty acids (BCFAs) and polyunsaturated fatty acids (PUFAs) [42]. On the other part, the recovered solid water-insoluble residue was submitted to a multistep lignocellulose deep eutectic solvent-mediated fractionation, which allowed the recovery of a cellulose-enriched and a lignin-enriched fractions. The latter product was tested as a potential precursor for the development of cement water reducers with encouraging results. In literature, BSG has also been employed for enzymes synthesis (such as cellulases, amylases) [43] and for the preparation of high-value compounds like xylitol [44], lactic acid [45], prebiotics [46], and ethanol [47].

In the present work, the authors present a simple and cost-effective alternative method to isolate high purity L-arabinose from an abundant agri-food waste through a careful and well-controlled fractionation in dilute acid hydrolysis of BSG. In the described process, the acid hydrolysis has been used to selectively cleave the glycosidic bonds between L-arabinose and D-xylose residues, leaving the

hemicellulose structure and the rest of the biomass unaltered, permitting to operate at mild conditions. In addition, the selective release of L-arabinose allowed its recovery only by a simple crystallization step, reducing the overall costs and steps of purification. Furthermore, theoretically, the presented methodology allows to valorize quite the full biomass because in the global process vision only, very few wastes would be discarded. In fact, almost each organic fraction could be subsequently employed for a further valorization, through microbial fermentations for the production of high-value fatty acids, or submitted to successive fractionation processes for cellulose and lignin separation and recovery (see Fig. 1, gray part).

2 Experimental part

2.1 Materials and general methods

All chemicals were purchased from Zentek s.r.l. (Milano, Italy) or Merck (Merck Life Science S.r.l., Milano, Italy) and used without further purification. Deuterated solvents were purchased from Eurisotop (Saint-Aubin, France). The employed solvents were of analytical or HPLC grade when necessary. All air- and moisture-sensitive reactions were carried out using dry solvents and under a static atmosphere of nitrogen. TLC analyses were performed on Kieselgel 60 F254 plates purchased from Merck. BSG was kindly provided by the Brewery "L'Orso Verde" (Busto Arsizio, Italy) from the Pilsner beer production process.

2.2 Biomass storage

BSG was obtained as wet residue directly from the brewery within 12 h after a batch of beer production. This material was brought to dryness in an oven (BO-50NL/TCN-50 PLUS, Argolab, China) set at 60 °C for 24 h and was finely ground with the help of an electric mixer. The obtained biomass contained 4.3% residual humidity and was stored sealed and refrigerated (4 °C).

2.3 Biomass composition analysis

BSG composition has been determined with a known multistep procedure with minor modifications [48]. BSG (1 g, value a) was suspended and stirred in deionized water (150 mL) at 100 °C for 1 h. Then, after filtration, the solid was washed with deionized water (300 mL), dried in an oven at 80 °C, and weighted (value b). The solid was treated with 1 N of H_2SO_4 (150 mL) at 100 °C for 1 h. Then, the suspension was filtered again and washed with water, and the solid was dried and weighted (value c). The solid was mixed with 72% H_2SO_4 (10 mL), kept at r.t for 4 h, and then treated with 1 N of H_2SO_4 (150 mL) under a reflux for 1 h. After cooling, the solid was isolated by filtration, dried, and weighted (value d). The fractions of the different components were quantified with the following equations:

$$\text{Soluble fraction}(\%): \ 100 \times (a - b);$$
$$\text{Hemicellulose}\ (\%) = 100 \times [(b - c)/a];$$
$$\text{Cellulose}\ (\%) = 100 \times [(c - d)/a];$$
$$\text{Lignin}\ (\%) = 100 - \text{soluble fraction} - \text{hemicellulose} - \text{cellulose}.$$

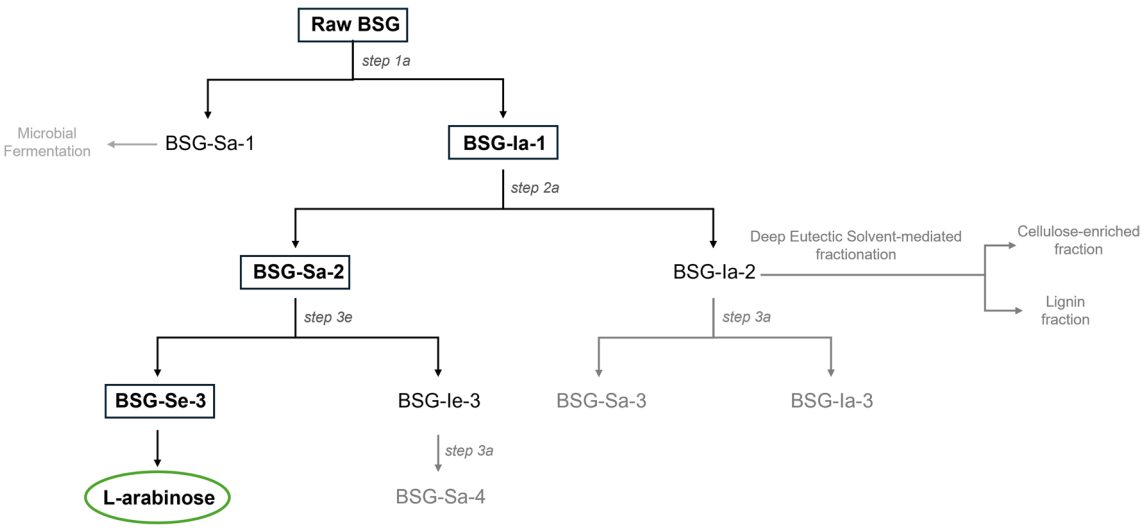

Fig. 1 Scheme of the fractionation process. S, soluble; I, insoluble; a, aqueous step; e, step in ethanol. In bold are reported the fractions that lead to the L-arabinose recovery, in regular the obtained wastes, and in gray the steps not involved directly in L-arabinose purification but required for a complete BSG valorization and analysis

2.4 Biomass fractionation process

The *step 1a* was performed in a temperature-controlled three-necked round bottom flask. 25 grams of raw BSG were mixed with H_2O (500 mL) at 100 °C for 1 h. The suspension was then filtered to recover a filtrate (BSG-Sa-1) and a solid (BSG-Ia-1) which was washed and dried in an oven for 24 h at 60 °C (20 g, 80% w/w).

In the *step 2a*, BSG-Ia-1 (20 g) was mixed with 150 mL H_2SO_4 or H_3PO_4 0.5 M for a maximum of 2 h in a range of temperature from 50 to 100 °C. The suspension was then filtered. The aqueous phase BSG-Sa-2 was neutralized with $CaCO_3$, the insoluble calcium salt was filtered off, and the aqueous phase was dried under reduced pressure (5 g). The solid BSG-Ia-2 was washed and dried in an oven (16.25 g, 65% w/w).

In the *step 3e*, BSG-Sa-2 (5 g) was suspended in ethanol (25 mL) for 3 h at 100 °C, and the soluble (BSG-Se-3) and insoluble (BSG-Ie-3) fractions were recovered by filtration and dried. BSG-Se-3 was further purified by chromatography and dried under reduced pressure (1.25 g). Subsequently, it was dissolved in boiling ethanol for 3 h, and L-arabinose was recovered by crystallization and filtration (0.5 g, 2% w/w raw BSG).

In the *step 3a*, BSG-Ia-2 (16.25 g) and BSG-Ie-3 (2.8 g) were mixed with 150 mL H_2SO_4 0.5 M for 3 h at reflux. The two suspensions were filtered, and the aqueous phases (BSG-Sa-3 and BSG-Sa-4 respectively) were neutralized with $CaCO_3$, the insoluble calcium salt was filtered off, and the aqueous phase was dried under reduced pressure. BSG-Ie-3 was completely solubilized during the hydrolysis, and no solid fractions were recovered. On the other hand, the solid fraction BSG-Ia-3 obtained by BSG-Ia-2 was dried in an oven (8.1 g, 50% w/w BSG-Ia-2).

2.5 Thin-layer chromatography

Thin-layer chromatography (TLC) Merck silica gel 60 F254 plates (Merck Millipore, Milan, Italy) were used for analytical TLC. Detection was performed by staining with a solution of cerium molybdate or an anisaldehyde solution (0.5 mL anisaldehyde in 50 mL glacial acetic acid and 1 mL of sulfuric acid 97%). The eluent was constituted by *n*-butanol/*i*PrOH/H_2O 6:4:1.

2.6 NMR analyses

^{1}H, ^{13}C NMR, DEPT, COSY, and HSQC spectra were recorded on a 400-MHz Bruker Avance spectrometer (Milano, Italy) at 302 K. Acquisition and data treatment were performed with Bruker TopSpin 3.2 software. Chemical shifts were reported in δ units (ppm), relative to tetramethylsilane (TMS) as internal standard, and all spectra were recorded in D_2O.

^{1}H NMR L-arabinopyranose: δ_H 5.19–5.17 (d, 1H, O-*CH*OH), 4.0–3.91 (m, 2H, *CH*OH-CH_2-O; CHOH-*CH_2*-O), 3.89–3.80 (m, 1H, CHOH-*CH*OH-CHOH), 3.77–3.74 (m,1H, O-CHOH-*CH*OH), and 3.63–3.57 (m, 1H, CHOH-*CH_2*-O).
^{13}C NMR: δ_C 92.6 (1C, O-*CH*OH), 68.6 (3C, O-CHOH-*CH*OH; CHOH-*CH*OH-CHOH; *CH*OH-CH_2-O), and 62.49 (1C, CHOH-*CH_2*-O).
^{1}H NMR L-arabinofuranose: δ_H 4.46–4.44 (d, 1H, O-*CH*OH-CHOH), 3.89–3.980 (m, 2H, CHOH-*CH*-CH_2OH; CH-*CH_2*-OH), 3.63–3.57 (m, 2H, CHOH-*CH*OH-CH; CH-*CH_2*-OH), and 3.47–3.42 (m, 1H; O-CHOH-*CH*OH).
^{13}C NMR: δ_C 96.79 (1C, O-*CH*OH), 72.49 (1C, CHOH-*CH*OH-CH), 71.89 (1C, O-CHOH-*CH*OH), 68.6 (1C, CHOH-*CH*-CH_2OH), and 66.39 (1C, CH-*CH_2*-OH).

2.7 Gas-chromatography/mass spectrometry

The gas-chromatography/mass spectrometry (GC/MS) apparatus was a HP-6890 gas chromatograph equipped with a 5973 mass detector and a HP-5MS column (30 m × 0.25 mm, 0.25-μm film thickness; Agilent Technologies Italia S.p.A., Cernusco sul Naviglio, Italy). The analysis of the monosaccharides was performed through the separation of their alditole acetates (prepared as reported below) with the following temperature program: 120 °C (3 min)—12 °C/min—195 °C (10 min)—12 °C/min—300 °C (10 min); carrier gas: He; constant flow 1 mL/min; and split ratio: 1/30. Less volatile compounds were analyzed by GC/MS with the following silylation procedure. A mixture of 25 μL of pyridine, 250 μL of dioxane, and 75 μL of silylation mixture composed of N,O-bis(trimethylsilyl)trifluoroacetamide was incubated with 1 mg of sample heated in a thermomixer (1.5 mL vial Eppendorf Thermomixer Comfort) at 70 °C and 600 rpm for 30 min. At the end, 100 μL of the mixture was withdrawn and added to 100 μL of a standard solution of benzaldehyde (0.49 mM final concentration). Compound identification was preliminary performed by comparing the MS data with the National Institute of Standards and Technology (NIST) database, and, then, selected peaks were confirmed with known standards (comparing both mass spectrum and chromatographic coordinate).

2.8 Alditole preparation and acetylation for GC/MS sugars identification

Seventy milligrams of sample was dissolved in 20 mL of H_2O, then 100 mg of $NaBH_4$ were added, and the suspension was led stirring at r.t. for 2 h. Then the reaction was quenched by the addition of glacial acetic acid, and the solvent was removed by evaporation. The residue (70 mg) was treated with pyridine (2.5 mL) and acetic anhydride (12 mL), stirring at r.t. for 24 h. Then the solvents were removed, and the sample was dissolved in water and extracted with ethyl acetate. The organic phase was dried and analyzed by GC/MS. The retention time of the alditole acetates of the corresponding commercial sugars was as follows: D-rhamnose 14.03 min, D-fucose 14.30 min, L-arabinose 14.46 min, D-xylose 14.88 min, D-mannose 21.55 min, D-glucose 21.65 min, and D-galactose 21.96 min.

2.9 Optical rotation analysis

Optical rotation analyses were performed using a Propol digital automatic polarimeter using a sodium lamp (λ 589 nm, D-line), and optical rotation values were determined at 20 °C in water. Concentrations (c) were reported in grams per 100 mL. The optical rotation for commercial L-arabinose is: $[\alpha]_D^{20}$: + 204 (c = 2, H_2O); for crystallized L-arabinose: $[\alpha]_D^{20}$: + 245 (c = 2, H_2O).

3 Results and discussion

3.1 BSG composition

As soon as received from the brewery, BSG biomass was put in a ventilated oven (60 °C for 24 h) and finely ground with an electric mixer. The aspect of the biomass during the different steps of drying and grounding is illustrated in Fig. 2. The grinding process is necessary to homogenize the starting material and also makes the hydrolysis process replicable on different BSG batches.

As already mentioned, BSG is an intricate biomass where all the components are closely related to each other, and it mainly contains fibers (hemicellulose, cellulose, and lignin), proteins, and fats. The quantification of the main components present in the biomass has been performed using a multistep method based on successive water and acid treatments. The composition of the presently studied biomass is reported in Table 1 and appears in agreement with those reported in literature [49].

Moreover, the chemical composition of the isolated hemicellulose fraction, reported in Table 1, has been determined by GC/MS analysis after total hydrolysis, reduction, and acetylation of the samples following a known procedure [42]. The two main components of BSG hemicelluloses were L-arabinose and D-xylose with a low amount of D-galactose and D-rhamnose, and traces of D-fucose (see Table 2).

In the present work, the selective recovery of L-arabinose (30% w/w of hemicellulose, 10% w/w raw BSG) is proposed from BSG hemicellulose, which is mainly composed of arabinoxylans (AXs). AXs are attached to the cellulosic fibers with hydrogen bonds, and they are composed of β-(1,4)-linked D-xylose residues, which can be substituted with L-arabinose ones, which could be in turn esterified with ferulic acid or substituted with other molecules, such as hexoses, uronic acid, and acetyl groups as reported in Fig. 3 [44, 50]. Complete AXs hydrolysis leads to the isolation of the two monosaccharides D-xylose and L-arabinose [51]. The particular substitution patterns in the biomasses from different agri-food sources are responsible for a diverse ratio of L-arabinose to D-xylose, which plays an important role in depicting the molecular structure of AXs and in the setup of the strategy for their fractionation. Due to the complex structure, the selective release of one of the two monosaccharides from the hemicellulosic matrix results is laborious, and for this reason, the overall hemicellulose hydrolysis is favorite and mainly investigated.

In fact, in literature, different methods for the complete hemicellulose acid hydrolysis have been reported, with

Fig. 2 Pictur of raw BSG as received (**A**), dried BSG (**B**), and ground BSG (**C**)

Table 1 BSG composition reported as % (weight component/weight BSG)

Sample	Hemicellulose (% w/w)	Cellulose (% w/w)	Lignin (% w/w)	Soluble part (% w/w)
BSG	28	25	22	25

Table 2 Relative abundance of monosaccharides derived from BSG hemicellulose hydrolysis

L-arabinose	D-xylose	D-galactose	D-rhamnose	D-fucose
28.97%	65.24%	2.06%	2.48%	1.20%

the final aim to recover a mixture of both L-arabinose and D-xylose, or only the latter one, but no examples of selective L-arabinose release are described. For example, in 2005, Mussatto and Roberto used this kind of hydrolysis to recover D-xylose for its conversion to xylitol [52], and in 2006, they hydrolyzed the 76.2% of BSG hemicellulose to recover both D-xylose and L-arabinose with 67% and 97.8% efficiency respectively [53]. Also, the patent literature presented a methodology for the separation of a mixture of L-arabinose and D-xylose extracted from beet pulp exploiting the selectivity in their transformation to ketals [24]. However, all these methodologies are not specific for L-arabinose release from hemicellulose, but they all investigate the complete AXs hydrolysis and the subsequent sugars purification, a process that requires many chemical steps, and the employment of hazardous solvents and compounds. Our final aim was to overcame all the issues of selectivity and purification by setting up a controlled partial hydrolysis of raw BSG for the selective release and recovery firstly of L-arabinose in high purity, leaving cellulose and lignin unaltered, and exploitable for further applications.

3.2 BSG fractionation process

The detailed fractionation process is reported in Fig. 1 and will be described in details here below.

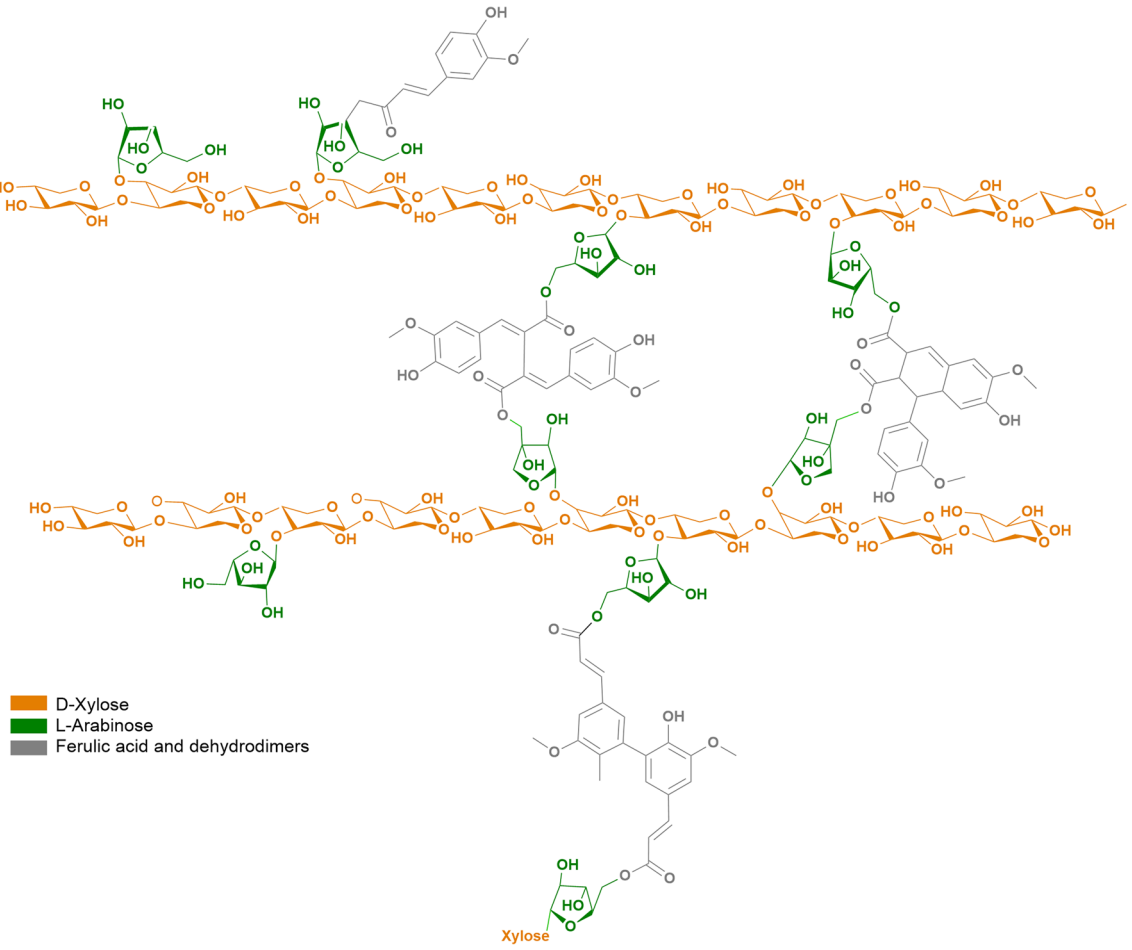

Fig. 3 Arabinoxylans structure

3.2.1 Step 1a

The first step of the process (*step 1a*) consisted in mixing raw BSG with water at 100 °C for 1 h in order to remove starch and β-glucans, to facilitate the subsequent L-arabinose recovery. In this way, a solid fraction called BSG-Ia-1 was obtained and an aqueous one called BSG-Sa-1 enriched in polymers/oligomers and with a negligible amount of monosaccharides. This latter was in this section hydrolyzed in H_2SO_4 0.5 M for 1 h at 100 °C and then analyzed by GC/MS to characterize its polymeric structure. The spectrum validated the presence of D-glucose at high percentages (73.75%), with minor amount of L-arabinose (20.13%) and D-xylose (6.11%). This result was in accordance with previously reported data [41], where raw BSG (called in that case BSG untreated: BSG_U) was submitted to a hydrothermal treatment in autoclave at 120 °C for 20 min, and a solid fraction (BSG treated: BSG_T) and an aqueous phase were recovered. This last sample was analyzed by GC/MS, showing the presence of mainly D-glucose, with some traces of L-arabinose and D-xylose (see Supplementary information). The solid recovered after separation of BSG-Sa-1, indicated as BSG-Ia-1, accounted for 80% (w/w) of the starting biomass.

3.2.2 Step 2a

BSG-Ia-1 was dried and subjected to a dilute acid hydrolysis (*step 2a*) testing different reaction conditions to obtain an aqueous phase, BSG-Sa-2 (20% w/w raw BSG), and an insoluble one, BSG-Ia-2 (65% w/w raw BSG). The use of a diluted acid is one of the most efficient protocols for this purpose, since it is generally employed to deconstruct hemicellulose and release a sugar mixture (mainly D-xylose and L-arabinose), leaving lignin and cellulose unaltered. During the dilute acid hydrolysis, the total hemicellulose structure cleavage is provoked by the proton release, which breaks the heterocyclic ether bonds between the sugar monomers in the polymeric chains formed by hemicellulose and cellulose. The reaction conditions of the treatment have a great influence on the molecular weight of the obtained oligomers and on the relative amount of released saccharides. In the present work, the parameters of the well-known acid hydrolysis have been deeply investigated, resulting in a complete new application: the selective L-arabinose release from hemicellulose. Thanks to this process, hemicellulose remained intact and could be subsequently valorized for D-xylose recovery. In fact, during *step 2a*, the exploited conditions allowed the recovery of only some oligomers and L-arabinose as monosaccharide, which were easily separable by employing their different solubility in ethanol.

3.2.3 Step 3e

Therefore, BSG-Sa-2 was submitted to *step 3e*: the sample was refluxed in ethanol for few hours in order to solubilize only the monosaccharide released in *step 2a* and obtain the BSG-Se-3 fraction (5% w/w raw BSG). On the other hand, the ethanol-insoluble fraction, BSG-Ie-3 (11% w/w raw BSG), contained only the oligomers/polymers obtained by the dilute acid hydrolysis of BSG (*step 2a*). After the *step 3e*, BSG-Se-3 was submitted to a chromatographic purification to remove possible low molecular weight dimers/oligomers, and, finally, L-arabinose was obtained by purification. TLC, GC/MS, and NMR confirmed the presence only of the monosaccharide in the sample soluble in ethanol.

3.2.4 Step 3a

Step 3a was necessary to deeply investigate BSG-Ia-2 and BSG-Ie-3 composition, in order to verify that the majority of L-arabinose was recovered. In details, these two fractions were completely hydrolyzed by using sulfuric acid 0.5 M at 100 °C for 3 h with the final aim to disrupt all the oligomeric chains still present in the samples and finally to completely transform the hemicellulose fraction into monosaccharides. Thanks to this step, BSG-Sa-3 (45% w/w BSG-Ia-2; 29% w/w raw BSG) and BSG-Ia-3 (55% w/w BSG-Ia-2; 33% w/w raw BSG) were obtained from BSG-Ia-2 and BSG-Sa-4 (100% w/w BSG-Ie-3; 11% w/w raw BSG) from BSG-Ie-3. Both the BSG-Sa-3 and -4 appeared particularly enriched in D-xylose, with some traces of L-arabinose, which probably remained linked to D-xylose in the previous steps, D-glucose and D-galactose.

3.3 Influence of different parameters on step 2a

The controlled release of L-arabinose required well-defined operating conditions, and for this reason, a screening in terms of typology of selected acid, temperature, and time of the *step 2a* was set-up because small changes in one of these parameters were shown to drastically influence the final result. In detail, a kinetic of hydrolysis was performed on BSG-Ia-1 by using both sulfuric (pKa1: − 2.8, pKa2: 1.99) and phosphoric acid (pKa1: 2.15, pKa2: 7.20, pKa3: 12.35) with a maximum of 80 °C for 1 h for sulfuric acid and 2 h for phosphoric acid, by analyzing the sample every 15 min (see Fig. 4).

For what concern the sulfuric acid utilization, 15–20% of total sugars were recovered by the *step 2a*, and D-xylose started to be released after 45 min. In fact, at 45 min, the temperature reached 70 °C, and only L-arabinose was recovered in BSG-Sa-2 as monosaccharide (excluding high molecular weight compounds), while, after 1 h at 80 °C, the monosaccharidic fraction was composed of 90% of L-arabinose and

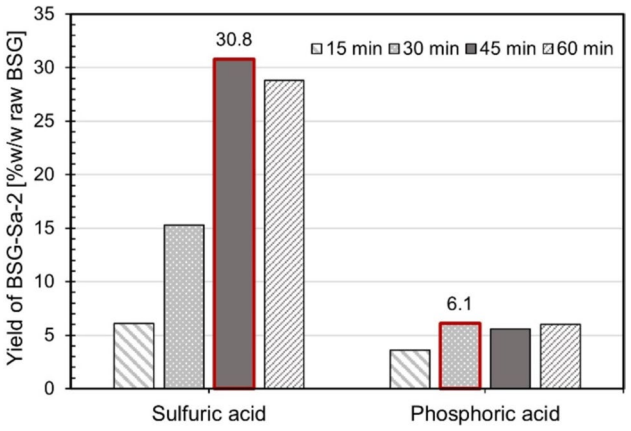

Fig. 4 Yield of BSG-Sa-2 (% w/w raw BSG) released over the time (15, 30, 45, 60 min) during the treatment in sulfuric acid 0.5 M. The best results in terms of BSG-Sa-2 are highlighted in red color

10% of D-xylose (see Table 3). Until 45 min, it appeared that only the covalent bonds between L-arabinose and D-xylose were selectively cleaved, since they are "external" and more accessible in the 3D lignocellulosic matrix, while the bonds that compose the xylan polymer remained untouched and were broken only at stronger conditions.

In order to demonstrate this assessment, the hydrolysis of BSG-Ia-1 was tested for 1 h at 80 °C, 90 °C, and 100 °C with H_2SO_4 0.5 M (Table 4), and it appeared clearly that the temperature setting was a crucial factor for the selective release of L-arabinose (Fig. 5). Indeed, by increasing the temperature, the monosaccharidic composition of BSG-Sa-2 changed, and was enriched in the percentage of released D-xylose and D-glucose, probably because the higher temperatures promoted the xylan polymeric chains rupture (see Supplementary information). In fact, at reflux, the L-arabinose released decreased to 32%, while D-xylose increased to 59%. In this condition, also D-glucose signal increased to almost 9%, proving that at stronger conditions also cellulose was damaged (see Table 4 and Fig. 5).

Table 3 Kinetic of BSG-Sa-1 fractionation in sulfuric acid 0.5 M (parameters and yields)

Time (min)	Temp (°C)	Yield of BSG-Sa-2 (% w/w raw BSG)	% L-arabinose in BSG-Sa-2[a]	% D-xylose in BSG-Sa-2[a]
15	50	6.1	100	/
30	65	15.3	100	/
45	70	30.8	100	/
60	80	28.8	95	5

[a]The data have been obtained by GC/MS analysis and are related only to the monosaccharidic fractionation present in BSG-Sa-2

Table 4 Data of BSG-Sa-2 obtained by fractionation in sulfuric acid 0.5 M for 1 h at 80, 90, and 100 °C (parameters and yields)

Time (min)	Temp (°C)	% w/w BSG-Sa-2/ raw BSG	% L-arabinose in BSG-Sa-2[a]	% D-xylose in BSG-Sa-2[a]
60	80	28.8	95	5
	90b	30	90	8
	100b	45	32	59

[a]The data have been obtained by GC/MS analysis and are related only to the monosaccharidic fraction present in BSG-Sa-2. [b]Traces of hexoses

According to Carvalheiro et al. [54], bonds between L-arabinose and D-xylose exhibit a higher thermal sensitivity than those that link together D-xylose units, and, for this reason, it is released first from the hemicellulose structure. The selection of the right conditions is crucial for not releasing also D-xylose at high concentrations. It resulted that the optimal reaction condition was 45 min at maximum 70 °C, which allowed to recover the fraction BSG-Sa-2 (20% w/w raw BSG), with a final composition of 100% w/w of L-arabinose (see Fig. 6). In addition, almost 60–70% w/w raw BSG (as BSG-Ia-2) was recovered.

Regarding the exploitation of phosphoric acid, this hydrolysis was a tentative of using a less hazardous acid to obtain the same result. The same kinetic of sulfuric acid was repeated, and at 1 h, only L-arabinose was recovered. For that reason, the hydrolysis was extended until 2 h, with an analysis also at 1.30 h. In both of them, D-xylose was released together with L-arabinose (see Supplementary information), and, in general, the yields of BSG-Sa-2 were much lower (maximum 6.1% w/w raw BSG) than with sulfuric acid. For all these reasons, the final choice was set on the use of sulfuric acid, because it allowed to recover higher yield of L-arabinose in less time, permitting in this way also a lower consumption of electricity and resources also in terms of purification.

3.4 Steps 3e and 3a: conditions and results

As the optimal hydrolysis conditions to maximize L-arabinose release in BSG-Sa-2 were set-up, NMR analysis showed that it contained not only L-arabinose but also oligo/polymeric sugars, and that there was the necessity to separate them from the sugar of interest. Therefore, in the *step 3e*, BSG-Sa-2 was dissolved in ethanol for 3 h at reflux in order to solubilize only the low molecular weight sugars present in the sample, while the ones at higher molecular weight remained insoluble, which were separated by filtration (BSG-Se-3 and BSG-Ie-3). For what concerned the ethanol-soluble fraction (5% w soluble/w raw BSG), it was

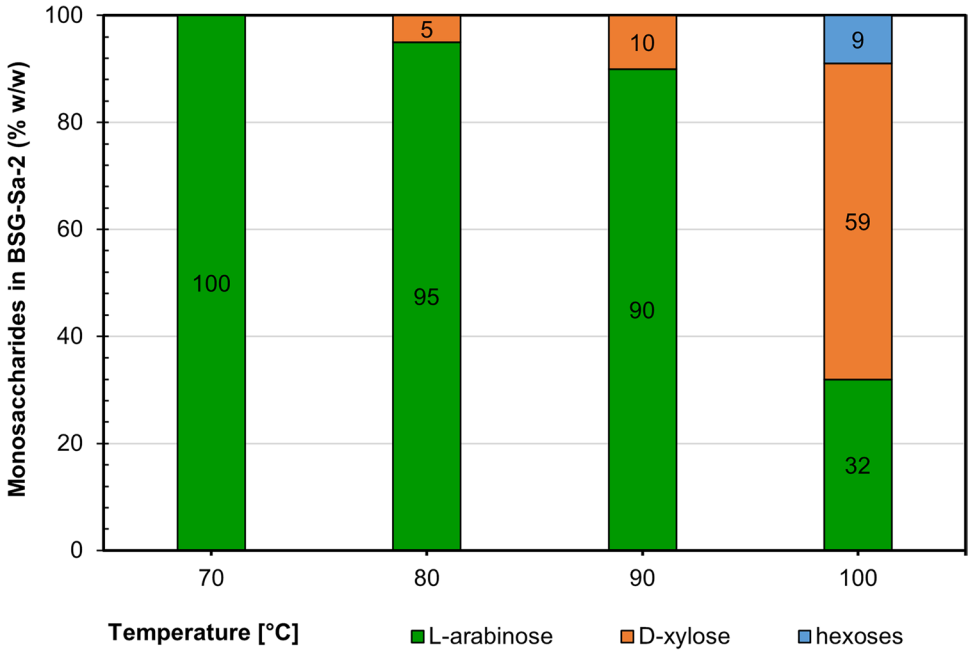

Fig. 5 Abundance of monosaccharides present in BSG-Sa-2 obtained by hydrolysis in sulfuric acid 0.5 M at 70, 80, 90, and 100 °C. The data have been obtained by GC/MS

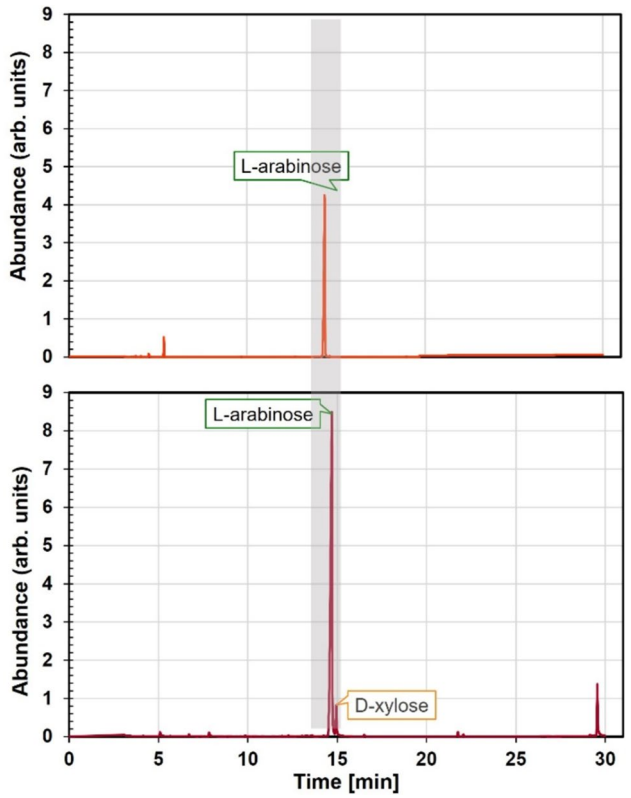

Fig. 6 Monosaccharides identified by GC/MS in BSG-Sa-2 obtained by hydrolysis in sulfuric acid 0.5 M for 45 min at 70 °C (light red) and for 1 h at 80 °C (dark red)

purified by chromatography to isolate only the monosaccharides in the sample and then analyzed by GC/MS, which confirmed the presence of only L-arabinose. On the other hand, BSG-Ie-3 was subsequently hydrolyzed (*step 3a*) in H_2SO_4 0.5 M for 3 h at reflux and analyzed by GC/MS. The spectrum showed a majority of D-xylose (42.66%) and D-glucose (32%), with small amounts of L-arabinose (23%) and D-galactose (1.99%). It should be pointed out that residues of D-glucose were probably due to a contamination of the cellulose fraction that appeared to be quite eliminated after the pretreatment of the biomass performed in the fractionation process.

The *step 3a* was applied also on BSG-Ie-3 derived from *step 2a* in sulfuric acid for 1 h at 80 °C, and the GC/MS analyses resulted in 96.7% D-xylose, 2.3% L-arabinose, and 1% D-glucose (see Fig. 7).

Moreover, *step 3a* on BSG-Ia-2 was performed, obtaining by filtration the samples BSG-Ia-3 (33% w/w raw BSG) and BSG-Sa-3 (29% w/w raw BSG), which were analyzed by GC/MS, showing the presence of 73.5% D-xylose, 17%% L-arabinose, 7.66% D-glucose, and 1.79% of D-galactose. As these results confirmed, the BSG-Ia-2 sample was enriched in xylans that were hydrolysed in *step 3a*, together with some L-arabinose still linked to the polymer. The experiment on BSG-Ia-2 was repeated after 1 h in H_2SO_4 0.5 M 80 °C, and, in this case, 83% of D-xylose was recovered, with small traces of L-arabinose (9%) and D-glucose (8%) (see Supplementary information).

The results obtained by strong hydrolysis of both BSG-Ie-3 and BSG-Ia-2 allowed to conclude that when *step 2a* was performed for 1 h at 80 °C, the almost complete release of L-arabinose from hemicellulose was accomplished, since only 2.3% of sugar was still recovered from the BSG-Ia-2 sample, while at 45 min at 70 °C, a small amount still remained attached to the polymer (17%). However, even if

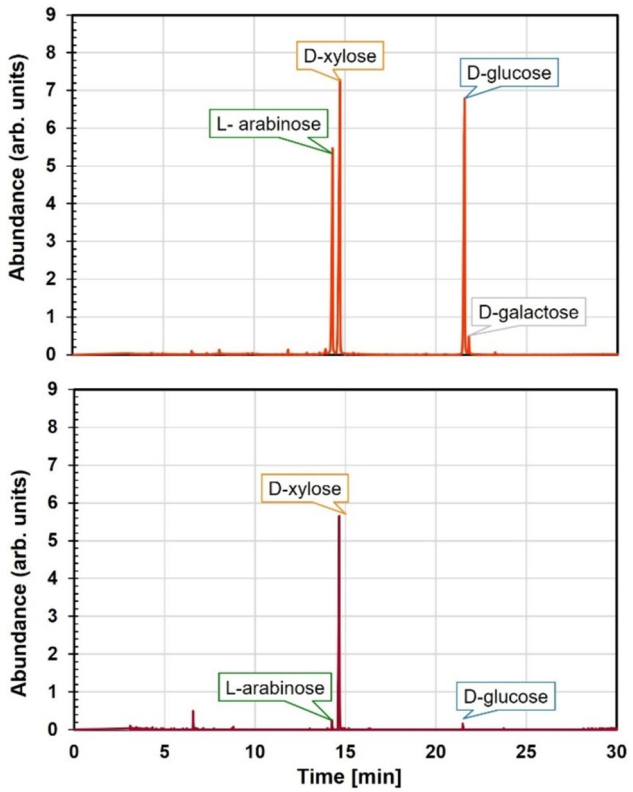

Fig. 7 Monosaccharides identified by GC/MS in BSG-Sa-4 derived from BSG-Sa-2 when *step 2a* is performed in sulfuric acid 0.5 M for 45 min at 70 °C (light red) and for 1 h at 80 °C (dark red)

L-arabinose was not totally recovered in *step 2a* at lower temperatures and times, we were confident in continuing performing the hydrolysis at those conditions because they were the only ones that permitted the selective release of only L-arabinose. In fact, at higher temperatures and times, major amount of L-arabinose was recovered, but together with D-xylose, which made the L-arabinose purification particularly difficult, laborious, and expensive. In fact, these two sugars present the same chemical/physical properties and are impossible to separate by the standard techniques, such as crystallization, distillation, and chromatography, but it would require previous chemical modifications, such as ketals formation and then chromatographic purification.

In the end, for what concern BSG-Sa-3 and BSG-Sa-4, both enriched in D-xylose, they could be exploited for microbial fermentation for arabitol production [42] or for the selective D-xylose purification through the chemical derivatization of the sample.

In addition, BSG-Sa-2 was derivatized by silylation in order to exclude the presence of molecules potentially derived from the degradation of lignin or sugar moieties (such as aromatics and furfurals) [55]. The GC/MS analysis demonstrated that neither lignin and sugars were modified by the process. Moreover, BSG-Sa-2 was acidified, extracted with ethyl acetate, and analyzed by ^{1}H NMR to confirm that no aromatics signals were present (see Supplementary information). These results confirmed that the hydrolysis performed with sulfuric acid at optimal conditions did not provoke lignin and sugars degradation.

3.5 L-arabinose purification

The next step of the process consisted in selectively purifying L-arabinose from BSG-Sa-2, which contained also some high molecular weight polysaccharides, mainly xylans, which were difficult to hydrolyzed and could be directly removed in order to obtain pure L-arabinose. As reported in the previous paragraph, the first step was the separation of the monosaccharide from the oligopolymers present in the sample, by performing the *step 3e*. After this solubilization in ethanol, BSG-Se-3 was enriched in L-arabinose, and it was further purified by a chromatographic separation, in order to eliminate all the dimer/oligomer residues solubilized in ethanol together with L-arabinose. In fact, if the crystallization was performed before purification, ^{1}H NMR spectrum presented signals between 3.4 and 3 ppm, which are characteristics of xylans (see Supplementary information). However, after this purification step, a crystallization of BSG-Se-3 was performed, and, at this point, only pure L-arabinose was obtained and characterized. Thanks to the described methodology, 20% w/w of the total L-arabinose present in BSG was recovered, which corresponded to a total of 2% on the starting material. The sample was analyzed by ^{1}H NMR, ^{13}C NMR, COSY, and HSQC, and its optical rotation was measured. By these analyses, performed in D_2O or water respectively, it had been possible to state that L-arabinose obtained by this fractionation process as well as commercial compound were present as L-arabinofuranose and L-arabinopyranose, with a higher stability in the furanosidic conformation. In fact, by both ^{1}H and ^{13}C NMR, the ratio between the anomeric signals of the two conformations was calculated, and it resulted that L-arabinose was 63% as furanosidic one (compound reported as *a* in Fig. 8) and 37% as pyranosidic form (compound named as *b* in Fig. 8, see Supplementary information).

3.6 Green metrics

This study resonates with the Green Chemistry principles firstly introduced by P. T. Anastas and J. C. Warner in 1998 [56, 57]. In this scenario, we calculated some of these parameters to prove the greenness of this technique. In particular, the E-factor (g of waste/g product) and the atom economy (Mw product/Mw reagents) have been evaluated for the final pure L-arabinose. In addition, the proposed method was compared to the one used to produce L-arabinose from gum Arabic [58] in terms of E-factor, atom

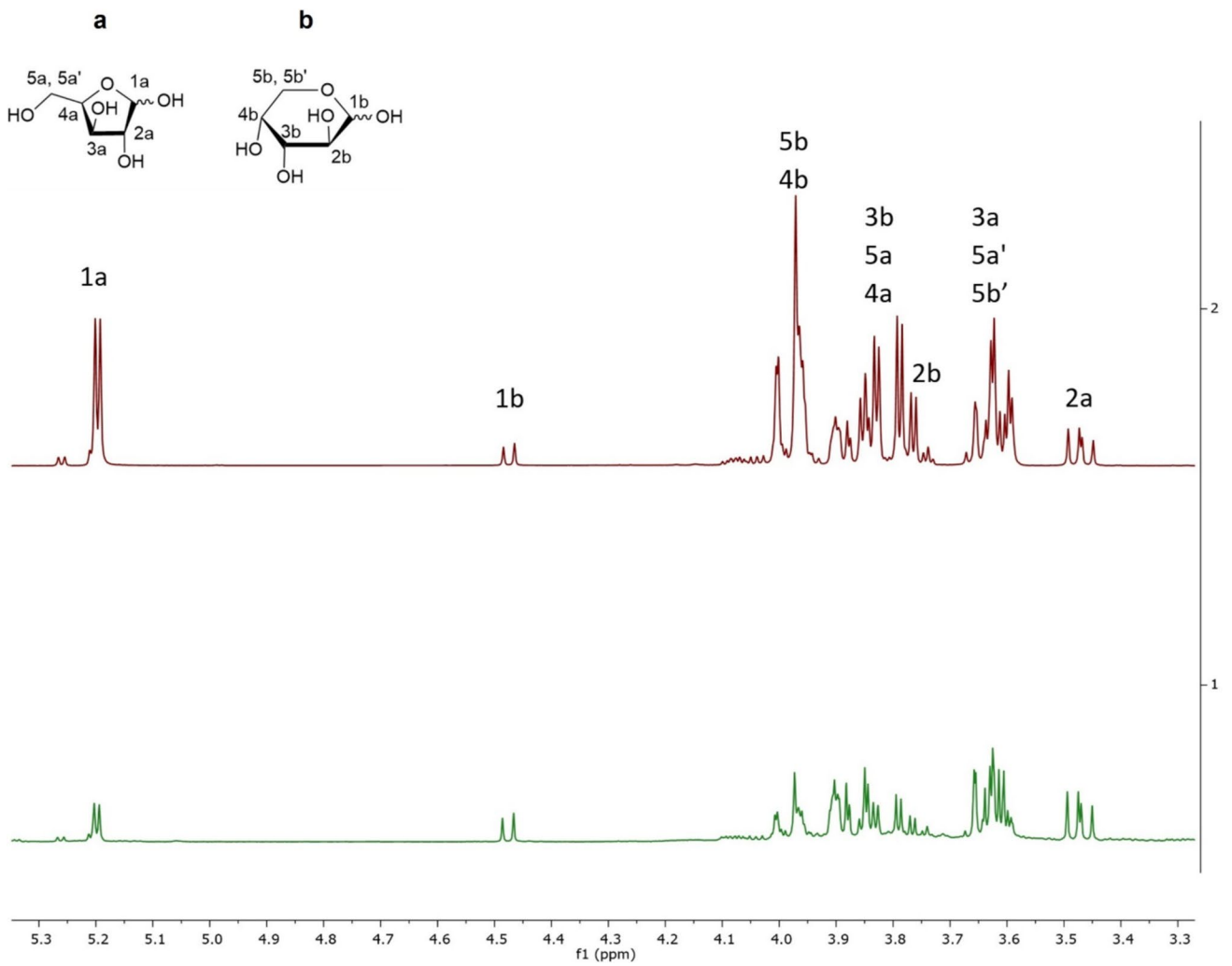

Fig. 8 ^{1}H NMR in D_2O. Up spectrum, commercial L-arabinose; down spectrum:, crystallized L-arabinose from BSG

economy, and efficiency of the overall system (see Supplementary information, page 18, for the two process schemes).

In particular, we evaluated the E-factor for every single step of our process (see Supplementary information) and a total E-factor for the overall process, compared to the one on gum Arabic, reported in Table 5. As we can see, the E-factor of the gum Arabic process is lower than the BSG one but only because gum Arabic contains more L-arabinose than BSG, and this consequently results in a major yield of recovered monosaccharide at the end of the overall process. However, we believe that the final yield couldn't be the only analyzed parameter, but also the overall process must be considered, since the gum Arabic one requires more steps, the utilization of toxic organic solvents (methanol, petroleum ether), and chemical transformations (ketal formation). On the other hand, even if with minor final yields, the process proposed in this paper is only based on BSG hydrolysis and product purification through ethanol purification and crystallization. Also, almost all the generated wastes were recycled or utilized for other purposes, such as microbial fermentation, D-xylose recovery, and cellulose/lignin separation. All of these considerations, to the practical side, are reflected also by the atom economy calculation. In fact, the one of the BSG process is clearly higher than gum Arabic one, which means that the proposed hydrolysis is more efficient in converting chemicals into the product.

Table 5 Green metric parameter calculations

Parameters	Gum Arabic	BSG
E-factor	112.01	327.1
Atom economy	51.38%	92.63%
Energy consumption for evaporation steps	140.1 W/h	103 W/h
Total cost	37.31 €/25 g	11.38 €/25 g

In addition, we also compared the rotavapor electricity consumption (W/h) considering each evaporation step as 1 h by using a Thermo Fisher tool, and the total prices of the two processes were calculated. As reported in Table 5, the recovery of arabinose from BSG requires in general less steps, which means fewer evaporation operations and so minor watts consumption (see Supplementary information). For what concern the total expenses, Kardofan gum Arabic costs 35 €/kg (https://www.goldleafsupplies.co.uk/gum-arabic-kordofan-1-kg/) while BSG, which is a discard from beer production, 35 €/t (https://doi.org/https://doi.org/10.1016/j.tibtech.2015.10.008), which means that gum Arabic costs 1000 times more than BSG. In general, the gum Arabic process requires more steps and reagents, which makes it costs in total 34.8 €/25 g. On the other hand, the BSG process costs in total 11.73 €/25 g. In addition, all the named chemicals in the paper have a very low cost if considered on an industrial point of view. In fact, $CaCO_3$ is marble dust, the industrial sulfuric acid has a very low cost, and ethanol is a fermentative product and could be self-produced. The evaluated costs in this work refer to chemicals bought from laboratory reagents sellers, and so the overall costs are obviously higher than the ones of a production plant. At this point, the real cost of the process relies in the energy consumption. Finally, in the present research, the BSG supplier was in the same Italian region (Lombardy) of the L-arabinose process plant, and that allowed to easily transport the raw material with low consumptions and carbon emissions. It is for this reason that this factor was not included in this study, even if, especially on an future industrial level where tons of biomass would be transported, the sustainability of the process also would rely on the distance between the raw material and the process plant.

4 Conclusions

The described work presents a sustainable cost-effective alternative to the common industrial process for the recovery of L-arabinose from gum Arabic. A well-controlled chemoselective hydrolytic process has been set-up, which allows the release of L-arabinose from the complex BSG matrix, permitting not only the recovery of the expensive natural sugar but also the valorization of an agri-food waste. In addition, this work represents an advance in the current state of the art, which is mainly focused on the application of acid hydrolysis for the complete D-xylose and L-arabinose release, without their separation and purification. On the other hand, thanks to the proposed process, a final recovery of L-arabinose was obtained which accounts for 2% w/w of dry biomass and for 20% of the total L-arabinose in BSG. This result is particularly encouraging on a circular economy perspective, since, considering the worldwide BSG production, it corresponds to 760,000,000 kg/year of L-arabinose. Furthermore, the proposed methodology reduces to the barely minimum purification steps allowing to keep the overall costs of production down. As a result, the process is compact and user-friendly and encapsulates the essence of the circular economy concept. Indeed, it focuses on the reuse of discarded organic materials from renewable sources to extract high-value organic products through environmentally friendly transformations. Furthermore, this method confirms that BSG is an industrial by-product with great potential for successive use in the food industry, and the present procedure constitutes a sustainable solution for both waste management and the production of a valuable bio-based sugar fitting perfectly within the scopes of the circular economy in an up-cycling perspective.

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Declarations

Conflict of interest The authors declare no competing interests.

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