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Bio-2,3-butanediol production from banana waste: Preliminary techno-economic evaluation of processing strategies

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ABSTRACT

This study evaluates different fermentation strategies to produce 2,3-butanediol (2,3-BD) from banana industry waste, such as whole bananas (fruit + peels) and banana peels, selecting the most favorable from a technical and economic point of view. Both residues have enough free sugars (17.8 %–35.8 %), glucan (11.0 %–14.2 %) and hemicellulose (2.8 %–6.3 %), to be promising substrates for 2,3-BD fermentation. Saccharification was studied by comparing enzymatic hydrolysis, hydrothermal pretreatment, and hydrothermal pretreatment followed by enzymatic hydrolysis. Different fermentation scenarios were also compared regarding the 2,3-BD yield and productivity: Separate Hydrolysis and Fermentation (SHF), Simultaneous Saccharification and Fermentation (SSF), and direct fermentation without prior saccharification using *Paenibacillus polymyxa* DSM-365 as the fermenting microorganism. The results showed that the pretreatment step was not necessary to improve the release of fermentable sugars. Enzymatic hydrolysis was the most effective alternative for maximizing sugar recovery, reaching sugar concentrations of 18.1 g/L (recovery: 92.5 %) for banana peels and 33.3 g/L (recovery: ~100 %) for whole bananas. The SSF strategy led to higher 2,3-BD concentrations of 15.0 g/L and 26.6 g/L for banana peels and whole bananas, respectively. The preliminary economic analysis indicated that SSF and direct fermentation could be the more cost-effective process alternatives for banana peels and whole bananas, respectively. Thus, it was demonstrated that banana waste is an interesting resource for the production of 2,3-BD. The bioprocess can be competitive when using a low-cost raw material and reducing the number of process steps compared to traditional technologies.

1. Introduction

The banana is one of the most consumed fruits in the world [1], reaching an annual production of up to 125 million tonnes in 2021 [2]. Colombia is one of the ten largest banana producers [3], being responsible for 2 % worldwide [4]. The Colombian banana industry can reject almost 70 % of the total production if it does not comply with export standards [5]. Banana wastes are generated mainly in farms, including rejected bananas and peels [6]. The whole banana (WB) and the banana peels (BP) have potential applications. For example, they can be used in low-value applications like combustion, contributing to greenhouse gas emissions [7]. In recent years, banana waste has been used as the raw material for high-value applications through the extraction of bioactive compounds [8] or the production of biofuels and commodities by the fermentation process [9,10].

A commodity gaining importance is 2,3-butanediol (2,3-BD) because of its industrial applications, especially as a precursor to other chemical products or fuel additives [11,12]. Applications for 2,3-butanediol include the production of plastics and solvents, the conversion to 1, 3-butadiene (a monomer for rubber synthesis) and its use as an octane enhancer in high-quality jet fuels [13]. Furthermore, 2,3-BD has a low melting point (-60 °C) and can be used as an antifreeze [11,13]. Bio-based 2,3-BD is a promising alternative to the petrochemical route [13]. The conventional method for producing 2,3-BD from oil involves hydrolyzing hydrocarbons at high temperatures and pressure, which is costly, complex, and has an environmental impact [14]. On the other

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hand, biological fermentation offers a more economical, simple, and scalable way of producing bio-based 2,3-BD [15,16]. Different bacteria, such as *Klebsiella*, *Bacillus*, *Enterobacter*, *Ralstonia*, *Paenibacillus*, *Serratia marcescens*, or even *Saccharomyces cerevisiae* mutant yeasts (i.e., YG01_SDBN and YPH499/pol3/BD_392), among others, can be used to produce 2,3-BD [13,17]. *Paenibacillus polymyxa* is highlighted among them since it is a non-pathogenic (class 1) strain with a high potential for 2,3-BD production. It could, therefore, be suitable for industrial-scale fermentation, as there is no biological safety level to consider [18].

The utilization of agro-industrial residues for 2,3-BD production can make bio-based processes sustainable. These resources contain complex sugars (cellulose, hemicellulose) not directly assimilable for *P. polymyxa.* A pretreatment step followed by enzymatic hydrolysis is usually required to release fermentable sugars from lignocellulosic materials [19]. The traditional bioprocess approach for 2,3-BD production is based on separate hydrolysis and fermentation (SHF) [20]. Because it is based on two steps, operating conditions can be better controlled, maximizing the product yields. However, the process stages can be combined for substantial improvement in energy efficiency, cost-effectiveness, and production enhancement [21]. Simultaneous fermentation and saccharification (SSF) is a one-step process combining enzymatic hydrolysis and fermentation. Thus, while enzymes break down polysaccharides into simple sugars, microorganisms can ferment them [22]. SSF minimizes the inhibition of byproducts and the risk of contamination [23], lowering the viscosity of the medium and leading to higher final product concentrations [22,24]. In addition, using the same reactor vessel simplifies the scaling up [25], reducing the capital cost [23]. SSF needs favorable conditions (*e.g.* temperature and pH) to be found for both the enzymes and microorganisms [26]. So, based on the above considerations, it is essential to select an adequate strategy to obtain fermentation products like 2,3-BD from food byproducts such as banana waste.

This study evaluates different fermentation strategies for 2,3-BD production from WB and BP to select the most favorable from a technical and economic point of view. To achieve this goal, it was proposed (i) to determine the composition of both raw materials, (ii) to study the saccharification of both feedstocks using different strategies (enzymatic hydrolysis (EH), hydrothermal pretreatment (HT), and hydrothermal pretreatment followed by enzymatic hydrolysis (HT + EH)) to select the most suitable to maximize the sugar recoveries, (iii) to compare different fermentation alternatives in terms of yield and productivity of 2,3-BD (SHF, SSF, and direct fermentation without previous saccharification), and (iv) to estimate the economic feasibility of producing 2,3-BD from WB and BP. This study represents the first investigation into 2,3-BD production from banana waste, encompassing a comparison of different process alternatives. The valorization of organic fruit waste contributes to the Sustainable Development Goal 12.3 [27].

2. Materials and methods

2.1. Raw material

BP and WB (Colombia variety: Cavendish) were kindly supplied by Gadis supermarket (Valladolid, Spain), which were discarded due to physical defects and nonconformity for the market. BP and WB were dried at 40 °C to avoid the caramelization of the raw material. This drying process was carried out in an oven (Heating Stove/Drying Stove U, Memmert, Germany) for 72 h until both substrates reached a homogeneous moisture content of approximately 9 % for BP and 20 % for WB. Subsequently, the dried raw material was milled to a particle size of 1–3 mm using a household grinder and then homogenized to ensure uniformity for characterization and experimental assays.

2.2. Saccharification

Three saccharification configurations were compared to select the most favorable alternative: hydrothermal pretreatment, enzymatic hydrolysis and hydrothermal pretreatment followed by enzymatic hydrolysis of the slurry. The experimental conditions are shown in Fig. 1A.

2.2.1. Hydrothermal pretreatment

The HT of the BP and WB was carried out in an autoclave (Model MED-12, Selecta, Spain) at 121 °C for 15 min, using 1000 mL ISO bottles at a substrate concentration of 5 % w/v. The slurry attained after pretreatment was subjected to EH (Fig. 1A, (2)). The sugar composition of the slurry was analyzed to determine the recovery of monosaccharides obtained in the pretreatment (Fig. 1A, (3)).

2.2.2. Enzymatic hydrolysis

The EH was carried out in 100 mL Erlenmeyer flasks in an orbital shaker (Optic Ivymen Systems, Comecta, Spain) using BP and WB with or without previous hydrothermal pretreatment (Fig. 1A, (1) and (2)). The enzymatic hydrolysis conditions were: substrate loading 5 % (w/v), 50 °C, 150 rpm, 24 h, and pH 4.8, employing water as the solvent (the initial pH (5.5) was adjusted to 4.8 with 10 M NaOH or 1 M H_2SO_4). The efficiencies for saccharification of two enzyme complexes, Cellic CTec2 (C) and Viscozyme L (V), kindly donated by Novozymes A/S (Denmark), were compared at different doses (10–15 FPU/g substrate), individually or blended. When BP and WB (without previous hydrothermal pretreatment) were employed, the enzymes were added separately (using an enzyme load of 15 FPU/g substrate for each enzyme) or combined (using an enzyme load of 10 FPU/g substrate and 15 FPU/g substrate of each enzyme). When the pretreated material (hydrothermal slurry) was used, only the mixture of enzymes was employed (the enzyme load of C + V was 10 + 10 FPU/g substrate). Samples were taken at 24 h, centrifuged (Mini Spin, Eppendorf, Germany), and the sugar content was analyzed. Tests were carried out with enzyme blanks to consider the sugar content in commercial enzymes. Monosaccharide recoveries were calculated considering the total sugar content in the banana wastes. All enzymatic hydrolysis tests were performed in duplicate, at least.

2.3. Microorganism and culture media

The microorganism employed in the 2,3-BD fermentation was *P. polymyxa* DSM-365 from the German collection of microorganisms (DSMZ, Leibniz, Germany). The strain was reactivated by inoculating the lyophilized cells into DSMZ liquid medium and grown overnight (12 h) at 30 °C in an orbital shaker (Optic Ivymen Systems, Comecta, Spain). The composition of the DSMZ liquid medium was (g/L): peptone, 5; meat extract, 3; and $MnSO_4.H_2O$, 0.01, at pH 7. Then, the strain was stored as glycerol stock (40 % (v/v) sterile glycerol) at – 80 °C until further use.

The inoculum was grown in the Häßler pre-culture media [28], whose composition was (g/L): 20 glucose, 10 yeast extract, 0.2 $MgSO_4$, 3 $(NH_4)_2SO_4$, 100 mM potassium phosphate buffer (pH 6), and 3 mL trace elements. The potassium phosphate buffer (pH 6) and trace element solutions were prepared separately and sterilized by filtration using 0.2 μm cellulose nitrate filters (Sartorius 254 stedim Biotech, Göttingen, Germany). The culture of *P.* polymyxa was grown in 250 mL Erlenmeyer flasks containing 100 mL medium until it reached an optical density between 0.7 and 0.8, and a cell concentration of 2 g/L 1 mL of *P. polymyxa* glycerol stock was inoculated. The cells were grown in a rotary shaker at 37 °C and 200 rpm for 24 h.

2.4. Fermentation

Three fermentation scenarios were compared once the most favorable saccharification alternative had been selected (Fig. 1B). Scenario 1: SSF; Scenario 2: SHF; Scenario 3: Direct fermentation without previous saccharification. The experimental conditions are summarized in Fig. 1B.

In the three scenarios, the Häßler pre-culture medium used in the

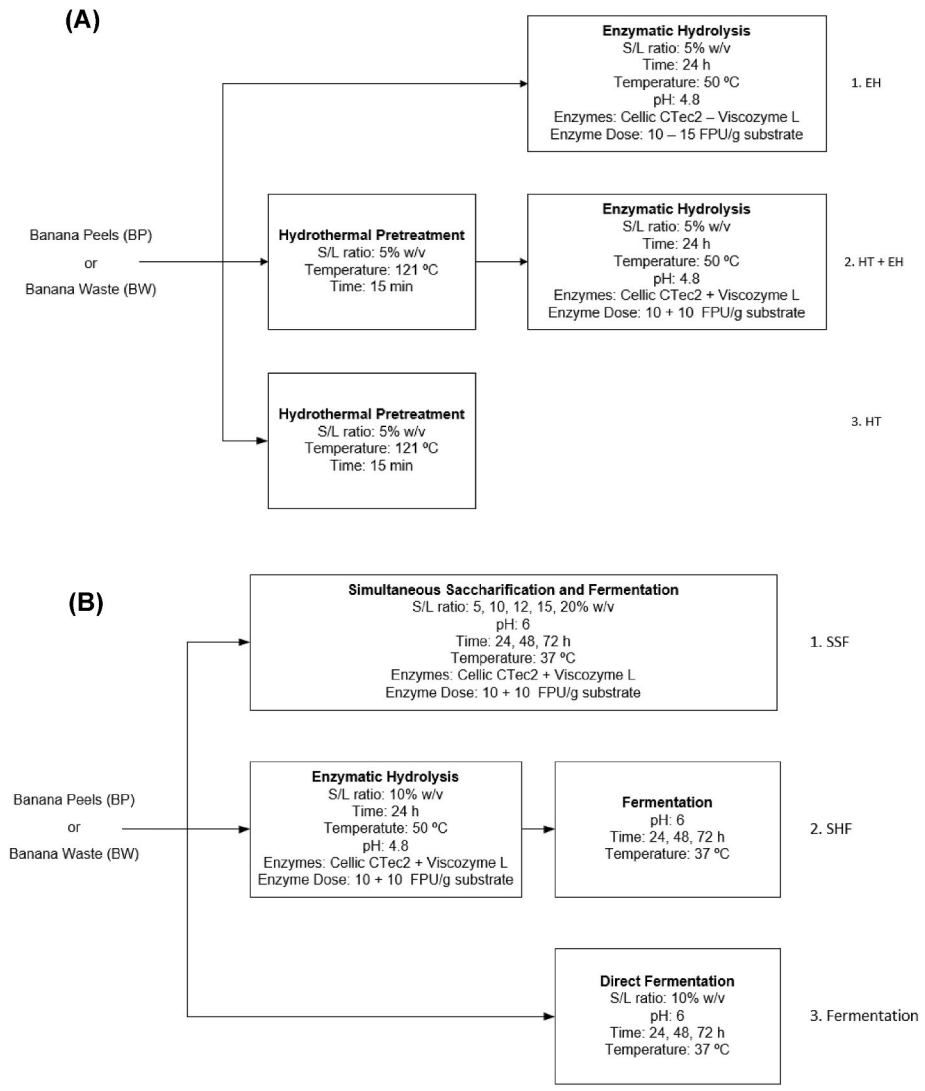

Fig. 1. Schematic process for the valorization of banana peels and whole banana for 2,3-butanediol production: (A) configurations for saccharification, (B) scenarios for fermentation.

inoculum preparation was employed as a supplement (except glucose), and the pH was adjusted to pH 6 with KOH 10 M. In the case of scenario 2, the enzymatic hydrolysate was previously pasteurized at 90 °C for 15 min. Fermentation assays were carried out in 250 mL Erlenmeyer flasks (containing 100 mL of medium) at 37 °C, 200 rpm, 144 h, and pH 6 in a rotary shaker (Miulab, China). Different substrate loadings (5–20 % of dry matter) were employed, using the enzymatic mixture of Cellic CTec2 and Viscozyme L at a dose of 10 FPU/g substrate of each. The inoculum loading was 10 % v/v, and no pH control was employed during the fermentation. Samples were withdrawn at 24, 48, 72 and 144 h, centrifuged (at 13,500 rpm for 10 min, (Mini Spin, Eppendorf, Germany)), and their content in sugars, 2,3-BD, ethanol, and acetoin were measured. All fermentation tests were performed, at least in duplicate.

The 2,3-BD yield (g 2,3-BD/g sugars consumed) was calculated as the relation between the 2,3-BD concentration (g/L) and the concentration of sugars (g/L) consumed during fermentation. As in scenarios 1 and 3, BP and BW were used directly, the concentrations of sugars obtained after EH under the same process conditions were considered as the initial sugars in SSF. On the other hand, the 2,3-BD productivity (g/L·h) was calculated as the ratio between the 2,3-BD concentration (g/L) and the fermentation time (h) at which this 2,3-BD concentration was measured.

2.5. Analytical methods

Analytical methods from the National Renewable Energy Laboratory (NREL) were employed to analyze the content of extractives [29], structural carbohydrates [30], acid-insoluble lignin [30], and ash [31] in BP and WB. High-Performance Liquid Chromatography (HPLC) was used to determine the content of sugars (glucose and other monosaccharides) and fermentation products (2,3-BD, ethanol, and acetoin), using a refractive index detector (Waters 2414), an Aminex HPX-87H column (at 60 °C), and 0.01 N H_2SO_4 (0.6 mL/min) as mobile phase. On the other hand, an acid hydrolysis step (120 °C, 3 % w/v H_2SO_4, 30 min) was applied to quantify the oligomeric sugar concentration in the liquid fractions resulting from the HT of BP and WB [30]. Macro, micronutrient and heavy metal concentrations were determined by ICP analysis, as described by Fernández-Delgado et al. [32]. All analytical determinations were carried out in triplicate, and the average results are shown.

2.6. Economic evaluation

A preliminary economic study of an industrial plant for 2,3-BD production from 100 kg/h of BP and WB was carried out to compare the three fermentation scenarios and assess their potential viability at a

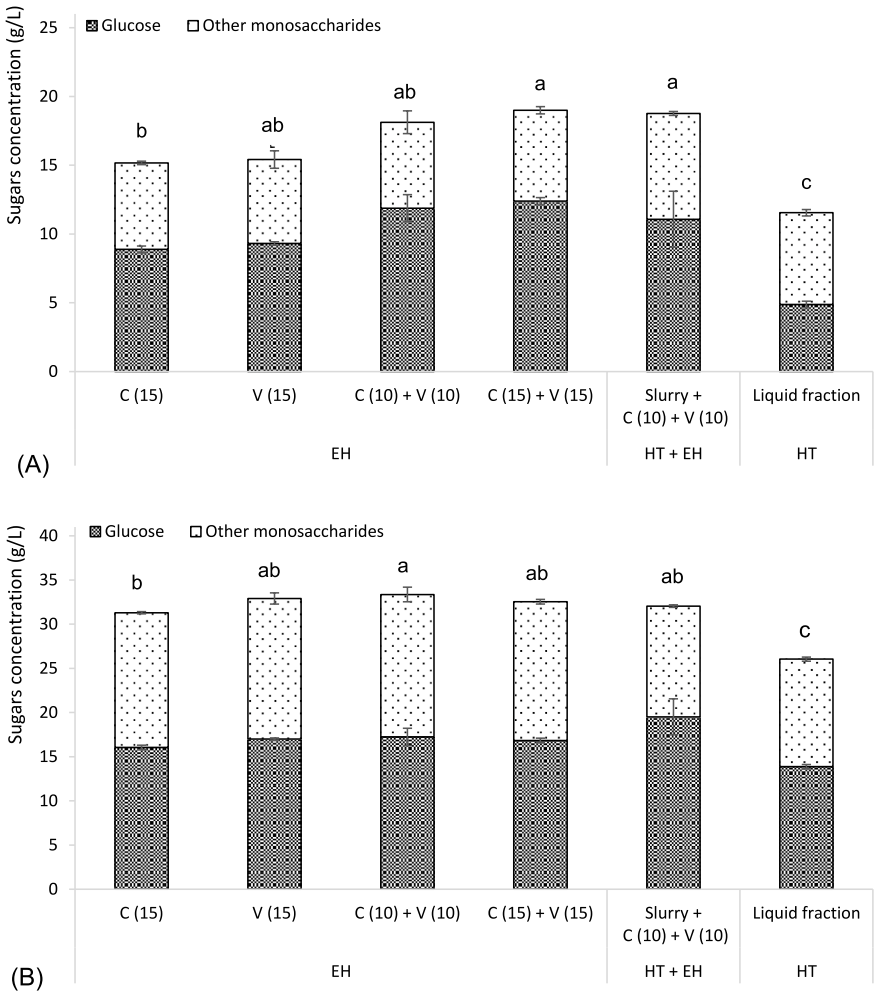

Fig. 2. Concentration of monosaccharides obtained from the saccharification of (A) banana peels and (B) whole banana. Treatments with the same letter are not significantly different ($p > 0.05$) referred to total sugars (Glucose + Other monosaccharides).Abbreviations: EH: Enzymatic Hydrolysis, HT: Hydrotermal Treatment, C: Cellic CTec2, V: Viscozyme L.

larger scale. The 2,3-BD flow rate was calculated based on the experimental yields solving the mass balance of the proposed processes. Two steps were considered to estimate the minimum selling price of 2,3-BD. First, the upstream (SSF, SHF or direct fermentation) was designed based on the laboratory data conditions. The associated purchased equipment cost (PCE) was estimated using Matches' online cost estimation tool. This database compiles purchase equipment prices [33]. PCE was adjusted to the year 2023 to ensure consistency and accuracy in the analysis. After that, the Lang factors method, extensively used in industrial engineering to calculate plant costs, was applied to calculate the Total Investment Cost (TIC) of the upstream process. A more detailed description of the method can be found in the literature [34–36]. Then, the downstream cost (recovery and purification) was estimated. The theoretical production of 2,3-BD was calculated, assuming that a vacuum distillation would recover 76.2 % of the 2,3-BD in the fermentation broth with a purity of 96.1 % since no downstream data was available [30]. This part of the process, being the most expensive of the plant, can range from 50 % to 70 % of the total plant costs [37–39]. A percentage of 70 % was applied to ensure a more conservative estimation that could account for potential unforeseen circumstances and contingencies.

The costs of the raw materials were obtained from the literature (process water, 3.16 €/m^3 [40]; and enzymes, 0.9 €/kg [41]). The enzyme cost considered was a generalized estimate of 0.9 €/kg, encompassing the range of enzyme prices from 0.9 €/kg to 2.1 €/kg reported [41,42]. Despite potential variations in enzyme costs due to different strains and production methods being acknowledged, uniformity was prioritized in the economic analysis. By selecting the lower end of the price spectrum, consistency across all scenarios was ensured, facilitating direct comparisons. The following assumptions were necessary to estimate the plant profits and the minimum selling price. A negligible cost was assumed for BP and WB. All scenarios and equipment amortization had a plant lifetime of 10 years. The annual production costs per L of 2,3-BD were estimated, considering the plant operates at 8000 h/y. Finally, the minimum sale price could be calculated, considering a net present value (NPV) of the plant of 0 € and an internal rate of return (IRR) of 10 % [35,43]. In this way, the minimum sale price could be compared with the selling price of 2,3-BD to determine the economic viability of the process.

2.7. Data analysis

The statistical software R (version 4.2.2. – Innocent and Trusting –

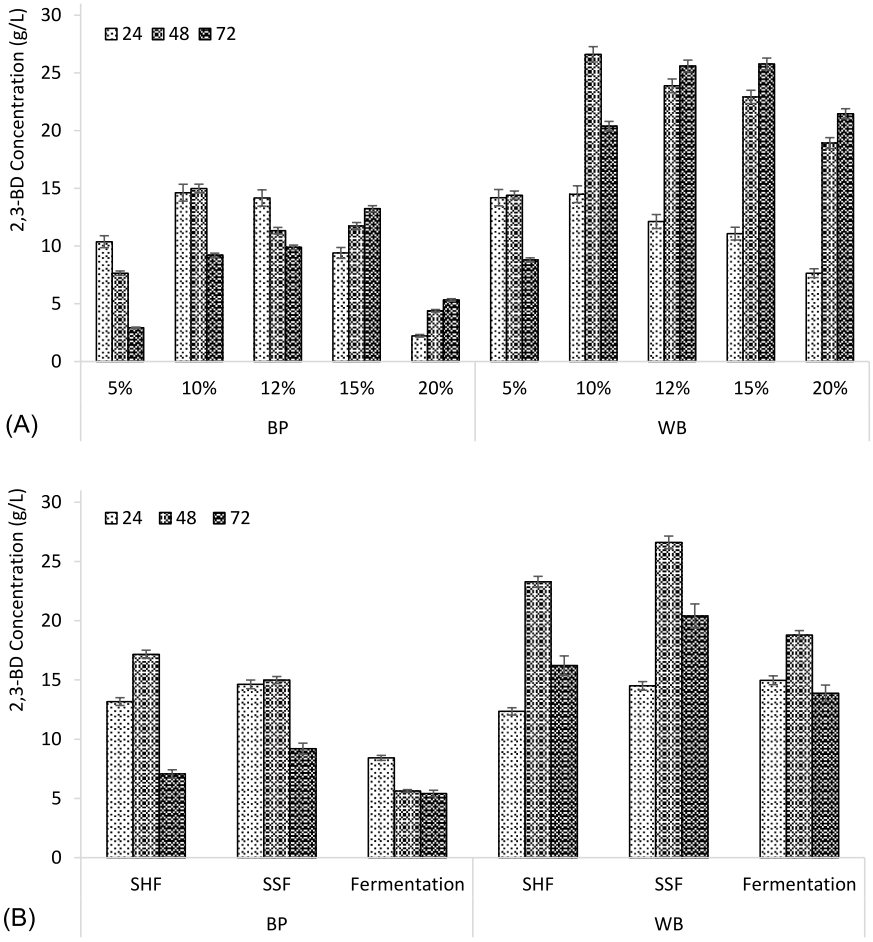

Fig. 3. 2,3-BD production from banana peels (BP) and whole banana (WB) for (A) SSF at different solid loadings (C + V (10 + 10)), and (B) comparison of the three fermentation strategies at a S/L ratio of 10 % w/v.

2022) was used to analyze the experimental data. Tukey's multiple-range tests analyzed the data to determine the statistically significant differences at a 95 % confidence level ($p < 0.05$).

3. Results and discussion

3.1. Characterization of banana wastes

The characterization of WB and BP (Table 1) reveals that both raw materials are abundant in monosaccharides, glucan and hemicellulose. The aqueous extract from WB (59.3 %) exceeds that obtained from BP (45.9 %). This difference can be attributed to the fact that the peel contains less free sugars than the whole banana, which includes both the peel and the fruit. The analysis of acid-soluble lignin content demonstrates relatively low values (BP: 2.0 %, WB: 2.1 %), not the content of the acid-insoluble lignin (BP: 16.7 %, WB: 8.9 %). Consequently, it can be inferred that both WB and BP offer significant potential for the enzymatic conversion of polysaccharides into simple sugars [26] although they could need pretreatment to free these sugars [44]. Compared with other studies, the BP composition aligns with the findings of Pathak et al. [45], who reported a hemicellulose content of 10.2 % for banana peels. Furthermore, Gupta et al. [1] reported a hemicellulose content ranging from 6.4 % to 9.4 % and lignin content from 6 to 12 % in banana peels. As indicated by Díaz et al. [46], most of the lignin can be found in the peel, which fits with the results obtained in this study (BP: 18.8 % vs. WB: 10.9 %). Oliveira et al. [47] presented a similar content of lignin (16.8 %) for banana peel, corroborating the BP composition data reported in Table 1. In addition, these previously published studies pointed out that glucose is one of the main sugar components in banana pulp and banana peel. The ash composition shows similar values for BP and WB (20.6 %–25.8 %). The K content is high in both raw materials. It also contains significant levels of Mg and

Table 1
Composition of banana peel and banana waste (Units: %, dry weight).

		Banana peel (BP)	Whole Banana (WB)
Glucan		14.2 ± 1.3	11.0 ± 0.2
Arabinan		2.2 ± 0.0	0.5 ± 0.0
Hemicellulose		6.3 ± 0.0	2.8 ± 0.1
Extractives in water		45.9 ± 1.8	59.3 ± 1.2
Composition of extractives in water			
	Glucose	9.3 ± 2.2	19.9 ± 0.1
	Arabinose	0.5 ± 0.2	0.8 ± 0.1
	Other monosaccharides	8.0 ± 3.1	15.1 ± 0.9
Lignin	Acid-soluble lignin	2.1 ± 0.0	2.0 ± 0.1
	Acid-insoluble lignin	16.7 ± 0.6	8.9 ± 0.8
Ash		20.6 ± 0.2	25.8 ± 0.3
Composition of Ash			
	K	5.5	2.4
	P	0.12	0.12
	Ca	0.20	0.09
	Mg	0.11	0.13
	Na	0.02	0.02

Table 2
Recovery of monosaccharides (%) from banana peels. Comparison of the saccharification alternatives.

Saccharification alternative	Enzyme (FPU/g substrate)	Glucose (%)	Other monosaccharides (%)	Total Sugars (%)
EH	C (15)	71.4	87.9	77.4
	V (15)	74.8	85.3	78.7
	C + V (10 + 10)	95.5	87.3	92.5
	C + V (15 + 15)	99.5	92.4	96.9
HT + EH	Slurry + C + V (10 + 10)	89.0	100.0	95.8
HT	Liquid fraction	45.7	100.0	63.7

*Whole Banana: Recovery around 100 % in all cases.
Enzyme dose in brackets (FPU/g substrate).
Abbreviations: EH: Enzymatic Hydrolysis, HT: Hydrotermal Treatment, C: Cellic CTec2, V: Viscozyme L.

Ca. However, it must be considered that the composition of banana waste depends on the production location, the soil pollution and the banana variety [48,49].

3.2. Saccharification of banana wastes

The efficiency of different saccharification alternatives based on hydrothermal and enzymatic hydrolysis was compared to select the most effective for WB and BP. Fig. 2A shows that the highest monosaccharide content obtained from BP corresponded to EH with an enzyme mixture of C + V (15 + 15), reaching 19.0 ± 0.5 g/L of total sugars, of which 12.4 ± 0.3 g/L were glucose, with a total sugar recovery of 96.9 % (Table 2). However, the enzyme mixture of C + V (15 + 15) did not present significant differences ($p > 0.05$) compared to when the mixture of enzymes C + V (10 + 10) was used, which led to a concentration of sugars of 18.1 ± 1.5 g/L (glucose: 11.9 ± 1.0 g/L) and 92.5 % of sugar recovery (Table 2). Finally, lower sugar concentrations were reached using a single enzyme. For both C (15) and V (15), the sugar concentrations were around 15.0 g/L, with a glucose concentration of 9 g/L, without significant differences ($p > 0.05$) between them.

Some references pointed out that applying an acid-diluted pretreatment [50] or hydrothermal pretreatment [51] is necessary before carrying out the enzymatic hydrolysis and fermentation of the banana waste. HT is a commonly employed alternative for pretreating lignocellulosic biomass in biofuel production processes, such as bioethanol and biobutanol [52]. HT effectively breaks down plant cell walls and releases sugars with a lower environmental impact than acidic pretreatments [53,54]. In the present study, the slurry obtained after HT reached a sugar concentration of 11.5 ± 0.5 g/L (glucose: 4.9 ± 0.2 g/L; Fig. 2A) and 63.7 % of sugar recovery (Table 2). These concentrations are lower than those obtained after EH with C + V (10 + 10) (11.5 ± 0.5 vs 18.1 ± 1.5 g/L). Moreover, Fig. 2A shows no significant improvement ($p > 0.05$) between the concentrations of total sugars obtained after EH with C + V (10 + 10) (18.1 ± 1.5 g/L) or HT + EH (18.8 ± 2.2 g/L). Comparing the three saccharification strategies for BP. It can be concluded that HT did not increase significantly ($p > 0.05$) sugar recovery. (Fig. 2A; Table 2). Therefore, the selected alternative for BP saccharification was configuration 1, using a blend of Cellic CTec2 and Viscozyme L enzymes at a dose of 10 FPU/g substrate of each.

Regarding results with WB (Fig. 2B), high sugar concentrations were obtained after EH with C + V (10 + 10) (33.3 ± 0.6 g/L). This value was similar ($p > 0.05$) to the concentration found after HT + HE (32.0 ± 0.2 g/L) and significantly higher ($p < 0.05$) than after HT (26.1 ± 1.0 g/L). As observed with BP, the application of a thermal pretreatment before EH did not enhance significantly ($p > 0.05$) sugar concentration. If compared to BP, the use of WB led to sugar concentrations that were almost two-fold higher than those obtained with the peel (17 vs. 33 g/L). These findings are consistent with the characterization of the residues, which revealed that WB presented a higher monosaccharide content than BP. Finally, it can be concluded that the best saccharification alternative for WB is configuration 1, as observed with the banana peel.

Other studies reported high sugar recoveries (greater than 90 %) with a blend of enzymes without previous pretreatment using agro-industrial wastes such as carrot discards [52], orange bagasse [55], spent coffee grounds [56], bananas, apples, mangos, and papayas [57]. Favaretto et al. [57] found that the blending of enzymes reduces the enzymatic hydrolysis time. López-Linares et al. [52] compared different types of enzymes and mixtures to recover fermentable sugars from carrot discards. They concluded that blending Cellic CTec2 and Viscozyme L improved the sugar concentration, recovering up to 76 % after 24 h EH. Baltaci and Hamamci [55] hydrolysated orange bagasse using cellulolytic and pectinolytic enzymes. The final sugar recovery was higher than

Table 3
Fermentation of the hydrolysates obtained from banana peels (BP) and whole banana (WB). 2,3-BD yield ($Y_{2,3\text{-BD}}$/sugars expressed as g/g sugars consumed); and 2,3-BD productivity (defined as g L^{-1} h^{-1} at the optimum time (h)).

Raw material	Time (h)	$Y_{2.3\text{-BD}}$ (g/g)	$P_{2.3\text{-BD}}$ (g/(L· h))	Sugar uptake (%)	Ethanol (g/L)	Acetoin (g/L)
SSF						
BP 5 %	24	0.48	0.43	100.0	1.6 ± 0.1	1.5 ± 0.1
BP 10 %	48	0.33	0.31	100.0	3.1 ± 0.2	3.3 ± 0.2
BP 12 %	24	0.39	0.59	70.6	2.6 ± 0.1	0.7 ± 0.1
BP 15 %	72	0.19	0.18	98.1	1.8 ± 0.3	2.8 ± 0.2
BP 20 %	72	0.10	0.07	60.3	2.4 ± 0.3	1.0 ± 0.1
WB 5 %	24	0.43	0.59	98.0	2.7 ± 0.1	1.2 ± 0.1
WB 10 %	48	0.36	0.55	99.3	4.5 ± 0.4	1.7 ± 0.3
WB 12 %	72	0.33	0.36	100.0	4.0 ± 0.1	3.5 ± 0.3
WB 15 %	72	0.35	0.36	78.4	3.7 ± 0.5	1.4 ± 0.4
WB 20 %	72	0.25	0.30	68.6	3.8 ± 0.4	1.3 ± 0.2
SHF						
BP 10 %	48	0.47	0.36	100.0	1.4 ± 0.1	6.0 ± 0.3
WB 10 %	48	0.49	0.49	83.3	2.4 ± 0.1	4.0 ± 0.4
Fermentation						
BP 10 %	24	0.38	0.35	97.7	2.2 ± 0.1	3.5 ± 0.1
WB 10 %	48	0.41	0.39	98.9	2.2 ± 0.1	4.4 ± 0.2

80 % after 24 h. Results show that high sugar concentrations and recoveries can be reached from fruit and vegetable waste after EH without applying a pretreatment. This is essential for the profitability of the global 2,3-BD production process from agro-industrial residues.

3.3. Fermentation processes

3.3.1. Simultaneous saccharification and fermentation (SSF)

Fig. 3A shows the 2,3-BD concentrations achieved for each substrate loading at different fermentation times (24, 48 and 72 h) for both BP and BW residues. As shown in this figure, when substrate loading increased from 5 % to 10 %, an increase in the 2,3-BD concentration of 40.9 % and 84.7 % took place for BP and WB, respectively at the time of maximum 2,3-BD production (24 h for BP and 48 h for WB). However, it is worth noting that a further rise in the solid percentage (12–20 %) did not yield a commensurate increase in 2,3-BD concentration without significant differences ($p > 0.05$) among them. As depicted in Fig. 3A, for substrate loadings higher than 10 %, the concentration of 2,3-BD reached similar concentrations and even exhibited a decline at 20 % substrate loading.

The maximum 2,3-BD concentrations were attained at different times, depending on the substrate loading and type of residue. For low solid loads (5 %–12 %), the optimal timeframe ranged from 24 to 48 h; whereas, for higher loads (15 %–20 %), the optimal period extended to 72 h (Fig. 3A; Table 3). This phenomenon can be attributed to the availability of fermentable sugars within the fermentation medium. So, in the SSF experiments performed at lower solid loads, sugars are released and metabolized earlier [58].

For both raw materials, the highest 2,3-BD concentration was attained at a solid loading of 10 % after 48 h, leading to a concentration of 15.0 ± 0.3 g/L for BP and 26.6 ± 0.6 g/L for BW (Fig. 3A). Consequently, this resulted in a 2,3-BD yield and productivity for BP of 0.33 g/g and 0.31 g/(L·h), respectively, and for BW, 0.36 g/g and 0.55 g/(L·h), respectively. Furthermore, in both cases, the consumption of sugars reached almost 100 % (Table 3).

3.3.2. Comparative of SSF process with separate hydrolysis and fermentation (SHF) and single-fermentation processes

The SSF fermentation of BP and BW, at the substrate loading where the higher 2,3-BD concentration was attained (10 % w/v), was compared with the processes SHF and direct fermentation, also using the mixture of enzymes previously selected (C + V (10 + 10)).

Fig. 3B compares the three strategies, revealing that the maximum 2,3-BD concentration was reached at 48 h in all cases (except for the direct fermentation of BP, where the highest 2,3-BD value was obtained after 24 h fermentation).

Fig. 3B shows that the 2,3-BD concentrations obtained after SHF (17.2 ± 0.3 g/L) and SSF (15.0 ± 0.2 g/L) of BP were significantly different ($p < 0.05$), being slightly higher in the case of SHF. Direct fermentation yielded a concentration of 2,3-BD that was much lower (8.4 ± 0.2 g/L). On the other hand, if the productivities and production yields are compared (Table 3), the SHF results ($P_{2,3\text{-BD}} = 0.36$ g/(L h); $Y_{2,3\text{-BD}} = 0.47$ g/g) were higher than those attained by direct fermentation ($P_{2,3\text{-BD}} = 0.35$ g/(L·h); $Y_{2,3\text{-BD}} = 0.38$ g/g) and SSF ($P_{2,3\text{-BD}} = 0.31$ g/(L·h); $Y_{2,3\text{-BD}} = 0.33$ g/g). Moreover, it is worth mentioning that the totality, or almost the totality (97.7 %–100 %), of sugars were consumed in all cases. So, no inhibition took place using a 10 % substrate loading.

Regarding WB, Fig. 3B shows that the SSF strategy yielded the highest 2,3-BD production (26.6 ± 0.6 g/L), with the highest and significant values at 48 h for the three tested strategies. In this way, at 48 h, the SSF enhanced 2,3-BD concentration by 1.14 times compared to SHF (26.6 ± 0.6 vs. 23.3 ± 0.5 g/L), although without significant differences ($p > 0.05$), and up to 1.41 times compared to direct fermentation (26.6 ± 0.6 vs. 18.8 ± 0.4 g/L), observing significant differences ($p < 0.05$) between them. This same behavior was also observed in 2,3-BD productivities (Table 3), being higher for the SSF strategy than the SHF (0.55 vs. 0.49 g/(L·h)) and direct fermentation (0.55 vs. 0.39 g/(L·h)). However, when comparing 2,3-BD yields (Table 3), the separate process (SHF) showed a higher yield (0.49 g/g) than the simultaneous process (SSF) and single fermentation (0.36 and 0.41 g/g, respectively).

Considering the results obtained for BP and WB, as well as the several advantages of the simultaneous processes, such as the prevention of end-product inhibition by enzymatic saccharification and the use of the same reactor vessel [59], from an industrial point of view, the SSF process could be a more effective process for 2,3-BD production. On the other hand, although the strategy of direct fermentation could be more profitable from an industrial point of view, since enzymes were not added, this strategy was not effective for BP and quite limited for WB, despite the significant amount of free sugars in the raw materials. Moreover, by comparing the fermentation results for both raw materials, WB achieved better results regarding concentration, yield, and productivity of 2,3-BD. This is probably due to the higher free sugar content in WB, which improves fermentation.

Besides 2,3-BD, a considerable content of acetoin was measured in all fermentation tests. In this way, between 0.7 ± 0.1 and 6.0 ± 0.3 g/L of acetoin were achieved for BP, while from 1.2 ± 0.1 up to 4.4 ± 0.2 g/L were attained for BW. Ethanol was another byproduct found in the fermentation broths, with concentrations ranging from 1.4 ± 0.1–3.1 ± 0.2 to 2.2 ± 0.1–4.5 ± 0.4 g/L for BP and BW, respectively (Table 3). Ethanol and acetoin are byproducts originating in 2,3-BD fermentation from pyruvate intermediate. Acetoin is formed through successive α-acetolactate synthase and 4,α-acetolactate decarboxylase pathways, while ethanol is produced by successive pyruvate–formate lyase, acetaldehyde dehydrogenase, and ethanol dehydrogenase pathways [60]. Considerable acetoin generation took place at longer fermentation times when sugar concentrations in the fermentation medium were low, the 2,3-BD produced also being considerably assimilated by the microorganism. According to Maina et al. [11], 2,3-BD can be converted to

Table 4
Operation conditions of proposed fermentation scenarios.

Raw material	Operation condition	Scenario 1 (SSF)	Scenario 2 (SHF)	Scenario 3 (Fermentation)
BP	S/L ratio (%)	10	10	10
	Time (h)	48	EH:24 Ferm: 48	24
	Temperature (°C)	37	EH: 50 Ferm: 37	37
	Enzymes	C + V	C + V	–
	Enzyme Dose (FPU/g substrate)	10 + 10	10 + 10	–
	$Y_{2,3\text{-BD}}$ (g/g)	0.333	0.470	0.382
WB	S/L ratio (%)	10	10	10
	Time (h)	48	EH:24 Ferm: 48	48
	Temperature (°C)	37	EH: 50 Ferm: 37	37
	Enzymes	C + V	C + V	–
	Enzyme Dose (FPU/g substrate)	10 + 10	10 + 10	–
	$Y_{2,3\text{-BD}}$ (g/g)	0.39	0.49	0.41

Abbreviations: EH: Enzymatic Hydrolysis, Ferm: Fermentation, C: Cellic CTec2, V: Viscozyme L; BP: Banana Peel; WB: Whole Banana.

Table 5
Preliminary economic evaluation of the proposed scenarios for fermentation of WB and BP for 2,3-BD production.

	2,3-BD Global Yield	PCE	TIC	Production Cost		Minimum Selling Price
Units	L/100 kg dry matter	€	€	€/year	€/L	€/L
Banana Peels						
Scenario 1 (SSF)	8.5	293000	1400000	1580000	23.3	34.5
Scenario 2 (SHF)	13.2	391000	1800000	1820000	17.2	26.2
Scenario 3 (Fermentation)	4.8	293000	1400000	980000	25.7	43.4
Whole Banana						
Scenario 1 (SSF)	15.1	293000	1400000	1580000	13.1	19.4
Scenario 2 (SHF)	13.5	391000	1800000	1820000	16.6	25.3
Scenario 3 (Fermentation)	10.6	293000	1400000	980000	11.4	19.5

acetoin, regenerating the NADH and keeping a continual oxidation-reduction state.

When comparing our results with those obtained from other agricultural residues, there is a significant variation in 2,3-BD production depending on the specific raw material used, the process configuration, and the microorganisms employed [12,13]. For instance, the SSF configuration was used for 2,3-BD production by Enterobacter cloacae sp.SG1 from oil palm front, yielding 30.7 g/L of 2,3-BD with a productivity of 0.32 g/(L·h) [61]. 20.6 g/L of 2,3-BD were also attained from corncob residue after 36 h SSF, using *Enterobacter cloacae* UV4 [62]. López-Linares et al. [63] attained 18.8 g/L 2,3-BD, with a 2,3-BD yield of 0.43 g/g, from carrot discard employing the same microorganism used in this work (*P. polymyxa* DSM 365) and an SHF configuration. Using the SHF configuration, *P. polymyxa* DSM 365 was also used to obtain 2,3-BD (23–32 g/L) from wheat straw [64]. According to Xie et al. [65], P. polymyxa DSM 365 is considered one of the 2,3-BD producer microorganisms able to generate the highest production titer and productivity of 2,3-BD. Therefore, this indicates that the 2,3-BD production potential is influenced not only by the type of substrate, but also by the specific microorganism used and the fermentation conditions.

3.4. Preliminary economic evaluation for 2,3-BD fermentation scenarios

A preliminary economic study to compare the 2,3-BD production strategies from BP and WB was carried out. As a basis for calculation, 100 kg/h of dry waste (BP or WB) with 75 % humidity was assumed. The overall mass balance was carried out at the selected conditions for the fermentation strategies (SSF, SHF and fermentation; Table 4). All

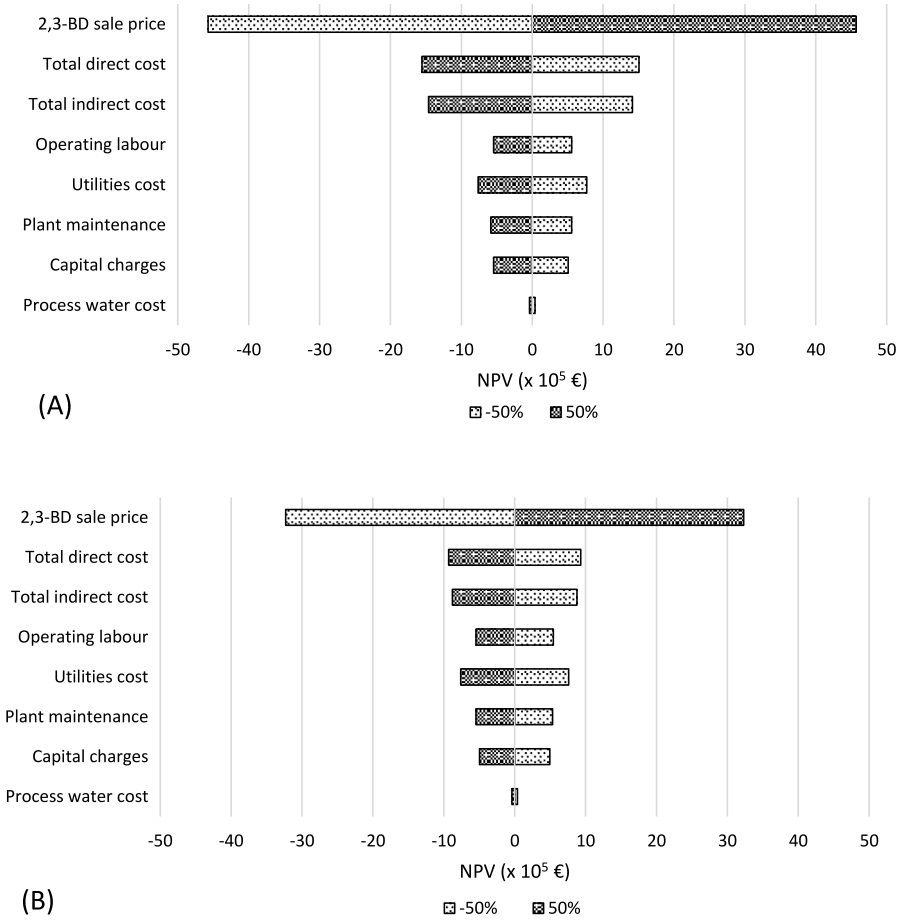

Fig. 4. Sensitivity analysis for the best economic scenarios. (A) SSF for Banana Peels and (B) Direct Fermentation for Whole Banana.

assumptions and methods used to make the economic assessment were detailed in section 2.6. Economic evaluation.

3.4.1. Investment and production costs

Table 5 summarizes the results of the Lang Factor method for the six proposed scenarios. As seen in Table 5, the global production 2,3-BD yields from BP were lower than from WB (4.8–13.2 vs. 10.6–15.1; L 2,3-BD/100 kg dry waste). In the case of BP, the highest 2,3-BD yield was obtained in scenario 2 (SHF) with 13.2 L/100 kg of dry matter. On the other hand, WB brought the best result in scenario 1 (SSF) with 15.1 L/100 kg of dry matter. Scenario 3 (fermentation) got lower 2,3-BD yields in both cases. The PCE of scenarios 1 and 3 are identical (293000 €) and independent of the raw material used. This is because the necessary equipment is the same in these scenarios. However, the PCE of scenario 2 is higher (391000 €). This increase in PCE is mainly due to the necessity of having two vessels, one for enzymatic hydrolysis and one for fermentation. According to the Lang factor method, the PCE directly affects the total investment cost (TIC), resulting in a higher TIC for scenario 2 (1800000 €) than for scenarios 1 and 3 (1400000 €). The production costs depend to a large extent on the reagents required. The main difference is the use of enzymes during the fermentation process. Scenario 3 has the lowest production cost (980000 €), since it does not require enzymes. In addition, Scenario 2 requires more energy to maintain the optimal temperature of each stage, leading to the highest production cost (1820000 €). Otherwise, the production cost per liter of 2,3-BD was associated with the annual production cost, the amount of 2,3-BD produced and its subsequent recovery. In the case of BP, scenario 2 yielded the lowest production costs per liter of 2,3-BD (17.2 €/L) due to the higher production yield of 2,3-BD. On the other hand, in the case of WB, scenario 3 emerged as the most cost-effective, with a production cost of 11.4 €/L. This result is in accordance with the lower annual cost of scenario 3. Finally, the minimum selling price for the 2,3-BD, shown in Tables 5 and is the selling price at which the plant would begin to be profitable. As can be seen, for BP, the lowest selling price for the 2,3-BD is associated with scenario 2, with 26.2 €/L, corresponding to the process with the higher 2,3-BD yield (13.2 L/100 kg dry waste). For WB, although scenarios 1 and 3 have similar minimum selling prices (19.4–19.5 €/L), direct fermentation (scenario 3) could be considered the best scenario from a technical and economic point of view (single fermentation with lower operating and fixed costs). In any case, these prices are above the estimated costs for the sale of 2,3-BD found in the market, indicating that the proposed processes are not profitable. For example, Maina et al. [11] point out that the price of petrochemically-derived 2,3-BD has an average of 1.4 €/L. In Europe, for the Quarter Ending June 2023, the price fluctuated around 2.3 €/L [66], between 8 and 11 times less than the prices estimated in this analysis. Process improvements are essential to reduce the minimum sale price of 2,3-BD produced by fermentation.

3.4.2. Sensitivity analysis

Based on the economic evaluation, a sensitivity analysis was performed to analyze the influence of the most critical parameters that could affect the NPV (Fig. 4).

The best scenarios selected were SSF for BP and direct fermentation for WB. The fundamental parameters significantly affecting the NPV are the 2,3-BD selling price and the total direct and indirect costs. In addition, process water is insignificant in the NPV variation in both scenarios (Fig. 4).

Concerning the plant profits, the only income is generated from selling 2,3-BD, which is the most influential factor on NPV. For example, a 50 % increase in the 2,3-BD sale price can increase the NPV by 32.3 M€ to 45.7 M€ for WB and BP, respectively (Fig. 4). However, the increment in the 2,3-BD price is unfeasible from an economic point of view, and it is necessary to reduce the selling price to be competitive.

4. Conclusions

This study addressed the valorization of banana wastes as feedstocks for producing 2,3-BD. The research evaluated three fermentation scenarios, SHF, SSF and direct fermentation, to determine the most favorable alternative from technical and economic standpoints. The characterization of the raw material revealed substantial amounts of monosaccharides and carbohydrates in both BP and WB. Enzymatic saccharification exhibited high sugar recoveries, indicating efficient sugar release without a previous pretreatment. Regarding 2,3-BD production, the results demonstrated the viability of both SSF and SHF for converting sugars into 2,3-BD from both feedstocks. Higher 2,3-BD concentrations were observed after 48 h, reaching up to 15.0 g/L for BP and 26.6 g/L for WB after SSF. Comparable concentrations were obtained through SHF, with 13.2 g/L for BP and 18.8 g/L for WB. From an economic perspective, a preliminary analysis considering investment and production costs alongside reagent prices indicated SSF and direct fermentation to be more cost-effective for BP and WB, respectively. However, they must be optimized to achieve a competitive and economically viable production. The findings demonstrate the potential of using banana wastes to produce high-value products such as 2,3-BD. Future research could explore ways to increase the process yield through continuous fermentation, investigate other microorganisms, find novel commercial applications for 2,3-BD, and produce other products to make the valorization process of banana wastes economically viable.

CRediT authorship contribution statement

Marina Fernández-Delgado: Writing – original draft, Methodology, Investigation. **Mercedes Rodríguez-Sarmiento:** Writing – original draft, Methodology, Investigation. **Jesus David Coral Medina:** Writing – review & editing, Supervision, Conceptualization. **Susana Lucas:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **M. Teresa García-Cubero:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Mónica Coca:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Juan Carlos López-Linares:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

Data availability

Data will be made available on request.

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