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Original article

Enzyme-assisted extraction of phenolic compounds and proteins from sugarcane bagasse using a low-cost cocktail from *Auricularia fuscosuccinea*

Romina O. Coniglio,[1,2*] Gabriela V. Díaz,[1,2] Ramona C. Barua,[1,2] Edgardo Albertó[3] & Pedro D. Zapata[1,2]

1 Universidad Nacional de Misiones, Facultad de Ciencias Exactas Químicas y Naturales, Instituto de Biotecnología Misiones "Dra. María Ebe Reca" (INBIOMIS), Laboratorio de Biotecnología molecular, Misiones, Argentina
2 CONICET, Buenos Aires, Argentina
3 Laboratorio de Micología y Cultivo de Hongos Comestibles y Medicinales, Instituto Tecnológico de Chascomús, Universidad Nacional de San Martín-CONICET. Chascomús, Buenos Aires, Argentina

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Abstract Sugarcane bagasse is the major by-product of the sugarcane industry, and it can serve as a substrate for biotechnological processes for obtaining value-added products. This study gave multiple adding values to sugarcane bagasse using it in two separate bioprocesses. Sugarcane bagasse was used as a substrate for enzymatic cocktail production from *Auricularia fuscosuccinea* LBM 244 and as a source of proteins and phenolic compounds. *A. fuscosuccinea* LBM 244 enzyme cocktail-assisted extraction, commercial enzyme assisted extraction and conventional extraction were compared. Enzymatic-assisted extractions released 557–827% more protein content than those at 0 h. *A. fuscosuccinea* LBM 244 enzyme cocktail released 50% and 30% phenolic compounds more than conventional and commercial enzyme extraction, respectively. These phenolic compounds were represented mainly by ρ-coumaric and ferulic acids. On top of that, the cost of the enzymes in enzyme-assisted extraction was reduced fourfold using the *A. fuscosuccinea* LBM 244 enzyme cocktail.

Keywords *Auricularia* mushrooms, cassava bagasse, ferulic acid, sugarcane bagasse, β-glucosidases, ρ-coumaric acid.

Introduction

Scenarios analysed for the coming decades show a growing global demand for the use of by-products from the food industries as a source of new bioactive components (Dueñas *et al*., 2005). Phenolic compounds are natural antioxidants and represent an important group of bioactive compounds in foods that may prevent the development of many chronic diseases (Dueñas *et al*., 2005). Among phenolic compounds, the demand for phenolic acids is very high in industries as they have antioxidant potential and work for precursors of other significant bioactive molecules which are needed on regular basis for therapeutic, cosmetics and food industries (Kumar & Goel, 2019).

Cassava (*Manihot esculenta*) bagasse (CB) is generated from the roots of the cassava, after the production of flour or starch, forming tonnes of cassava bagasse as solid waste (Woiciechowski *et al*., 2002). Sugarcane (*Saccharum officinarum* L.) bagasse (SCB) is a fibrous material that remains of the sugarcane, after juice extraction (George *et al*., 2010). As a kind of natural product, the healthcare value of SCB should not be ignored (Zheng *et al*., 2017). In this way, some chemical processes have been employed to obtain bioactive compounds from SCB, such as alkaline and acid hydrolysis (Leal *et al*., 1994; Xu *et al*., 2005), steam explosion (Martín *et al*., 2007), ultrasound (Zheng *et al*., 2017) and pyrolysis (Naron *et al*., 2019). In this sense, the enzymatic-assisted extraction (EAE) represents an eco-friendly approach and commercial enzymes were used to improve the aqueous extraction of proteins and bioactive fractions from very different agricultural by-products including grape residues (Gómez-García *et al*., 2012), watermelon rind (Mushtaq *et al*., 2015) and sesame bran (Görgüç *et al*., 2019).

On the contrary, SCB and CB are very good substrates for producing hydrolytic enzymes since consist mainly of hemicellulose, cellulose and lignin. These hydrolytic enzymes can be used instead of commercial enzymes in the EAE process and consist mainly of

*Correspondent: E-mail: rominnaconiglio@hotmail.com.

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β-glucosidase (BGL, EC 3.2.1.21), 1,4-β-D- cellobiohydrolase (CBH, EC 3.2.1.91), endo-1,4-β-xylanase (EX, EC 3.2.1.8), xylan-1,4-β-xylosidase (BXL, EC 3.2.1.37 and 1,4-β-D-endoglucanase (EG, EC 3.2.1.4) (Gil López *et al*., 2019).

Paranaense rainforest, one of the most biodiverse ecosystems of the word, is a refuge for a great diversity of fungal species growing on standing trees and fallen branches producing enzymes responsible for wood biodegradation (Wright *et al*., 2008). These species can be isolated from the rainforest and used for producing hydrolytic enzymes in liquid media for an easier downstream recovery (Coniglio *et al*., 2017; Díaz *et al*., 2019). In this sense, it is well-known that using generally recognised as safe fungi and their enzymes provide strategies to improve nutraceutical properties and produce bioactive phenolic ingredients (Martinez-Avila *et al*., 2014).

We wanted to add value to agro-industrial by-products by producing a low-cost enzymatic cocktail from *Auricularia fuscosuccinea* and using this cocktail for the extraction of phenolic compounds and proteins from SCB.

Materials and methods

Feedstock material and mushrooms strains

SCB was provided by the Sugar Mill of San Javier (Misiones, Argentina), and CB was provided by San Alberto Cooperative (Misiones, Argentina). These materials were dehydrated at 60°C for 12 h, pounded to 40-mesh and stored at room temperature. Five mushroom strains of *A. fuscosuccinea* were used, LBM 242, LBM 243, LBM 244, LBM 245 and LBM 246, and are deposited in the culture collection of the Biotechnology Institute of Misiones 'María Ebe Reca', National University of Misiones, Argentina. Stock cultures were maintained on malt extract agar (MEA) plates at 4°C.

Culture conditions in liquid culture

To obtain the cell-free supernatant, two culture media optimised for the production of hydrolytic enzymes by Díaz *et al*. (2019) were tested. The culture media consisted of Czapek minimal medium supplemented with peptone 2.50 g l^{-1}, yeast extract 2 g l^{-1} and sugarcane bagasse 15 g l^{-1}; or peptone 2.19 g l^{-1}, urea 2.84 g l^{-1} and cassava bagasse 40 g l^{-1}. The five strains were reactivated on MEA at 28 ± 2°C for 7 days, and 3 plugs of MEA covered with fresh mycelium of each strain were inoculated into the liquid culture media and growth at 28°C and 100 rpm. The media were sampled every 5 days for one month and centrifuged at 3000 rpm for 20 min to obtain the supernatant. BGL activity was determined to select the optimal strain, culture medium and time of incubation for producing the enzymatic cocktail. Once the strain and optimal conditions were selected, the enzymatic cocktail was obtained and used to determine the enzymatic activities detailed below.

Enzymatic activities

BGL, CBH and BXL activities were assayed by the method described by Herr *et al*. (1978), using 500 μM p-nitrophenyl-β-D-glucopyranoside, p-nitrophenyl-β-D-cellobioside or p-nitrophenyl-β-D-xylopyranoside (SIGMA, MO, USA) respectively. One unit (U) of enzyme activity was defined as the amount of enzyme necessary to release 1 μmol of p-nitrophenol per minute.

EX and EG activities were quantified according to Miller (1959) using beechwood xylan (SIGMA, MO, USA) or carboxymethyl cellulose (CMC) respectively. One IU was defined as the amount of enzyme that released 1 μmol of product per minute under the assay conditions.

Conventional extraction

For conventional extraction (CE) of SCB, 0.50 g of samples were individually extracted with 5 ml of ethanol 56% according to Gullón *et al*. (2019). The extracts were centrifugated at 1500 rpm for 20 min, and the supernatants were evaporated to dryness, dissolved in ethanol 56% and stored at −20°C until analysis.

Enzymatic-assisted extractions

For enzymatic-assisted extraction (EAE), hydrolysis of SCB 2% was carried out by Coniglio *et al*. (2020), followed by a conventional one-step extraction. Enzymatic cocktails from *A. fuscosuccinea* LBM 244 or Viscozyme L (Novozymes, Denmark) were used at 6 IU g^{-1} $biomass_{dw}$ (dry weight). A buffer-assisted extraction (BAE) was done as a control. BAE was an extraction carried out with sodium acetate 0.05 M, pH 4.8 buffer instead of enzymatic extract. Sodium azide 0.20% was supplemented as antibacterial. After eight hours of incubation at 50°C and 20 rpm, EAE and BAE reactions were finished by boiling for 5 min and filtered through filter paper, and supernatants were stored at 4°C until used. For all assays, SCB was dry at 40°C for 15 h and then was extracted again at 50°C and 120 rpm for 225 min, using aqueous ethanol as solvent (56%). After centrifugation at 1500 rpm for 20 min, the supernatants were combined with those obtained from enzymatic hydrolysis, evaporated to dryness, dissolved in ethanol 56% and stored at −20°C until analysis.

Control reactions without substrate and without enzyme were also included, to subtract possible proteins and phenols present in the enzymatic extract or released from the substrate as a consequence of the incubation.

Total phenolics content

Total phenolics content (TPC) was analysed following Singleton *et al.* (1999) using 1 N Folin/Ciocalteu reagent (Biopack, Argentina). TPC was expressed in mg equivalents of gallic acid per ml against a standard curve.

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl radicals

Scavenging ability on 1,1-diphenyl-2-picrylhydrazyl (DPPH, SIGMA, MO, USA) radicals was determined according to the method by Yamaguchi *et al.* (1998). Readings were compared with a standard, containing 100 µl of distilled water instead of extract. The scavenging ability was calculated by the following equation:

$$Scavenging\ ability\ :\ \left\{\left(Abs_{517}^{standard} - Abs_{517}^{extract}\right)/Abs_{517}^{standard}\right\} \times 1000 \quad (1)$$

Where: $\boldsymbol{Abs_{517}^{standard}}$ is the absorbance at 517 nm of the standard and $\boldsymbol{Abs_{517}^{extract}}$ is the absorbance at 517 nm of the reactions containing the extracts.

Total protein content

Protein content was determined following Bradford (1976) and was expressed in mg ml^{-1} against a bovine serum albumin standard curve.

Identification of phenolic acids released from SCB by HPLC-UV

Phenolic acids released from SCB by the different extraction strategies were identified by HPLC-UV using an Ultimate 3000 RSLC (Dionex, Thermo Scientific) with UV-Vis Detector VWD-3400RS (Thermo Scientific) and a C18 Hypersil-GOLD analytical column (Thermo Scientific) at 15°C. The isocratic mobile phase consisted of A: H_2O MQ/0.10% formic acid and B: ACN/0.10% formic acid at a flow rate of 02 ml min^{-1}. The concentration of phenolic acids was quantified against patterns curves of vanillic acid (VCO), syringic acid (SCO), 4-OH-benzaldehyde acid (4OH), syringaldehyde acid (SLD), p-coumaric acid (COU) and ferulic acid (FCO) and vanillin (VNA) (SIGMA, USA).

Statistical analyses

All tests were conducted in triplicate and the results were expressed as mean ± standard deviation. Statistical differences among samples were estimated using Student's t-test and ANOVA (repeated measures ANOVA and Tukey's Multiple Comparison Test) using the software GraphPad Prism 5.0 (Graph Pad Software Inc., San Diego, CA).

Economic analysis

The economic analysis was carried out by comparing the cost in single extractions using the enzymatic cocktail from *A. fuscosuccinea* or commercial enzyme. In addition, a comparison of the cost for obtaining 1 mg of FCO or COU was performed.

Results and discussion

Evaluation of BGL activity of A. fuscosuccinea strains in liquid medium

In Figure 1, it can be observed that as time went on, BGL activity increased in all trails. There were no significant differences in BGL activities between media containing CB or SCB for the strains LBM 243, LBM 245 and LBM 246. BGL activity was higher in strain LBM 242 growing on medium containing SCB than in medium with CB at days 25 ($P < 0.01$) and 30 ($P < 0.001$). BGL activity was also higher in strain LBM 244 growing on medium containing SCB than in medium with CB at days 20, 25 and 30 ($P < 0.001$). The highest values of enzymatic activity ($P < 0.01$) were obtained at day 30 with the strains LBM 242 (383.40 U l^{-1}) and LBM 244 (393.10 U l^{-1}), without significant differences between them ($P > 0.05$). Additionally, the strain LBM 244 presented higher values of enzymatic activity ($P < 0.001$) than strain LBM 242 at days 20 (248.40 U l^{-1}) and 25 (277 U l^{-1}) (Fig 1).

While soluble phenolics can easily be released from the plant matrix, the extraction of insoluble-bound phenolic compounds is difficult because they are covalently bound to plant cell wall structural elements such as cellulose, hemicellulose, lignin and structural protein or polysaccharides (Wang *et al.*, 2017). In this context, BGL-assisted extraction significantly enhances the release of insoluble-bound phenolics content of vegetal matrices (Wang *et al.*, 2017). However, BGLs are generally in low quantity in many commercial preparations (Sørensen *et al.*, 2013).

To obtain an enzymatic cocktail with high BGL activity, we carried out a new trial using the optimal independent factors, *A. fuscosuccinea* LBM 244, medium enriched with SCB and 30 days of culture.

Production of enzymatic cocktail by A. fuscosuccinea LBM 244

Since the most commonly used enzymes for the extraction of bioactive compounds in the vegetal matrix are

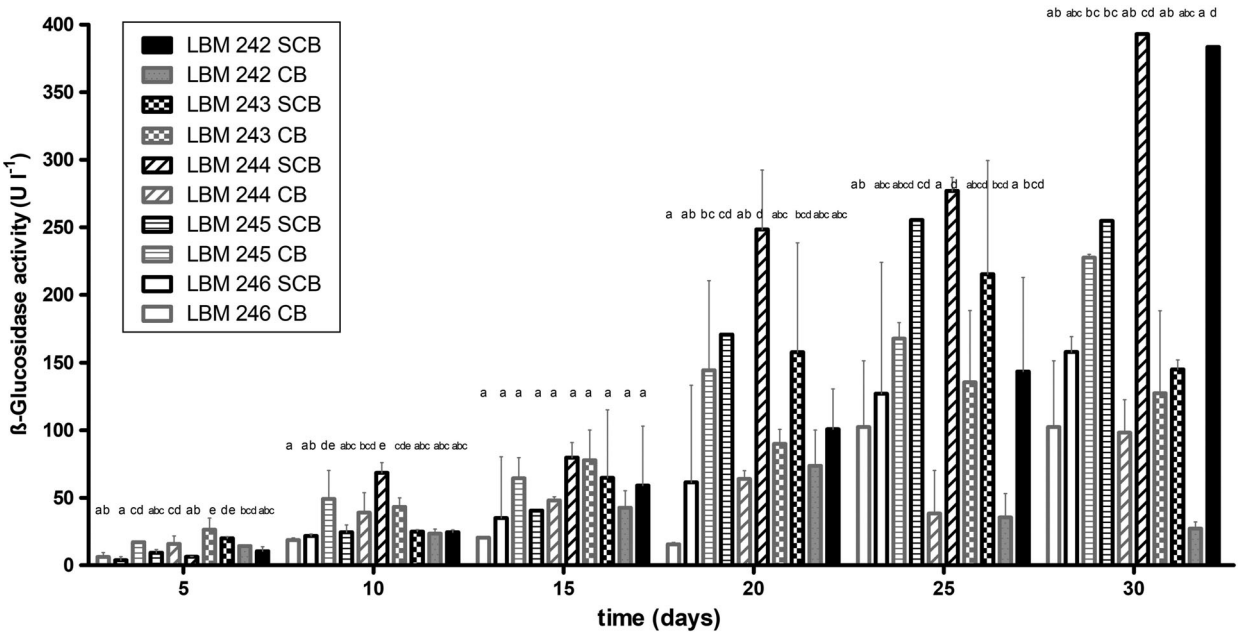

Figure 1 Effect of medium containing SCB and CB on BGL activity by strains *A. fuscosuccinea* LBM 242, LBM 243, LBM 244, LBM 245 and LBM 246 at 5, 10, 15, 20, 25 and 30 days of culture. The error bars represent the standard deviation of the triplicates. SCB: sugarcane bagasse; CB: cassava bagasse. Means with different letters are significantly different ($P > 0.05$).

Table 1 Cellulase and hemicellulose activities in 30-day cocktail from *A. fuscosuccinea* LBM 244 grown on SCB. The values represent the means of the triplicates ± standard deviation

Activity	Substrate	U l^{-1}
β-glucosidase	pNPG	205.90 ± 6.80
xylan-1,4-β-xylosidase	pNPX	15.90 ± 0.10
1,4-β-D- cellobiohydrolase	pNPC	16.80 ± 0.20
endo-1,4-β-xylanase	beechwood xylan	288.90 ± 51
1,4-β-D endoglucanase	CMC	186.10 ± 16.80

CMC, carboxymetylcellulose; pNPC, p-nitrophenyl-β-D-cellobioside; pNPG, p-nitrophenyl-β-D-glucopyranoside; pNPX , p-nitrophenyl-β-D-xylopyranoside; U, International units.

cellulases, xylanases and pectinases (Marathe *et al.*, 2017), enzymatic activities in cell-free supernatant of 30-day culture media with SCB of *A. fuscosuccinea* LBM 244 were determined. This study has shown that the enzymatic cocktail from *A. fuscosuccinea* LBM 244 had a broad spectrum of activities relating to SCB cell wall hydrolysis (Table 1).

The highest activity of the enzymatic cocktail from *A. fuscosuccinea* LBM 244 was EX, followed by BGL activity, but CBH, EG and BXL activities were also present, and therefore, this cocktail can be described as a 'cellulase–hemicellulose' blend. Agricultural wastes such as SCB have more xylan than softwoods (Van Dik and Pletschke, 2012). This could be the reason for high yields of EX produced by *A. fuscosuccinea* LBM 244 grown on SCB.

The use of enzymatic cocktails is transversal to most of the works focused on the EAE of phenolic acids (Nadar *et al.*, 2018) since the synergy between enzymes can improve the extraction yields due to the disruption of the bonds between the phenolics and the cell wall components of the SCB matrix. This disruption can promote the release of insoluble-bound phenolics into soluble forms and cause an increment in total phenolics content and antioxidant activity.

Obtention of bioactive compounds from SCB using A. fuscosuccinea LBM 244

The enzymatic cocktail from *A. fuscosuccinea* LBM 244 was evaluated in an EAE of bioactive compounds (EAE_{Aur}) and compared with an EAE using Viscozyme L (EAE_{Vis}). TPC, antioxidant activity and total protein content in the extracts were determined. Since there were not significant ($P > 0.05$) differences between BAE (control) and CE strategies, the analysis was carried out by comparing EAEs strategies and CE. A positive effect of enzymatic strategies on the extraction of phenolic compounds from SCB was observed with an incubation period of 8 h. The highest release of phenolics of SCB was obtained with EAE_{Aur} ($P < 0.05$), releasing about 50% phenolic compounds more than CE and 30% more than EAE_{Vis} (Fig 2a).

EAE is a green approach (Marathe *et al.*, 2017) and has been evaluated in citrus residues (Li *et al.*, 2006), black currant pomace (Kapasakalidis *et al.*, 2009) and winemaking by-products (de Camargo *et al.*, 2016). However, all of these works focused on the optimisation and comparison of EAE using commercial enzymes, which greatly increases the cost of the global EAE process. In this work, we demonstrated that the extraction of phenolic compounds from SCB can be improved by a homemade enzymatic cocktail. The observed increase in TPC with EAE_{Aur} was due to the release of free phenolics caused by the action of carbohydrate metabolising enzymes (Vattem & Shetty, 2002), which are described in Table 1. Moreover, the application of food-grade enzymes in the recovery of phenolic acids from biomass can hold their nutraceutical properties, which can be smoothly adopted by the food industry, allowing to obtain these antioxidant nutrients from renewable sources at a low cost (Costa *et al.*, 2020).

Plants with rich phenolic contents can be a valuable source of antioxidants; thus, all the extracts of SCB in this study were tested for their antioxidant activity. EAE_{Aur} was throughout a higher inhibition percentage than EAE_{Vis} strategy ($P < 0.05$). However, no significant difference was observed ($P < 0.05$) for scavenging capacity with DPPH free radical between the extracts obtained with EAE_{Aur} and CE strategy ($P > 0.05$) (Fig 2b).

Proteins are other important bio-actives as nutritional and dietary supplements (Marathe *et al.*, 2017). Among the extraction strategies, EAEs treatments greatly improved protein yield from SCB, releasing 827% (EAE_{Vis}) and 557% (EAE_{Aur}) more protein content than those at 0 h (126,88 mg l^{-1}); this value represents the proteins contained in the enzyme solutions. Otherwise, CE strategy released only 128% more protein content than those at 0 h (Fig 2c).

Identification of phenolic acids released from SCB by HPLC-UV

Extracts obtained using EAE_{Aur}, EAE_{Vis}, BAE and CE strategies were analysed by UHPLC-UV. The main phenolic compounds identified in the four extracts were ρ-coumaric (COU) and ferulic acids (FCO) (Table 2).

FCO and COU are abundant, valuable and renewable aromatic compounds with great potential as antioxidants in food and nutrition industries; moreover, COU exhibited *in vitro* and *in vivo* antiplatelet activity, having health benefits (Luceri *et al.*, 2007). The current methods used for their isolation vary greatly, and they are dependent upon the class of phenolic compounds and the nature of the matrix. Previous studies (Xu *et al.*, 2005; Ou *et al.*, 2009) reported the isolation of FCO, COU and related phenolic compounds from

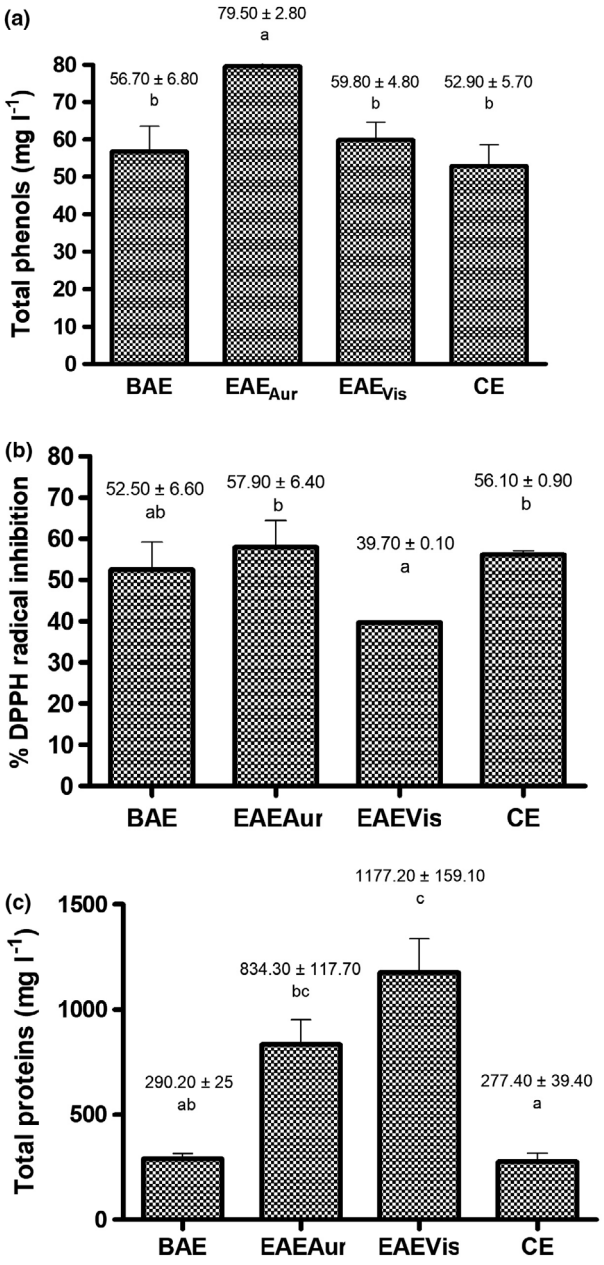

Figure 2 Comparison of the effect of different treatments. EAE_{Aur}: enzymatic-assisted extraction (*A. fuscosuccinea* LBM 244); EAE_{Vis}: Enzymatic-assisted extraction (Viscozyme L); BAE: buffer-assisted extraction; CE: conventional extraction) on (a) total phenols content, (b) free radical scavenging activity (DPPH assay), (c) total protein content. Data were recorded as the mean value ± standard deviation of three replicates. Means with different letters are significantly different ($P > 0.05$).

SCB by alkali and acid hydrolysis. However, chemical extraction could allow for low yields of phenolic compounds due to their sensitivity to oxidation and

Table 2 Abundance of phenolic acids released from SCB through conventional and enzyme-assisted extractions. The values represent the means of the duplicates ± standard deviation. Abundance is expressed in μg ml^{-1}

	VCO	4-OH	SCO	VNA	SLD	COU	FCO
EAE_{Aur}	4.50 ± 0.50 b	5 ± 0 b	4 ± 0 a	2.50 ± 0.50 b	3.50 ± 0.50 a	28.50 ± 2.50 c	17 ± 2 b
EAE_{Vis}	4 ± 0 b	5.50 ± 0.50 b	3 ± 0 a	2 ± 0 b	2.50 ± 0.50 a	41.50 ± 0.50 bc	12.50 ± 1.50 c
BAE	1.50 ± 0.50 a	5.50 ± 0.50 b	3 ± 0 a	2 ± 0 b	2.50 ± 0.50 a	24.50 ± 1.50 a	3.50 ± 1.50 b
CE	1 ± 0 a	3 ± 0 a	2.50 ± 0.50 a	1 ± 0 a	6.50 ± 0.50 a	15.50 ± 0.50 ab	10.50 ± 1.50 a

Means in columns without letters in common differ significantly ($P < 0.05$).
4-OH, 4-OH-benzaldehyde acid; BAE, buffer-assisted extraction; CE, conventional extraction; COU, p-coumaric acid; EAE_{Aur}, enzymatic-assisted extraction (*A. fuscosuccinea* LBM 244); EAE_{Vis}, enzymatic-assisted extraction (Viscozyme L); FCO, ferulic acid; SCO, syringic acid; SLD, syringaldehyde acid; VCO, vanillic acid; VNA, vanillin.

Table 3 Enzymatic cocktail from *A. fuscosuccinea* LBM 244 versus commercial enzyme, a cost-effectiveness study

	Enzymatic cocktail from *A. fuscosuccinea* LBM 244			Commercial enzyme
Medium components	**Unit cost (USD kg^{-1})**	**(g l^{-1})**	**Total cost (USD l^{-1})**	**Total cost (USD l^{-1})**
$NaNO_3$	115.35	2	0.24	
KH_2PO_4	363.65	1	0.37	
KCl	171.71	0.50	0.09	
$MgSO_4{\cdot}7H_2O$	336.49	0.50	0.17	
$FeSO_4{\cdot}7H_2O$	218.84	0.01	0.00	
Peptone	185.13	2.50	0.04	
Yeast extract	331.85	2	0.68	
SCB	0.01	15	0.00	
Water	0.07	1000	0.07	
Subtotal		1000	1.66	
Operational parameters	Unit cost (USD $year^{-1}$)	(Days l^{-1})	Total cost (USD l^{-1})	
Fixed asset investment	25.80	30	2.15	
Utilities (electricity, water and natural gas)	5.88	30	0.49	
Consumables	2.78	30	0.23	
Labour/fixed cost	5.38	30	0.45	
Subtotal			3.32	
Total			4.98	6171
Enzyme activity (U l^{-1})	200			6000
U $reaction^{-1}$	3			3
Volume used (ml $reaction^{-1}$)	15			0.05
Cost of enzyme (USD $reaction^{-1}$)	0.07			0.31
Cost of enzyme for 1 mg of COU	USD 4.39			USD 24.67
Cost of enzyme for 1 mg of FCO	USD 2.62			USD 7.43

COU, p-coumaric acid; FCO, ferulic acid; SCB, sugarcane bagasse; U, International Units; USD, United States dollar.

hydrolysis. Therefore, it becomes necessary to develop a green quantitatively method for isolating FCO, COU and related phenolic compounds from cell walls of plants and apply them for industrial use and human health. In this work, phenolic acids, such as FCO and COU, were released from SCB using an enzymatic cocktail from the edible mushroom *A. fuscosuccinea* LBM 244, which represents a green approach.

Although COU and FCO were the main phenolic compounds, the presence of small quantities of vanillic acid, 4-OH-benzaldehyde acid, syringic acid, vanillin and syringaldehyde acid was also recorded in the samples. The presence of these seven compounds in extracts endorsed the EAE strategy as a potential candidate for the recovery of phenolic acids from underutilised agro-industrial by-products and residues. Specifically, the observed increase in phenolic acid concentration using EAEs strategies demonstrates the importance of the enzyme activities for the release of these compounds from SCB.

Economic analysis

The cost of using enzymes for EAE of phenolic acids from SCB was examined by an economic analysis (Table 3). The prices of using commercial enzyme or

the enzymatic cocktail from *A. fuscosuccinea* LBM 244 produced with media containing SCB were compared.

The general cost in enzymes for one reaction was fourfold less expensive using the enzymatic cocktail from *A. fuscosuccinea* LBM 244 than using a commercial enzyme. On the contrary, the enzymatic cocktail from *A. fuscosuccinea* LBM 244 was more efficient for FCO extraction, while Viscozyme L was more efficient for COU extraction. However, there was a great difference in the cost of enzymes for producing 1 mg of both FCO and COU. The cost of the required enzymes for producing 1 mg of FCO with the enzymatic cocktail from *A. fuscosuccinea* LBM 244 was 2.83 times cheaper than producing 1 mg of FCO using Viscozyme L. On the contrary, the cost of the required enzymes for producing 1 mg of COU with the enzymatic cocktail from *A. fuscosuccinea* LBM 244 was 5.61 times cheaper than producing 1 mg of FCO using Viscozyme L.

Taking into account that the value of 1 mg of FCO (SIGMA-Aldrich, MO, USA) is 1,681.90 dollars, the cost of the commercial enzyme would represent 0.44% of the price, while the cost of the cocktail from *A. fuscosuccinea* LBM 244 would be 0.15%.

Conclusions

For the first time, we gave multiple adding values to SCB using it in two separate bioprocesses. SCB acts as an inducer for the production of a 'cellulase–hemicellulose' blend from *A. fuscosuccinea* LBM 244. This enzymatic blend involved the following enzymatic activities: β-glucosidase, xylan-1,4-β-xylosidase, 1,4-β-D- cellobiohydrolase, endo-1,4-β-xylanase and 1,4-β-D-endoglucanase. On the contrary, we demonstrated that the utilisation of enzymes successfully assisted the extraction of proteins and phenolic compounds from SCB. We also reported quantitative data of phenolic compounds from SCB being FCO and COU the main compounds identified in all extracts. More important, the cost of the enzymes used in EAE could be reduced 4 times using the enzymatic blend from *A. fuscosuccinea* LBM 244.

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Author Contribution

Romina Olga Coniglio: Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Software (equal); Supervision (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Gabriela Verónica Díaz:** Conceptualization (equal); Formal analysis (equal); Investigation (equal); Methodology (equal); Software (equal); Validation (equal); Visualization (equal); Writing – original draft (equal); Writing – review & editing (equal). **Ramona Celeste Barua:** Formal analysis (supporting); Methodology (supporting); Visualization (supporting); Writing – original draft (equal); Writing – review & editing (equal). **Edgardo Albertó:** Conceptualization (equal); Formal analysis (equal); Supervision (equal); Writing – review & editing (equal). **Pedro Darío Zapata:** Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Project administration (equal); Visualization (equal); Writing – review & editing (equal).

Ethical guidelines

Ethics approval was not required for this research.

Conflict of interest disclosure

There is not a potential conflict of interest in the work.

Peer review

The peer review history for this article is available at https://publons.com/publon/10.1111/ijfs.15477.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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