Revista Argentina de Microbiología
ISSN: 0325-7541
ram@aam.org.ar
Asociación Argentina de Microbiología
Argentina

Sepúlveda, Leonardo; de la Cruz, Reynaldo; Buenrostro, José Juan; Ascacio-Valdés, Juan Alberto; Aguilera-Carbó, Antonio Francisco; Prado, Arely; Rodríguez-Herrera, Raúl; Aguilar, Cristóbal Noé

Effect of different polyphenol sources on the efficiency of ellagic acid release by Aspergillus niger

Revista Argentina de Microbiología, vol. 48, núm. 1, 2016, pp. 71-77
Asociación Argentina de Microbiología
Buenos Aires, Argentina

Rev Argent Microbiol. 2016;48(1):71–77

REVISTA ARGENTINA DE MICROBIOLOGÍA

www.elsevier.es/ram

ORIGINAL ARTICLE

Effect of different polyphenol sources on the efficiency of ellagic acid release by *Aspergillus niger*

Leonardo Sepúlveda[a], Reynaldo de la Cruz[a], José Juan Buenrostro[c], Juan Alberto Ascacio-Valdés[a], Antonio Francisco Aguilera-Carbó[b], Arely Prado[c], Raúl Rodríguez-Herrera[a], Cristóbal Noé Aguilar[a,*]

[a] *Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila, 25280 Saltillo, Coahuila, Mexico*
[b] *Animal Nutrition Department, Universidad Autónoma Agraria Antonio Narro, 25315, Buenavista, Saltillo, Coahuila, Mexico*
[c] *Department of Biotechnology, Departamento de Biotecnología, Universidad Autónoma Metropolitana, 09340 Iztapalapa, Mexico*

Received 7 December 2014; accepted 5 August 2015
Available online 23 February 2016

KEYWORDS
Polyphenols;
Aspergillus niger;
Solid-state culture;
Ellagitannase

Abstract Fungal hydrolysis of ellagitannins produces hexahydroxydiphenic acid, which is considered an intermediate molecule in ellagic acid release. Ellagic acid has important and desirable beneficial health properties. The aim of this work was to identify the effect of different sources of ellagitannins on the efficiency of ellagic acid release by *Aspergillus niger*. Three strains of *A. niger* (GH1, PSH and HT4) were assessed for ellagic acid release from different polyphenol sources: cranberry, creosote bush, and pomegranate used as substrate. Polyurethane foam was used as support for solid-state culture in column reactors. Ellagitannase activity was measured for each of the treatments. Ellagic acid was quantified by high performance liquid chromatography. When pomegranate polyphenols were used, a maximum value of ellagic acid (350.21 mg/g) was reached with *A. niger* HT4 in solid-state culture. The highest amount of ellagitannase (5176.81 U/l) was obtained at 8 h of culture when cranberry polyphenols and strain *A. niger* PSH were used. Results demonstrated the effect of different polyphenol sources and *A. niger* strains on ellagic acid release. It was observed that the best source for releasing ellagic acid was pomegranate polyphenols and *A. niger* HT4 strain, which has the ability to degrade these compounds for obtaining a potent bioactive molecule such as ellagic acid.

* Corresponding author.
E-mail address: cristobal.aguilar@uadec.edu.mx (C.N. Aguilar).

http://dx.doi.org/10.1016/j.ram.2015.08.008

PALABRAS CLAVE
Polifenoles;
Aspergillus niger;
Cultivo en estado sólido;
Elagitanasa

Efecto de las diferentes fuentes de polifenoles sobre la eficiencia de liberación de ácido elágico por *Aspergillus niger*

Resumen La hidrólisis fúngica de los elagitaninos produce ácido hexahidroxidifénico, considerado como una molécula intermedia en la liberación de ácido elágico. El ácido elágico tiene importantes y deseables propiedades benéficas para la salud humana. El objetivo de este trabajo fue identificar el efecto de la fuente de elagitaninos sobre la eficiente liberación de ácido elágico por *Aspergillus niger*. La liberación de ácido elágico se realizó con tres cepas de *A. niger* (GH1, PSH y HT4) en presencia de diferentes fuentes de polifenoles (arándano, gobernadora y granada), usadas como sustrato. Se empleó espuma de poliuretano como soporte para el cultivo en estado sólido en reactores en columna. Se midió la actividad elagitanasa a cada uno de los tratamientos. El ácido elágico liberado se cuantificó por cromatografía líquida de alta resolución. Cuando se utilizaron los polifenoles de granada, se alcanzó un valor máximo de 350,21 mg/g de ácido elágico con *A. niger* HT4 en cultivo en estado sólido. La mayor actividad elagitanasa (5176.81 U/l) se obtuvo a 8 h de cultivo cuando se usaron los polifenoles de arándano como sustrato y *A. niger* PSH. Los resultados demostraron el efecto que tiene la fuente de polifenoles y la cepa de *A. niger* en la liberación de ácido elágico. Se observó que la mejor fuente para la liberación de ácido elágico fueron los polifenoles de granada y que la cepa *A. niger* HT4 posee la habilidad de degradar estos compuestos para la obtención de potentes moléculas bioactivas, como el ácido elágico.

Introduction

Polyphenols are a wide range of compounds considered to be secondary metabolites of different plant parts: leaves, stems, flowers, fruits, seeds, and others[2]. Polyphenols are divided into three main groups: complex tannins, condensed tannins and hydrolyzable tannins[17]. Ellagitannins are a class of hydrolyzable tannins. These compounds are essentially formed by a hexahydroxydiphenic acid (HHDP) group linked by an ester bond to a glucose[8]. There are over 500 different structures of ellagitannins reported in the literature[13]. Ellagitannins are mainly obtained from the bark and trunks of trees such as oak (*Quercus* spp.)[18] and eucalyptus (*Eucalyptus* spp.)[19]. Ellagitannins are abundant in some red berries[24], plants of the Mexican desert[6] and pomegranate fruit[22]. Ellagitannins have various beneficial health properties. It has been shown that the ellagitannins present in pomegranate juice can induce apoptosis by activation of the leukemic cell cycle[11]. Punicalagin is an ellagitannin from the pomegranate peel, which has been linked to apoptosis in HT-29 colon cancer cells[23]. Ellagitannins present in leaf extracts, flower extracts, peel, juice and seeds of pomegranate have beneficial effects on the control of obesity, diabetes, cardiovascular disease, hypertension and hypercholesterolemia[5]. Moreover, these polyphenols in pomegranate leaves, obtained by macroporous resin column chromatography have a high antioxidant activity[29]. Ellagitannins can be hydrolyzed by chemical or enzymatic methods to release the HHDP group[15]; this group undergoes a spontaneous molecular arrangement forming a new molecule called ellagic acid (EA)[3]. EA is a secondary metabolite of high industrial interest due to various beneficial effect on human health such as antiviral[16], anticarcinogenic[20], antioxidant[26], and anti-inflammatory activities[10]. Industrially, EA is obtained with the use of chemicals, involving high production costs and recovery as well as environmental damage. Therefore, it is necessary to develop methods for the biotechnological production of secondary metabolites. There are few studies about obtaining EA by bioprocessing agroindustrial residues such as pomegranate peel and microorganisms that are capable of degrading the ellagitannin compounds present in the waste by solid-state culture (SSC) to generate EA[25]. The aim of this work was to evaluate the effect of different polyphenol sources: cranberry (CP), creosote bush (CBP) and pomegranate (PP) on the hydrolytic efficiency of three different xerophilic *Aspergillus niger* strains, denominated A-GH1, A-PSH and A-HT4, to obtain EA by SSC.

Materials and methods

Polyphenol extraction and purification

Cranberries (*Vaccinium macrocarpon* Ait.) were originally from Wisconsin, USA, and were received in vacuum packed bags (350 g) in the month of January of the year 2012. Pomegranates (*Punica granatum* L. var. Wonderful) were originally from California, USA, and were received in cardboard boxes (25 kg), in the same session and year. These fruits were purchased at a supermarket in Saltillo, Coahuila, Mexico. Creosote bush plants (*Larrea tridentate* Sesse & Mocino ex DC. Coville) were manually collected in Federal Highway 57 along 15 km of the Saltillo-Monclova stretch, in Coahuila State, Mexico in the second semester of the year 2011. Pomegranate peel, cranberry and creosote bush

leaves were dehydrated at 60 °C for 48 h, then pulverized in a grinder (PULVEX® Mini 100) to obtain a particle size of 600 μm and taken to constant weight[25]. Polyphenols were extracted according to the methodology proposed by Ascacio-Valdés et al[6]. Substrate was hydrated at 1:5 ratio (100 g substrate/500 ml distilled water) and incubated at 60 °C for 30 min. The substrate mass was filtered through muslin cloth and the extract obtained was centrifuged at 3000 rpm for 15 min. The supernatant was purified by chromatography (Amberlite XAD-resin 16®). The extracts were eluted with distilled water (dH_2O) to remove soluble compounds. The polyphenolic compound-rich fraction was eluted with 96 % ethanol. Finally, the ethanol was evaporated in a heating oven (NAPCO® Model 322) at 60 °C for 24 h. The CP CBB and PP were obtained as a fine powder.

Support preparation

The use of polyurethane foam (PUF) as inert support for polyphenol biodegradation was assessed. The PUF was cut and ground to a particle size of 0.85 mm, then washed (3×) with hot water (80–90 °C) for 10 min, filtered to eliminate and dried until constant weight[21].

Microorganisms and culture media

A. niger GH1, *A. niger* PSH and *A. niger* HT4 strains were supplied by DIA-UAdeC (Departamento de Investigación en Alimentos, Universidad Autónoma de Coahuila). These strains have the ability to degrade substrates rich in polyphenols[3,12,22]. The strains were activated in potato dextrose agar (PDA-Bioxon), and incubated at 30 °C for 5 days. Spores were harvested with 0.01 % Tween-80 and counted in a Neubauer® chamber. Pontecorvo culture medium having the following composition (g/l): $NaNO_3$ 6.0, KH_2PO_4 1.52, KCl 0.52 $MgSO_4{\cdot}7H_2O$ 0.52, $ZnSO_4$ 0.001, FeCl 0.85 and 1 ml trace metals was used. The trace metal solution contained (mg/l) $Na_2B_4O_7{\cdot}10H_2O$ 10, $MnCl_2{\cdot}4H_2O$ 50, $Na_2MoO_4{\cdot}2H_2O$ 50 and $CuSO_4{\cdot}5H_2O$ 250. The carbon source (30 g/l) was supplemented with either CP, CBP or PP[7] and the initial culture medium pH was adjusted to 6.5 with 1 M NaOH. An inoculum size of 2×10^7 spores per g of culture material was used.

SSC conditions

The SSC was prepared as follows: 0.5 g of PUF was homogenized with 1.16 ml of culture medium (70 % humidity) and packed in columns (0.5 cm × 5 cm) at a packing density of 0.08 g/cm^3. The solid-state culture was performed for 32 h at 30 °C, with sampling every 8 h (two columns by treatment)[9]. The enzymatic extract of the culture was obtained by adding 1.16 ml of 50 mM citrate buffer (pH 5) within each column, the mass culture was mixed and the extract was obtained by manual pressing. To recover the EA, an ethanol–formic acid (1.16 ml of 0.01 %) solution was added[25] and the extracts obtained were filtered (0.45 μm) and frozen until analysis.

Ellagitannase activity assay

Ellagitannase activity was carried out according to the methodology suggested by De la Cruz et al[12]. Ellagitannins 1 mg/ml in 50 mM citrate buffers, pH 5, were used as enzyme substrate. A substrate control (1 ml ellagitannins and 50 μl of 50 mM citrate buffers, pH 5), enzyme control (1 ml of 50 mM citrate buffers, pH 5 and 50 μl of enzymatic extract) and the reaction mixture (1 ml of ellagitannins and 50 μl of enzymatic extract) were prepared. The reaction was done in a water bath for 10 min at 60 °C. The reaction was stopped by adding 1050 μl of absolute ethanol. Then, samples were sonicated for 25 min, filtered through 0.45 μm nylon membrane and collected in vials. One ellagitannase enzymatic unit was defined as the enzyme amount needed to release 1 μmol of EA per min, under the conditions described.

HPLC analysis

Quantification of EA was performed on a Varian ProStar model 2301 HPLC. A column Pursuit XRs 5um C18 (150 mm × 46 mm) and, (A) methanol (washing phase), (B) acetonitrile and (C) acetic acid 3 % (mobile phase). The flow rate was 1 ml/min, at a column temperature of 30 °C. Standard curve of EA (≥95 %, Sigma–Aldrich®) from 0 to 500 μg/ml was prepared[7].

Data analysis

The effect of the different polyphenol sources and strains on EA accumulation was assessed under a 3 × 3 factorial design with two replicates. Data were analyzed by ANOVA using SAS 9.0 and means were compared by Tukey's multiple range procedure ($p \leq 0.05$) when needed.

Results

Kinetics of EA accumulation

Culture kinetics of A-GH1, A-PSH and A-HT4 strains and three culture media enriched with CP, CBP and PP were studied. The kinetic profile when using CP as polyphenolic substrate (Fig. 1) showed the highest EA accumulation (74.56 mg/g) with the A-PSH strain at 8 h of culture followed by the A-HT4 strain (69.55 mg/g) at 32 h of culture and A-GH1 (64.35 mg/g) at 32 h of culture. The A-HT4 strain produced 13.69 % more EA than the A-GH1 strain; however the former strain also required a 4-fold increase in the culture time required by the A-GH1 strain. Therefore, in terms of process time, A-GH1 resulted in higher EA accumulation than A-HT4. Fig. 2 shows the EA accumulation profile when using CBP. Total EA accumulation by the different strains was 182.92, 158.54 and 125.97 mg/g using A-GH1, A-HT4 and A-PSH strains respectively; however, A-HT4 reached 158.54 mg/g in an 8 h culture while A-GH1 reached in 32 h culture. Once again, a lower EA accumulation value (158.54 mg/g) could be observed in one of the strains tested (A-HT4) but a culture time 4 times lower than in strain A-GH1 that showed the highest EA accumulation (182.92 mg/g). In contrast, when using PP as substrate, EA

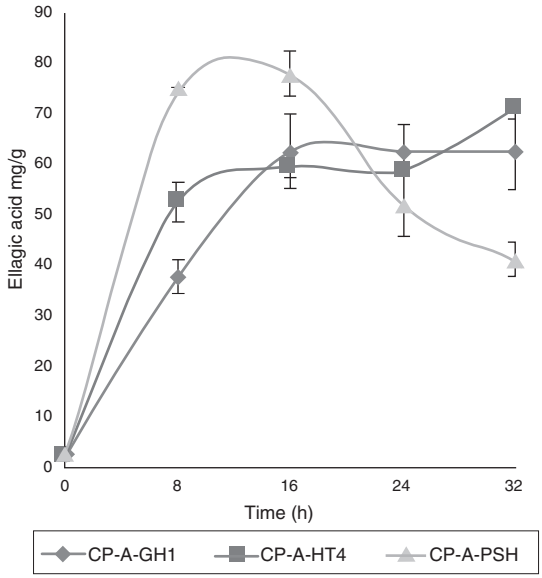

Figure 1 EA accumulation kinetics using: (♦) CP-A-GH1; (■) CP-A-HT4; (▲) CP-A-PSH in SSC.

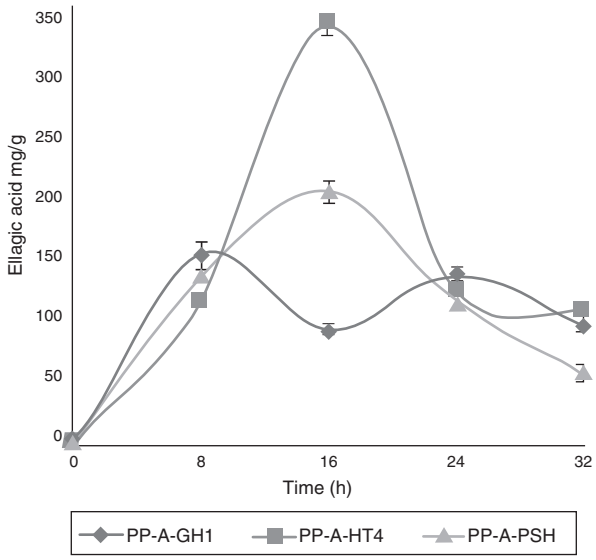

Figure 3 EA accumulation kinetics using: (♦) PP-A-GH1; (■) PP-A-HT4; (▲) PP-A-PSH in SSC.

accumulation was 163.03, 350.21 and 200.33 mg/g for A-GH1 (8 h culture), A-HT4 (16 h of culture) and A-PSH (16 h culture) respectively; the highest EA accumulation occurred at 16 h of culture when using A-HT4 and no EA increase was observed at any other time (Fig. 3).

Ellagitannase activity and productivity

Ellagitannase activity reached maximum value of 5176.81 U/l when using CP as carbon source and A-PSH at 8 h of SSC (Fig. 4). Other strains reached maximum values in the first 8 h and then these values were kept constant. Table 1 shows effect of substrates, strains and interaction on ellagitannase activity and EA productivity. EA productivity was defined as the ratio among the maximum accumulation of EA per culture time. In Fig. 5, the values of EA productivity are displayed at a time of 16 h, which corresponds to the time of maximal EA accumulation obtained. In Table 2, the individual effect of each variable, validated by a Tukey test on ellagitannase activity and EA productivity is shown. Then the highest productivity (21.89 mg/g/h), was obtained when using the *Aspergillus* strain coded as A-HT4 and CP as source. It was observed that when A-HT4 and CP were used, a maximum productivity of 21.89 mg/g/h was reached.

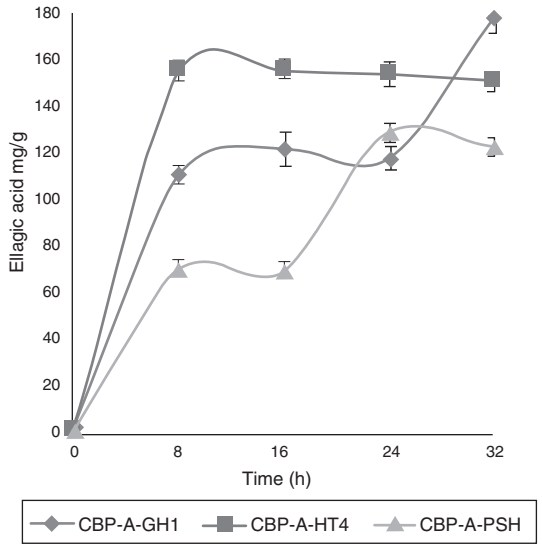

Figure 2 EA accumulation kinetics using: (♦) CBP-A-GH1; (■) CBP-A-HT4; (▲) CBP-A-PSH in SSC.

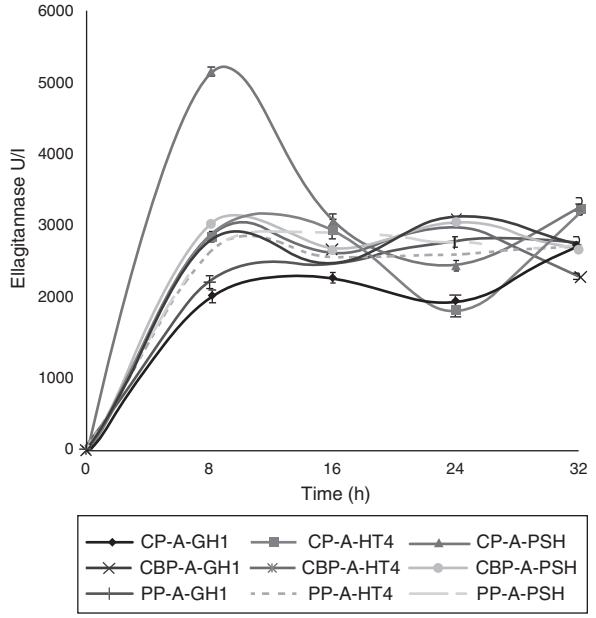

Figure 4 Values of ellagitannase activity, (♦) CP-A-GH1; (■) CP-A-HT4; (▲) CP-A-PSH; (X) CBP-A-GH1; (*) CBP-A-HT4; (●) CBP-A-PSH; (|) PP-A-GH1: (-) PP-A-HT4; (– -) PP-A-PSH.

Table 1 Analysis of variance for EA productivity and ellagitannase activity

Responses	Source of variation	df[a]	MS[b]	*p*-Value	*F*-test
EA productivity	Replicate	1	0.00	0.98	0.00
	S[c]	2	1252.51	<.0001	858.68
	X[d]	2	487.89	<.0001	334.43
	*S*X*	4	382.99	<.0001	262.52
$R^2 = 0.997$					
Ellagitannase activity	Replicate	1	17 034.93	0.26	1.50
	S[c]	2	43 399.04	0.07	3.82
	X[d]	2	230 928.25	<.0001	20.32
	*S*X*	4	112 108.11	<.0001	9.86
$R^2 = 0.917$					

*S*X* = combined effect of substrate and strains.
[a] Degree of freedom.
[b] Mean square.
[c] Substrate (polyphenols).
[d] Strains (*A. niger*).

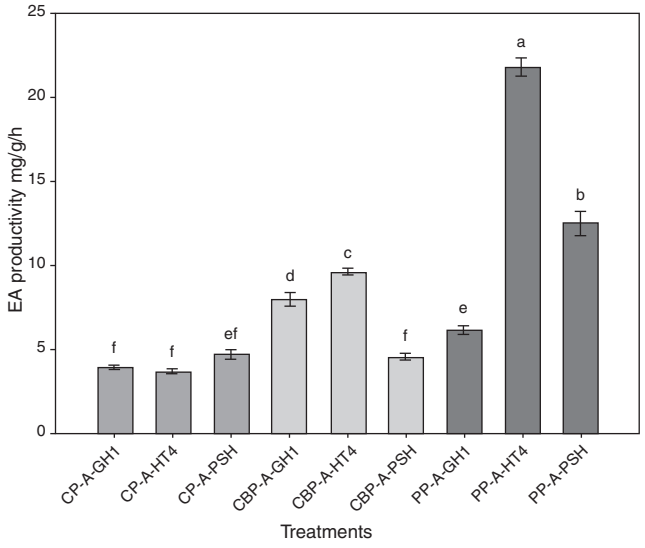

Figure 5 EA productivity, ( ) CP-A-GH1; CP-A-HT4; CP-A-PSH; ( ) CBP-A-GH1; CBP-A-HT4; CBP-A-PSH; ( ) PP-A-GH1; PP-A-HT4; PP-A-PSH.

Table 2 Substrate and strain effect on EA productivity and ellagitannase activity

Substrate	EA productivity (mg/g/h)	Ellagitannase activity (U/l)
PP	40.59 a	2721.38 a
PCB	22.06 b	2671.36 a
PC	12.13 c	2837.17 a
Strains		
A-HT4	35.11 a	2733.28 b
A-PSH	21.76 b	2944.31 a
A-GH1	17.92 c	2552.33 c

Means with the same letter, in the same column, are not significantly different according to the Tukey's multiple range test ($p < 0.05$).

Discussion

The A-PSH strain has been reported to have the ability to produce EA by hydrolysis of the pomegranate peel by SSC[22]. Moreover, this strain also showed the ability to degrade hydrolyzable tannins from extracts of creosote bush (*L. tridentata*) and tarbush (*Flourensia cernua*) releasing EA and gallic acid by SSC[28]. Furthermore, the A-GH1 strain has been reported for the hydrolysis of polyphenolic compounds from pomegranate peel, reaching 17 mg/g of EA at 32 h by SSC[4]. Vattem and Shetty (2003) reported the use of cranberry pomace and *Lentinus edodes* strain for EA production reaching a maximum accumulation of 350 μg/g of EA by dried cranberry pomace and they attributed the EA accumulation to the β-glucosidase enzyme[27]. In this work, when A-HT4 strain and PP are used, a maximum of 350.21 mg/g of EA at 16 h is reached. It has also been reported that the pomegranate peel is an excellent carbon source for EA production, using an *A. niger* strain[1]. Recent studies mention that there is a possible set of enzymes that are responsible for the release of the HHDP group and formation consequence of EA[7].

The values of ellagitannase activity obtained in this study are 166-fold higher than those reported by Aguilera-Carbó et al[4]. These authors reported values of 44.5 U/l using pomegranate peel as carbon source and A-GH1 at 48 h of culture time. Similar studies obtained maximum values of ellagitannase activity (2189.94 U/l) at 30 h of SSC using PP as carbon source and several fungal strains (*A. niger* PSH, *A. niger* GH1, *A. niger* HT4 and *A. niger* HC2)[12]. Several enzymes responsible for ellagitannin hydrolysis have been reported; however, the authors found that only ellagitannase, clearly associated with EA accumulation on SSC, reached maximum values (200.04 U/l) at 12 h of SSC using PP and *A. niger* GH1 strain[7]. The authors reported that EA production is higher using partially purified polyphenols, since these molecules have a glucose core, making them more susceptible to attack by microbial enzymes[14]. There are a few studies about the use of polyphenols from plants, barks or fruits SSC for obtaining secondary metabolites.

The productivity obtained with CP and the A-HT4 strain is higher compared with that in previous reports using valonia tannins as a carbon source, reaching values of 0.92 U/l/h[11]. The A-GH1 strain has been associated with the release of EA from creosote bush extracts by SSC, reaching a productivity of 1.22 U/l/h[4].

In conclusion, it was possible to obtain EA from rich substrates in polyphenolic compounds using *A. niger* strains in SSC. A-HT4 reached a maximum EA value (350.21 mg/g of PP at SSC). When CP and the A-PSH strain was used, high values of ellagitannase activity (5176.81 U/l) were obtained at 8 h of SSC. The EA release mechanism is unknown due to EA complexity; however ellagitannase is one of the enzymes that may be responsible for the hydrolysis of the ester link in the HHDP group to generate EA.

Conflict of interest

The authors declare that they have no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this investigation.

Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Acknowledgements

The authors would like to thank the Mexican Council of Science and Technology (CONACyT) for the scholarship granted for their postgraduate studies in the program of Food Science and Technology of the Autonomous University of Coahuila, Saltillo, México.

References

1. Aguilar CN, Aguilera-Carbó A, Robledo A, Ventura J, Belmares R, Martínez D, Rodriguez R, Contreras J. Production of antioxidant nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. Food Technol Biotechnol. 2008;46:218–22.
2. Aguilera-Carbó A, Augur C, Prado-Barragan L, Aguilar CN, Favela-Torres E. Extraction and analysis of ellagic acid from novel complex sources. Chem Pap. 2008;62:440–4.
3. Aguilera-Carbó A, Augur C, Prado-Barragan L, Favela-Torres E, Aguilar C. Microbial production of ellagic acid and biodegradation of ellagitannins. Appl Microbiol Biotechnol. 2008;78:189–99.
4. Aguilera-Carbó A, Hernández J, Augur C, Prado-Barragan L, Favela-Torres E, Aguilar C. Ellagic acid production from biodegradation of creosote bush ellagitannins by *Aspergillus niger* in solid state culture. Food Bioprocess Technol. 2009;2:208–12.
5. Al-Muammar MN, Khan F. Obesity: the preventive role of the pomegranate (*Punica granatum*). Nutrition. 2012;28:595–604.
6. Ascacio-Valdés J, Aguilera-Carbó A, Martínez-Hernández J, Rodríguez-Herrera R, Aguilar C. Euphorbia antisyphilitica residues as a new source of ellagic acid. Chem Pap. 2010;64:528–32.
7. Ascacio-Valdés J, Burboa E, Aguilera-Carbó A, Aparicio M, Pérez-Schmidt R, Rodríguez R, Aguilar CN. Antifungal ellagitannin isolated from *Euphorbia antisyphilitica* Zucc. Asian Pac J Trop Biomed. 2013;3:41–6.
8. Ascacio-Valdés J, Buenrostro J, De la Cruz R, Sepúlveda L, Aguilera-Carbó A, Prado A, Contreras J, Rodríguez R, Aguilar CN. Fungal biodegradation of pomegranate ellagitannins. J Basic Microbiol. 2014;54:28–34.
9. Buenrostro-Figueroa J, Ascacio-Valdés J, Sepúlveda L, De la Cruz R, Prado-Barragán L, Aguilar-González M, Rodríguez R, Aguilar CN. Potential use of different agroindustrial by-products as supports for fungal ellagitanase production under solid-state fermentation. Food Bioprod Process. 2013;92:376–82.
10. Chao P-C, Hsu C-C, Yin M-C. Anti-inflammatory and anti-coagulatory activities of caffeic acid and ellagic acid in cardiac tissue of diabetic mice. Nutr Metab. 2009;6:1–8.
11. Dahlawi H, Jordan-Mahy N, Clench M, McDougall GJ, Le Maitre CL. Polyphenols are responsible for the proapoptotic properties of pomegranate juice on leukemia cell lines. Food Sci Nutr. 2013;1:196–208.
12. De la Cruz R, Ascacio JA, Buenrostro JJ, Sepúlveda L, Rodríguez R, Prado-Barragán A, Contreras JC, Aguilera A, Aguilar CN. Optimization of ellagitannase production by *Aspergillus niger* GH1 by solid state fermentation. Prep Biochem Biotechnol. 2015;45:617–31.
13. Feldman KS, Sahasrabudhe K, Smith RS, Scheuchenzuber WJ. Immunostimulation by plant polyphenols: a relationship between tumor necrosis factor-alpha production and tannin structure. Bioorg Med Chem Lett. 1999;9:985–90.
14. Gross GG. Biosynthesis of ellagitannins: old ideas and new solutions. In: Quiedau S, Word Scientific Publishing, editors. Chemistry and biology of ellagitannins: an underestimated class of bioactive plant polyphenols. France: University of Bourdeux; 2009. p. 94–115.
15. Huang W, Ni J, Borthwick AGL. Biosynthesis of valonia tannin hydrolase and hydrolysis of valonia tannin to ellagic acid by *Aspergillus* SHL 6. Process Biochem. 2005;40:1245–9.
16. Kang EH, Kown TY, Oh GT, Park WF, Park SI, Park SK, Lee YI. The flavonoid ellagic acid from a medicinal herb inhibits host immune tolerance induced by the hepatitis B virus-e antigen. Antivir Res. 2006;72:100–6.
17. Khanbabaee K, van Ree T. Tannins: classification and definition. Nat Prod Rep. 2001;18:621–49.
18. Lei Z, Jervis J, Helm RF. Use of methanolysis for the determination of total ellagic and gallic acid contents of wood and food products. J Agric Food Chem. 2001;49:1165–8.
19. Li J, Xu H. Bioactive compounds from the bark of *Eucalyptus exserta* F. Muell. Ind Crop Prod. 2012;40:302–6.
20. Masamune A, Satoh M, Kikuta K, Suzuki N, Satoh K, Shimosegawa T. Ellagic acid blocks activation of pancreatic stellate cells. Biochem Pharmacol. 2005;70:869–78.
21. Mussatto SI, Aguilar CN, Rodrigues LR, Teixeira JA. Colonization of *Aspergillus japonicus* on synthetic materials and application to the production of fructooligosaccharides. Carbohyd Res. 2009;344:795–800.
22. Robledo A, Aguilera-Carbó A, Rodriguez R, Martinez J, Garza Y, Aguilar C. Ellagic acid production by *Aspergillus niger* in solid state fermentation of pomegranate residues. J Ind Microbiol Biotechnol. 2008;35:507–13.
23. Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other

polyphenols as found in pomegranate juice. J Nutr Biochem. 2005;16:360–7.
24. Seeram N, Adamas L, Zhang Y, Lee R, Sand D, Scheuller H, Heber D. Blackberry, black raspberry, blueberry, cranberry, red raspberry and strawberry extracts inhibit growth and simulate apoptosis of human cancer cells in vitro. J Agric Food Chem. 2006;54:9329–39.
25. Sepúlveda L, Aguilera-Carbó A, Ascacio-Valdés JA, Rodríguez-Herrera R, Martínez-Hernández JL, Aguilar CN. Optimization of ellagic acid accumulation by *Aspergillus niger* GH1 in solid state culture using pomegranate shell powder as a support. Process Biochem. 2012;47:2199–203.
26. Srivastava A, Jagan Mohan Rao L, Shivanandappa T. Isolation of ellagic acid from the aqueous extract of the roots of *Decalepis hamiltonii*: antioxidant activity and cytoprotective effect. Food Chem. 2007;103:224–33.
27. Vattem DA, Shetty K. Ellagic acid production and phenolic antioxidant activity in cranberry pomace (*Vaccinium macrocarpon*) mediated by *Lentinus edodes* using a solid-state system. Process Biochem. 2003;39:367–79.
28. Ventura J, Belmares R, Aguilera-Carbó A, Guitiérrez G, Rodríguez R, Aguilar CN. Fungal biodegradation of tannins from creosote bush (*Larrea tridentata*) and tar bush (*Fluorensia cernua*) for gallic and ellagic production. Food Technol Biotechnol. 2008;46:213–7.
29. Wang Y, Zhang H, Liang H, Yuan Q. Purification antioxidant activity and protein-precipitating capacity of punicalin from pomegranate husk. Food Chem. 2013;138:437–43.