



Revista Internacional de Investigación e Innovación Tecnológica

Página principal: www.riit.com.mx

Comparative study of polyphenol extraction from grapefruit (*Citrus paradisi*) peel using solid fermentation

Estudio comparativo de extracción de polifenoles de cáscara de toronja (*Citrus paradisi*) usando fermentación sólida

de la Rosa-Hernández, M.^a, Aguilar, C.N.^b, Reyes-Munguía, A.^a, Muñoz-Márquez, D.B.^{a*}, Wong-Paz, J.E.^{a*}

^a Laboratorio de Investigación en Alimentos. Facultad de Estudios Profesionales Zona Huasteca. Universidad Autónoma de San Luis Potosí; C.P. 79060, Ciudad Valles, San Luis Potosí.

^b Grupo de Bioprocesos y Bioproductos. Facultad de Ciencias Químicas. Universidad Autónoma de Coahuila; C.P. 25280, Saltillo, Coahuila.

martina.derosa@uaslp.mx; crisobal.aguilar@uadec.edu.mx; abigail.reyes@uaslp.mx; diana.marquez@uaslp.mx*; jorge.wong@uaslp.mx*

Technological innovation: Alternate release of polyphenols from grapefruit peel.

Industrial application area: Pharmaceutical and food industry.

Received: June 01th, 2022

Accepted: January 11th, 2023

Resumen

Tres cepas de hongos fueron utilizadas con el objetivo de liberar los polifenoles de la cáscara de toronja usando la fermentación en estado sólido (SSF). El bioproceso se realizó en biorreactores por triplicado a 30 ± 2 °C durante 5 días en condiciones estáticas. La cuantificación de los polifenoles totales se hizo con las técnicas de Folin-Ciocalteu y el ensayo n-Butanol/HCl/Fe³⁺. La caracterización de los extractos fermentados fue por HPLC-MS. En los resultados se obtuvieron mejores rendimientos de polifenoles liberados a las 96 h usando la fermentación con *A. niger*, alrededor de 518 ± 22 mg/g de muestra. Un menor rendimiento fue obtenido con *A. oryzae*, 196 ± 22 mg/g, mientras que *Penicillium digitatum* no mostró adaptación al sustrato en el tiempo de la evaluación. Además, se logró la identificación de ocho compuestos flavonoides, siendo la naringina y hesperidina las moléculas mayoritarias en los extractos fermentados. Nuestro estudio comparativo evidenció que la fermentación en estado sólido con *A. niger* permite la obtención de un mayor contenido fenólico que el control sin fermentar, en contraste, la fermentación con *A.*

oryzae permitió menor liberación durante todo el proceso de extracción. Los resultados demuestran que *A. niger* es mejor cepa fermentativa para la recuperación de metabolitos importantes aplicables el área farmacéutica y alimentaria usando residuos de toronja. No se descarta el uso de *A. Oryzae* en fermentaciones sólidas, pero se recomienda estudiar su crecimiento y adaptación con otros sustratos agroindustriales.

Palabras clave: *A. niger*, hongos-filamentosos, HPLC-MS, polifenoles, residuos-toronja.

Abstract

Three fungal strains were used to release polyphenols from grapefruit peel using Solid-State Fermentation (SSF). The bioprocess was carried out in bioreactors in triplicate at 30 ± 2 °C for 5 days under static conditions. Quantification of total polyphenols was done with the Folin-Ciocalteu and *n*-Butanol/HCl/Fe³⁺ assays. The characterization of fermented extracts was by HPLC-MS. In the results, better yields of polyphenols released at 96 h were obtained using the fermentation with *A. niger*, around 518 ± 22 mg/g of sample. A lower yield was obtained with *A. oryzae*, 196 ± 22 mg/g, while *Penicillium digitatum* did not show adaptation to the substrate at the time of evaluation. In addition, eight flavonoids compounds were identified, being naringin and hesperidin the major molecules in fermented extracts. Our comparative study, evidenced that Solid-State Fermentation using *A. niger* allows higher phenolic content to be obtained than the unfermented control, in contrast, fermentation with *A. oryzae* allowed less release during the whole extraction process. The results show that *A. niger* is a better fermentative strain for the recovery of important metabolites applicable in pharmaceutical and food industry using grapefruit residues. The use of *A. Oryzae* in solid fermentations is not discarded, but it is recommended to study its growth and adaptation using other agro-industrial substrates.

Keywords: *A. niger*, fungi-filamentous, HPLC-MS, polyphenols, residues-grapefruit.

1. Introduction

Solid-State Fermentation (SSF) to release of bioactive compounds is the use of fungal strains to release valuable biomolecules from plants, fruits and agroindustry waste almost without water. Recently, the studies using SSF are increasing due to some advantages showed in the method, such as simple, cheap, environmentally friendly, improved yields of extracted bioactive compounds and sometimes a biotransformation of bioactive compounds is observed with increased biological properties [1, 2]. SSF using fungal microorganisms is an alternative that is

replacing the expensive chemical methods for the extraction of bioactive compounds from natural products because it allows the recovery of secondary metabolites with several biological properties without the use of toxic solvents [3, 4, 5]. In this regard, SSF has been successfully used on several agro-industrial wastes including fruit peels (orange, lemon, banana, apple, pomegranate) [6, 7], bagasse, among other materials [8, 9, 10].

The grapefruit peel is a waste product considered as a potential source of bioactive

compounds such as terpenes as carotenoids and limonoids, vitamins A, E, C and polyphenolic compounds such as coumarins, flavonoids and phenolic acids. Polyphenolic compounds are a group of bioactive compounds widely distributed in plants, vegetables, and fruits with beneficial effects on human health [11, 12]. Recently, polyphenolic compounds in grapefruit are considered to play a crucial role in human health because they help in the prevention of chronic diseases, as cancer, diabetes, obesity, and cardiovascular diseases [13, 14]. In addition, citrus compounds are considered as powerful antioxidant and antimicrobial molecules [15, 16, 17], with several applications in the food and pharmaceuticals industries. However, at the present the grapefruit peel has not been intensively exploited for the recovery of these bioactive compounds and even less under SSF [18, 19]. Therefore, the aim of this study was to evaluate the release of polyphenolic compounds from grapefruit (*Citrus paradisi*) peels obtained by SSF using *Aspergillus niger*, *Penicillium digitatum* and *Aspergillus oryzae*.

2. Materials and methods

2.1. Grapefruit peel collection and treatment

A batch of pink grapefruit was obtained from a local market in Ciudad Valles, S. L. P., Mexico. The fruits were washed and peeled in the Laboratory (FEPZH-UASLP). The selected grapefruit peel was dehydrated at 70 °C during 72h in a convection stove. Then, the dried grapefruit peel was pulverized (Kitchenaid® bean mill P/beater), sieved and stored in hermetic bags.

2.2. Cell culture of fungal strains

Aspergillus niger and *Penicillium digitatum* were obtained from the fungal collection of the Food Research Laboratory (FEPZH-UASLP), and *Aspergillus oryzae* from the

collection of the Department of Research (DIA-UAdeC). All fungal strains were cryopreserved at -20 °C in media composed of glycerol and skimmed milk. Then, fungal spores were reactivated in PDA agar at 30 °C for 5 days. The spore suspension was prepared by washing with 0.01% Tween-80 and counted on a Neubauer® adjusting the inoculum to 2×10^7 spores/g dry sample.

2.3. Fungal Strains Selection: Radial growth kinetics

The grapefruit peels (humidity 70%) were distributed in Petri dishes by triplicates and inoculated with fungal spore suspension, then incubated at 30 ± 2 °C. Radial growth was measured every 24 h for 7 days and expressed in mm/h. The higher radial growth was selected for the kinetics of polyphenols release.

2.3.1. Polyphenol extraction by Solid State Fermentation (SSF)

Grapefruit peel (3 g) was impregnated with sterile water and spore solution to reach 70% of moisture content and 2×10^7 spores/g db. The inoculated material was deposited in Petri dishes as bioreactor model in triplicate. The SSF kinetics were controlled at 30 ± 2 °C during 4 days under static conditions. Substrate without inoculum under the same conditions of humidity and fermentation was used as a control. Polyphenols were recovered every 24 hours, using 20 ml of ethanol (70%) at 220 rpm for 10 min in a Shaker to extract the compounds from the vegetal fermented. Extracts were filtered and stored at -20 °C.

2.4. Analytical determinations

2.5.1. Hydrolysable tannins (HT)

HT were quantified by triplicate using the Folin-Ciocalteu method [20]. In this 400 µl of extract and 400 µl of Folin-Ciocalteu reagent were pipetted in a test tube and reacted during 5 min in the dark. After, 400 µl of Na_2CO_3

(0.01 M) and 2.5 ml of distilled water were added. The solution was read at 790 nm in spectrophotometer. The concentration was calculated using a standard curve of gallic acid in ethanol (70%). Concentrations were expressed in mg gallic acid equivalents by gr of dry basis (GAE mg/g of DB).

2.5.2. Condensed tannins (CT)

In this quantification, the n-Butanol/HCl/Fe³⁺ method was used [21]. Briefly, 250 µl of extract, 1.5 ml HCl-Butanol (95%) and 50 µl of ferric reagent (Ferric ammonium sulfate) were collocated in a test tube. These were exposed in a boiling water bath during 40 min, then cooled to room temperature and the absorbance was read at 550 nm. The concentration was calculated with Procyanidin C1 (Sigma-Aldrich) standard curve according to the n-Butanol/HCl/Fe³⁺ reaction method. Concentrations were expressed in mg procyanidin C1 equivalents by gr dry basis (C1PE mg/g of DB).

2.5.3. HPLC-MS Characterization

HPLC-MS analysis was performed using a Varian HPLC system including an autosampler (Varian ProStar 410, USA), a ternary pump (Varian ProStar 230I, USA) and a PDA detector (Varian ProStar 330, USA) coupled to a mass spectrometer (MS). Each extract was filtered with a syringe using acro-disperse filters. Samples (5 µL) were injected onto a Denali C18 column (150 mm × 2.1 mm, 3 µm, Grace, USA). The oven temperature was maintained at 30 °C. The eluents were formic acid (0.2%, v/v; solvent A) and acetonitrile (solvent B). The following gradient was applied: initial, 3% B; 0–5 min, 9% linear B; 5–15 min, 16% linear B; 15–45 min, 50% linear B. The column was then washed and reconditioned. The flow rate was

maintained at 0.2 mL/min and the elution was monitored at 245, 280, 320 and 550 nm. The whole effluent (0.2 mL/min) was injected into the mass spectrometer source, without splitting. All MS experiments were carried out in the negative mode [M-H]⁻¹. Nitrogen was used as the nebulizing gas and helium as the damping gas. The ion source parameters were: spray voltage 5.0 kV and, capillary voltage and temperature were 90.0 V and 350 °C, respectively. Data were collected and processed using MS Workstation software (V 6.9). The samples were firstly analyzed in full scan mode acquired in the *m/z* 50–2000 range. MS/MS analyses were performed on a series of selected precursor ions.

3. Discussion of the results

3.1. Fungal strain selection for the release of bioactive compounds

Figure 1 shows the kinetic of the radial growth by the three fungal strains on the solid substrate (grapefruit). Here, *A. oryzae* and *A. niger* showed the highest invasiveness compared to *P. digitatum*, so it was discarded due to having a lower radial growth. Based on this, *A. oryzae* and *A. niger* were selected for the SSF process. The growth of microorganism is the result of physiological responses of the fungi in function of the grapefruit peels (substrate) as an environment. *Aspergillus* genus is considered as resistant strain to adverse growth conditions due to its capacity to invade several types of substrates [22] including fruit residues. This last one is a disadvantage against *Penicillium digitatum*, considered a slow growing strain [23], despite to be a citrus-deteriorating fungus. Also, compounds present in the grapefruit are antifungal agents produced as natural defense mechanism of citrus fruit against *Penicillium* genus [24].

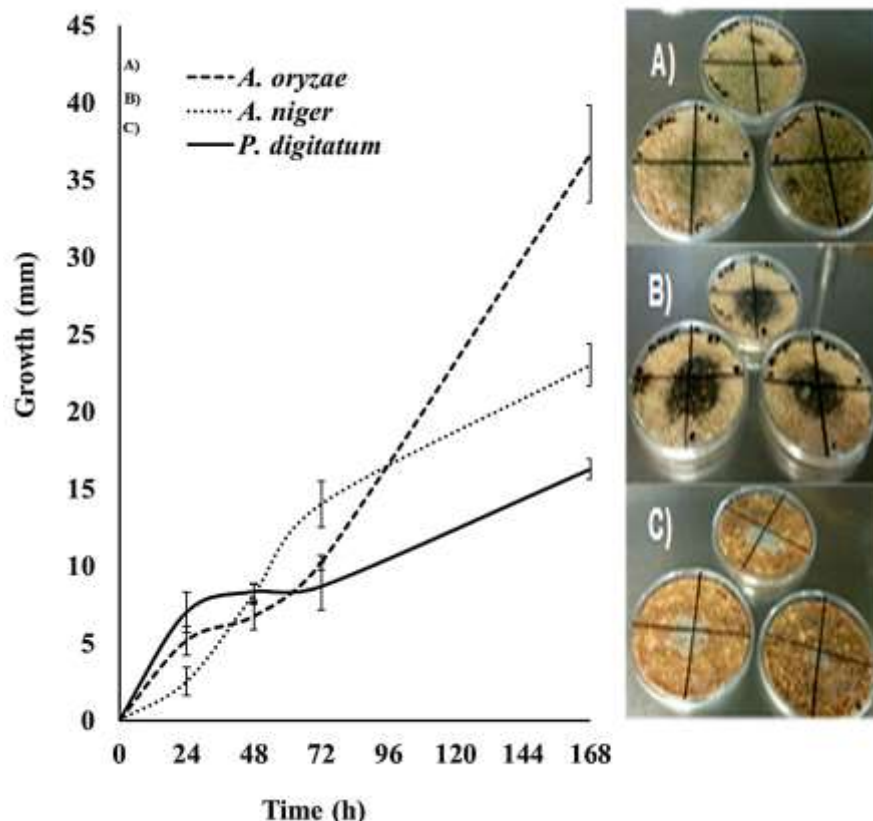


Figure. 1 Radial growth kinetics of all fungi studied on solid substrate (grapefruit).

In the release of compounds of grapefruit peels, *A. niger* presented greater ability to release polyphenolic compounds from grapefruit using the SSF (Figure 2). In contrast with the control without inoculum, an increment of phenolic content on grapefruit peel using *A. niger* was observed after 48h of processing. During all the fermentation until last 96 h, higher yields were obtained with *A. niger* than *A. oryzae*, 434.69 ± 22 mg GAE /g DB of hydrolysable tannins (Figure 2a) and 84.161 ± 6.11 mg PC1E/g DB of condensed tannins quantified

(Figure 2b). These results were better than those obtained by Šelo *et al.* (2022), using a substrate richer in antioxidant compounds, but could be improved by regulating experimental conditions as Larios *et al.* (2019).

All compounds were handled as Total Polyphenols (TC) from of the summary of CT and HT content obtained with both microorganisms during fermentative process, the values are shown in the table 1.

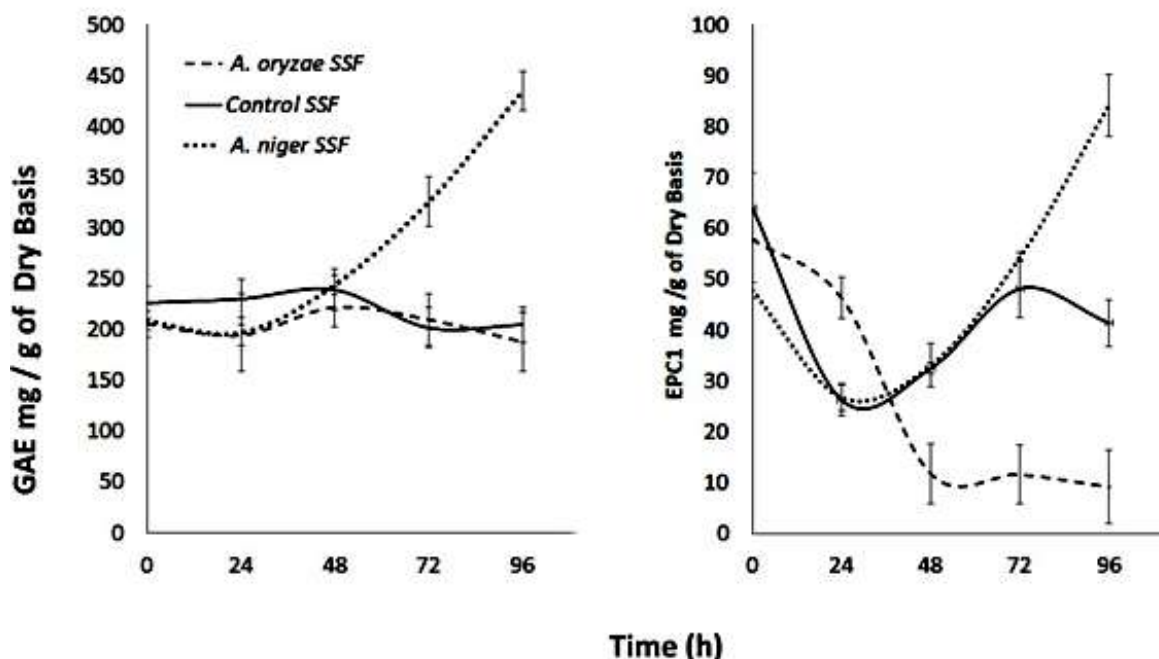


Figure. 2 Kinetics of Hydrolysable Tannins (left) and Condensed Tannins (right) extracted from grapefruit peels by SSF.

With these results obtained, it is confirmed that *A. niger* possesses a great metabolic activity [26, 27] to produce enzymes as cellulases [28, 29], pectinases [9] and hemicellulases [30, 31, 32], biological tools capable to degrading the polysaccharides of grapefruit peel allowing a better release of compounds from the substrate. Similar studies report the release of antimicrobial metabolites using *A. niger* metabolism from of grapefruit residue degradation [19, 33, 34]. Likewise, the activity of *A. niger* metabolism during the polyphenols release kinetics starts by consuming simple elements (monosaccharides) until their adaption, requiring greater enzymatic activity to consume complex polysaccharides such as protopectin, pectin and cellulose contained in epicarp and mesocarp of peels [35, 36, 37]. This happens with the genus *Aspergillus*, which has been regularly used to obtain enzymes, such as the work carried out by Zehra, Syed & Sohail (2020) using banana peels as substrate by FES, with the aim of obtaining pectinases from the degradation of

cell wall degradation of waste material with *Aspergillus fumigatus*. And although enzyme activity was not measured, the metabolic behavior of *A. niger* by FES has already been measured using grapefruit residues, so the activity of the microorganism could be like that of *A. niger* entering the adaptation and dormancy phase reported by Larios *et al.* (2016).

The activity of *A. niger* metabolism during the polyphenols release kinetics start by the consumption of simple elements (monosaccharides) until their adaption, requiring a greater enzymatic activity to consume the complex polysaccharides contained in epicarp and mesocarp of peels [19].

Regarding the low and high polyphenols concentrations obtained with *A. oryzae* and *A. niger* respectively could be related with tannase enzyme produced by *Aspergillus* genus [38, 39]. Tannase enzyme is used to obtain secondary metabolites (gallic acid)

[40, 41], so, its action represents an estimation of polyphenols release during the SSF. Consequently, polyphenols values are

the result of microorganism adaptation submitted to the SSF process and the effect on its enzymatic activities.

Table 1. Recovery of Total Polyphenols by SSF from of grapefruit (*Citrus paradisi*) peels at 96 h of process.

Analytical compounds	Fungal Extraction Assistant		
	Control SSF	<i>A. oryzae</i>	<i>A. niger</i>
HT (GAE mg/g of DB)	205±17 ^a	188±28 ^a	435±19 ^b
CT (C1PE mg/g of DB)	41±4 ^a	9±3 ^a	84±6 ^b
TP (mg/g of DB)	246±15 ^a	197±32 ^a	518±22 ^b

Abbreviations: HT: Hydrolysable tannins; CT: Condensed tannins; TP: Total polyphenols; GAE: Equivalents Gallic Acid; C1PE: Equivalents Procyanidin C1; DB: Dry Basis.

3.2. Phenolic profile by HPLC-MS

The phenolic profile obtained by HPLC-MS at 96 h by SSF is shown in the chromatograms of the samples analyzed (Fig. 3). 5 glycosidic flavonones were identified (peaks 1, 2, 3, 4 and 8) and 3 aglyconic flavonones (polymethoxylated) (peaks 5, 6 and 7). The chromatographic profile indicates that *A. niger* allowed the release of a better diversity of polyphenols, peaks 1 at 8 (Figure 3b) than *A. Oryzae*, peaks 1, 2, 3, 4 and 7 (Figure 3a) using the solid-state fermentation. A similar polyphenol profile was obtained by Sepúlveda *et al.*, (2016) where ellagic acid molecules are released using *A. niger* by SSF using pomegranate peels.

In our results, both profiles evidence the metabolism of genus *Aspergillus* to released different molecules from of grapefruit peels respect to the unfermented control. However, the chromatograms showed that the control had a major abundance of detected molecules (1600 mUA) (Figure 3c), while in the fermented treatments it decreases (1200-600 mUA), this probably due to phenolic components degradation by the enzyme tannase [39], although new compounds are apparently generated.

It is important to mention that variability of bioconversion yields between both strains depends of physiological growth conditions, fermentation parameters (temperature, pH,

heat transfer) and the matrix used [43, 44]. In *A. oryzae* case, these factors directly affect enzyme production activity. However, this microorganism has allowed the phenolic content in substrates such as wheat and other foodstuffs for human consumption to be increased [2, 45, 46].

In addition, naringin (naringenine-7-rhamnosidoglucoside) (peak 3) a predominant flavonoid in grapefruit peels [47], was the most abundant molecule present in unfermented control. In *A. niger* and *A. oryzae* extracts, hesperidin (hesperitin-7-rutinoside) (peak 4) and naringin (peak 3) were the most abundant molecules; this, indicates the fungal bioconversion diversity of the studied organisms to obtain bioactive molecules of interest from of same substrate. Some compounds detected in this study match to those reported in the literature [48]; however, SSF effect on the phenolic variety recovered from grapefruit peels fermented with two known organisms was considered better than without this extraction method.

According to chromatographic profiles analyzed, and the yields obtained, it can be said that *A. niger* has a greater enzymatic metabolism than *A. oryzae*, hence this microorganism is ideal to SSF using citrus residues. This may be one reason why *A. niger* has been preferred within the genus *Aspergillus* for bioactive molecules production by SSF with others agro-industrial

substrates [42, 49]. The profile showed that SSF is an effective and innovative technique to increase phenolic compounds from grapefruit peel, probably even better than traditional methods. These results are like those of Francis, Vodnar & Socaciu (2016)

who reported an increase in phenols of more than 30% for SSF with *Rhizopus oligosporus* and more than 21% for SSF with *A. niger* using plum by-products. In addition, these authors confirmed that the fermented extracts had a higher free radical scavenging activity.

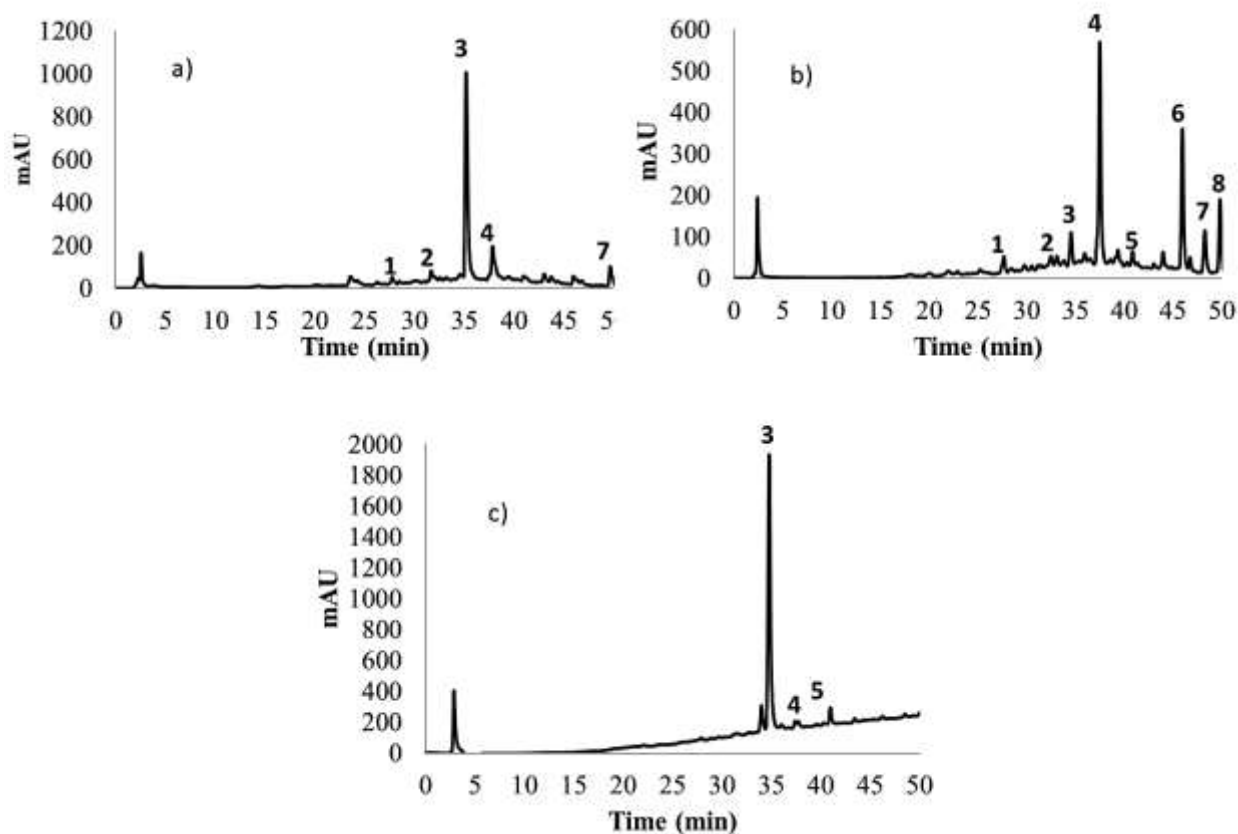


Figure 3. HPLC-MS Chromatograms obtained from the fermented grapefruit peel extract at 96 h of process: A) *A. oryzae*; B) *A. niger*; C) Control. Compounds identified: (1) Eriocitrin, (2) Isonaringine, (3) Naringin, (4) Hesperidin, (5) Naringenin, (6) Tangeretin, (7) Nobiletine, (8) Poncirin.

4. Conclusion

Obtaining bioactive biomolecules from grapefruit peels by SSF was successful applied. This work showed to *A. niger* has greater metabolism to degrade citrus wastes and release flavonoids compounds than *A. oryzae*, so it should not be ruled out for SSF. The molecules detected such as naringin, hesperidin and the others extracted by SSF are biological and functional ingredients that can be used as natural additives,

antimicrobials, and antioxidants in the development of pharmaceutical and food products. However, it is necessary to compare the biological properties of fermented products and to optimize the extraction process with fungal strain selected.

Acknowledgments

This work was funded by TecNM by project 6693.18-P Evaluation of antioxidant potential from fermented extracts from grapefruit peel

(*Citrus paradisi* L.). Also was funded by Facultad de Estudios Profesionales Zona Huasteca de la UASLP.

Conflict of interest

The authors declare no conflict of interest.

5. References

1. Aguilar, C. N., Aguilera-Carbo, A., Robledo, A., Ventura, J., Belmares, R., Martínez, D., Rodríguez-Herrera, R., & Contreras, J. (2008). Production of antioxidants nutraceuticals by solid-state cultures of pomegranate (*Punica granatum*) peel and creosote bush (*Larrea tridentata*) leaves. *Food Technol Biotechnol*, 46:218-222.
2. Duhan, J. S., Mehta, K., Sath, P. K., Saharan, P., & Surekha, C. (2016). Bio-enrichment of phenolics and free radicals scavenging activity of wheat (WH-711) fractions by solid state fermentation with *Aspergillus oryzae*. *African Journal of Biochemistry Research*, 1(2): 2-19. <https://doi.org/10.5897/AJBR2015.0854>
3. Koyani, R. D. & Rajput, K. S. (2015). "Solid State Fermentation: Comprehensive Tool for Utilization of Lignocellulosic through Biotechnology. *Journal of Bioprocessing and Biotechniques*, 5(10).doi:10.4172/2155-9821.1000258.
4. Buenrostro-Figueroa, J. J., Velázquez, M., Flores-Ortega O., Ascacio-Valdés, J. A., Huerta-Ochoa, S., Aguilar, C. N., & Prado-Barragán, L. A. (2017). "Solid state fermentation of fig (*Ficus carica* L.) by-products using fungi to obtain phenolic compounds with antioxidant activity and qualitative evaluation of phenolics obtained ". *Process Biochemistry*, 62, 16-23.
5. Meena, L., Sengar, A.S., Neog, R. *et al.* (2021). Pineapple processing waste (PPW): bioactive compounds, their extraction, and utilisation: a review. *J Food Sci Technol*. <https://doi.org/10.1007/s13197-021-05271-6>
6. Sepúlveda, L., de la Cruz, R., Buenrostro, J. J., Ascacio-Valdés, J. A., Aguilera-Carbó, A. F., Pradoc, A., Rodríguez-Herrera, R., & Aguilar, C. (2016). "Efecto de las diferentes fuentes de polifenoles sobre la eficiencia de liberación de ácido elágico por *Aspergillus niger* ". *Revista Argentina de microbiología* 48(1):71-77.
7. El-Sayed, E., Ahmed, A. and Al-Hagar, O. (2020), Agro-industrial wastes for production of paclitaxel by irradiated *Aspergillus fumigatus* under solid-state fermentation. *J Appl Microbiol*, 128: 1427-1439. <https://doi.org/10.1111/jam.14574>
8. Sindhu, R., Pandey, A., & Binod, P. (2015). Solid-state "Fermentation to produce Poly (hydroxyalkanoates). *Chemical and Biochemical Engineering Quarterly*, 29(2): 173-181.
9. Ahmed, I., Zia-Anjum, M., Azhar-Hussain, M., Akram, Z., Naveed, M. & Nowrouz, I. (2015). A Bioprocessing of citrus waste peel for induced pectinase production by *Aspergillus niger*; its purification and characterization. *Journal of Radiation Research and Applied Sciences*, 9(2). <https://doi.org/10.1016/j.jrras.2015.11.003>.

10. de la Rosa, O., Flores-Gallegos A. C., Muñiz-Marquez, D., Nobre, C., Contreras-Esquivel, J. C., Cristobal, N. A. (2019). Fructooligosaccharides production from agro-wastes as alternative low-cost source. *Trends in Food Science & Technology*, 91, 139-146, <https://doi.org/10.1016/j.tifs.2019.06.013>.
11. Zhang, B., Cai, J., Duan, C., Reeves, J. M., & He, F. (2015). Review of Polyphenolics in Oak Woods. *International Journal of Molecular Sciences*, 16(4). doi:10.3390/ijms16046978.
12. Arfaoui, L. (2021). Dietary Plant Polyphenols: Effects of Food Processing on Their Content and Bioavailability. *Molecules*, 26(10), 2959. <https://doi.org/10.3390/molecules26102959>
13. Agudelo, C., Barros, L., Santos-Buelga, C., Martínez-Navarrete, N., & Ferreira, I. C. (2017). Phytochemical content and antioxidant activity of grapefruit (*Star Ruby*): A comparison between fresh freeze-dried fruits and different powder formulations. *LWT-Food Science and Technology*, 80, 106-112.
14. Ahmed, W., Azmat, R., Mehmood, A. *et al.* (2021). Comparison of storability and seasonal changes on new flavonoids, polyphenolic acids and terpenene compounds of *Citrus paradisi* (grapefruit) cv. shamber through advance methods. *Food Measure*, 15, 2915–2921. <https://doi.org/10.1007/s11694-021-00815-y>
15. Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z. (2015). Antioxidant activity of Citrus fruits. *Food Chemistry*. 196; pp.885–896.
16. Lin, X., Cao, S., Sun, J., Lu, D., Zhong, B., & Chun, J. (2021). The Chemical Compositions, and Antibacterial and Antioxidant Activities of Four Types of Citrus Essential Oils. *Molecules (Basel, Switzerland)*, 26(11), 3412. <https://doi.org/10.3390/molecules26113412>
17. Saini, R. K., Ranjit, A., Sharma, K., Prasad, P., Shang, X., Gowda, K., & Keum, Y. S. (2022). Bioactive Compounds of Citrus Fruits: A Review of Composition and Health Benefits of Carotenoids, Flavonoids, Limonoids, and Terpenes. *Antioxidants (Basel, Switzerland)*, 11(2), 239. <https://doi.org/10.3390/antiox11020239>
18. Londoño, J., Sierra, J., Álvarez, R., Restrepo, A. M., & Passaro, C. (2012). Aprovechamiento de los subproductos cítricos en Cítricos: cultivo, poscosecha e industrialización. *Artes y Letras*.
19. Larios-Cruz, R., Buenrostro-Figueroa, J., Prado-Barragán, A. *et al.* (2019). Valorization of Grapefruit By-Products as Solid Support for Solid-State Fermentation to Produce Antioxidant Bioactive Extracts. *Waste Biomass Valor* 10, 763–769. <https://doi.org/10.1007/s12649-017-0156-y>.
20. FAO/IAEA. (2000). Quantification of Tannins in Tree Foliage, A laboratory manual for the FAO/IAEA, Working Document, IAEA, Vienna, Austria Sec. 3.05-4.09.

21. Porter, L. J., Hrstich, L. N., & Chan, B. G. (1986). The conversion of procyanidins to cyanidin and delphinidin. *Phytochem*, 25(1): 223-230.
22. Paulussen, C., Hallsworth, J. E., Álvarez-Pérez, S., Nierman, W. C., Hamill, P. G., Blain, D., Rediers, H., & Lievens, B. (2017). Ecology of aspergillosis: insights into the pathogenic potency of *Aspergillus fumigatus* and some other *Aspergillus* species. *Microbial biotechnology*, 10(2), 296–322. <https://doi.org/10.1111/1751-7915.12367>
23. Li, Y., Xia, M., He, P., Yang, Q., Wu, Y., He, P., Ahmed, A., et al. (2022). Developing *Penicillium digitatum* Management Strategies on Post-Harvest Citrus Fruits with Metabolic Components and Colonization of *Bacillus subtilis* L1-21. *Journal of Fungi*, 8(1), 80. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/jof8010080>
24. Chen, J., Shen, Y., Chen, C., & Wan, C. (2019). Inhibition of Key Citrus Postharvest Fungal Strains by Plant Extracts In Vitro and In Vivo: A Review. *Plants*, 8(2), 26. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/plants8020026>
25. Šelo, G., Planinić, M., Tišma, M., Grgić, J., Perković, G., Koceva Komlenić, D., & Bucić-Kojić, A. (2022). A Comparative Study of the Influence of Various Fungal-Based Pretreatments of Grape Pomace on Phenolic Compounds Recovery. *Foods*, 11(11), 1665. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/foods11111665>
26. Martins, S., Mussatto, S. I., Martínez-Avila, G., Montañez-Saenz J., Aguilar, C. N., & Teixeira, J. A. (2011). Bioactive phenolic compounds: Production and extraction by solid-state fermentation. A review. *Biotechnology Advances*, 29, 365-373.
27. Ooi C. K., Rasit N., Rafizah W., Abdullah W. (2021). Optimization of Protease from *Aspergillus Niger* under Solid-State Fermentation Utilizing Shrimp Shell Substrate. *Biointerface Research in Applied Chemistry*, 11 (6), 14809 – 14824. <https://doi.org/10.33263/BRIAC116.1480914824>
28. Persad, A. & Bisaria, S. V. (2014). Bioprocessing of Renewable Resources to Commodity Bioproducts. First Edition. Bisaria S. V. and Kondo A. Editors; *John Wiley & Sons*.
29. Dias, L., dos Santos, B., Albuquerque, C., Baeta, B., Pasquini, D. and Baffi, M. (2018), Biomass sorghum as a novel substrate in solid-state fermentation for the production of hemicellulases and cellulases by *Aspergillus niger* and *A. fumigatus*. *Journal of Applied Microbiology*, 124, 708-718. <https://doi.org/10.1111/jam.13672>.
30. Ottenheim, C., Verdejo, C., Zimmermann, W., & Wu, J. C. (2014). Hemicellulase production by *Aspergillus niger* DSM 26641 in hydrothermal palm oil empty fruit bunch hydrolysate and transcriptome analysis. *Journal of Bioscience and Bioengineering*, 118(6). <http://dx.doi.org/10.1016/j.jbiosc.2014.05.014>.

31. Upadhyaya, J., Joshi, D. R., Lekhak, B., Kumar Chaudhary, D., Raj Pant, B., Raj Bajgai, T., et al. (2020). Production, Characterization, and Industrial Application of Pectinase Enzyme Isolated from Fungal Strains. *Fermentation*, 6(2), 59. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/fermentationn6020059>
32. Li Q., Ray C., Callow N., Loman A., Islam A., Ju LK. (2020). *Aspergillus niger* production of pectinase and α -galactosidase for enzymatic soy processing. *Enzyme and Microbial Technology*, 134(1). <https://doi.org/10.1016/j.enzmictec.2019.109476>.
33. Scurria, A., Sciortino, M., Presentato, A., Lino, C., Piacenza, E., Albanese, L., Zabini, F., et al. (2020). Volatile Compounds of Lemon and Grapefruit IntegroPectin. *Molecules*, 26(1), 51. MDPI AG. Retrieved from <http://dx.doi.org/10.3390/molecules26010051>
34. Rojas, P., Perea, A., & Ortíz, C. (2007). Determinación por HPLC de flavanonas en jugos cítricos de variedades cultivadas en Santander. *Scientia Et Technica*, 13(33).
35. Yun, D. & Liu, J. (2022). Recent advances on the development of food packaging films based on citrus processing wastes: A review. *Journal of Agriculture and Food Research*, 9. <https://doi.org/10.1016/j.jafr.2022.100316>.
36. Zehra, M., Syed, M. N., & Sohail, M. (2020). Banana Peels: A Promising Substrate for the Coproduction of Pectinase and Xylanase from *Aspergillus fumigatus* MS16. *Polish journal of microbiology*, 69(1), 19–26. <https://doi.org/10.33073/pjm-2020-002>
37. Larios-Cruz, R., Buenrostro-Figueroa, J., Prado-Barragán, A., Rodríguez-Jasso, R., & Aguilar, C. 2016. Perfil respirométrico de *Aspergillus niger* GH1 por fermentación en estado sólido sobre residuos de toronja. *Agronomía Colombiana*, 34(1): S1232-S1235.
38. Abdel-Nabey, M. A., Sherief, A. A., & EL-Tanash, A.B. (2011). Tannin Biodegradation and Some Factors Affecting Tannase Production by Two *Aspergillus* sp. *Biotechnology*. 10(2):149-158. <http://dx.doi.org/10.3923/biotech.2011.149.158>.
39. Al-Manhel, A. & Al-fekaiki, D. (2019). Purification and characterization of tannase from the local isolate of *Aspergillus niger*. 7. 29-34. <http://dx.doi.org/10.7324/JABB.2019.70106>.
40. Jiménez, N., Esteban-Torres, M., Mancheño, J. M., de las Rivas, B., & Muñoz, R. (2014). Tannin Degradation by a Novel Tannase Enzyme Present in Some *Lactobacillus plantarum* Strains. *Applied and Environmental Microbiology Journal*, 80 (10):2991-2997
41. Reges de Sena A., Morgana de Melo-Lopes L., Jamesse-Gouveia M., Gouveia M., C., Rodrigues M., Campos-Leite T., Souza-Lima K., Aparecida-Moreira K. & Aparecida K. (2021) Tannin biodegradation by tannase from *Serratia marcescens*: optimization of production by response surface methodology and its partial characterization. *Chemical Engineering Communications*, 208:10, 1369-1384.

42. Sepúlveda, L., De la Cruz, R., Buenrostro-Figueroa, J., Ascacio-Valdés, J. & Aguilera-Carbó, A., Prado, L., Rodríguez, R. & Aguilar, C. (2016). Effect of different polyphenol sources on the efficiency of ellagic acid release by *Aspergillus Niger*. *Revista Argentina de microbiología*, 48, 1-7. <http://dx.doi.org/10.1016/j.ram.2015.08.008>.
43. Huynh N. T., Camp, J., Smagghe, G., & Raes, K. (2014). Improved Release and Metabolism of Flavonoids by Steered Fermentation Processes: A Review. *International Journal of Molecular Sciences*, 15: 19369-19388. <http://dx.doi.org/10.3390/ijms151119369>.
44. Wen, Q., Cao, X., Chen, Z., Xiong, Z., Liu, J., Cheng, Z., Zheng, Z., Long, C., Zheng, B. & Huang, Z. (2020). An overview of *Monascus* fermentation processes for monacolin K production. *Open Chemistry*, 18(1), 10-21. <https://doi.org/10.1515/chem-2020-0006>
45. Chuenjit-Chancharonpong, P., Chuan H., & Shyang-Chwen S. (2012). Enzyme Production and Growth of *Aspergillus oryzae* S. on Soybean Koji Fermentation. *International Journal of Bioscience, Biochemistry and Bioinformatics*, 2(4). <http://dx.doi.org/10.7763/IJBBB.2012.V2.106>
46. Adebo, O. A., & Medina-Meza, I. (2020). Impact of Fermentation on the Phenolic Compounds and Antioxidant Activity of Whole Cereal Grains: A Mini Review. *Molecules (Basel, Switzerland)*, 25(4), 927. <https://doi.org/10.3390/molecules25040927>
47. Stabrauskiene, J., Marksa, M., Ivanauskas, L., Bernatoniene, J. (2022). Optimization of Naringin and Naringenin Extraction from Citrus × paradisi L. Using Hydrolysis and Excipients as Adsorbent. *Pharmaceutics*, 14, 890. <https://doi.org/10.3390/pharmaceutics14050890> Academic Editors: Donatell
48. Xi, W., Zhang, G., Jiang, D. & Zhou, Z. (2015). Phenolic compositions and antioxidant activities of grapefruit (*Citrus paradisi* Macfadyen) varieties cultivated in China. *International Journal of Food Sciences and Nutrition*, 66(8). <http://dx.doi.org/10.3109/09637486.2015.1095864>
49. Ntana, F., Sarazin, C., Figge, R. & Mortensen, U. (2020). *Aspergillus*: A Powerful Protein Production Platform. *Catalysts*, 10. <http://dx.doi.org/10.3390/catal10091064>.
50. Francisc, D., Vodnar, D. & Socaciu, C. (2016). Effects of solid-state fermentation with two filamentous fungi on the total phenolic contents, flavonoids, antioxidant activities and lipid fractions of plum fruit (*Prunus domestica* L.) by-products. *Food Chemistry*, 209. <http://dx.doi.org/10.1016/j.foodchem.2016.04.016>.