Solid State Fermentation of Mexican Oregano (Lippia Berlandieri Schauer) Waste

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Abstract: Problem statement: Mexican oregano is recognized for their aromatic characteristics and flavor quality. Principal products obtained from the plant and marketing are the leaves and essential oil; however the extraction of the essential oil generates large amounts of agro industrial wastes; that can be used as support-substrates in Solid-State Fermentations (SSF). Approach: In this study a fungal bioprocess, as solid state fermentation using Mexican oregano wastes as support, for the use of these residues to obtain adds value products and/or molecules were developed. The fungal strain was selects by its adaptability to the support. The aqueous and non polar extracts were obtained kinetically until 120 h and then it was partially characterized (hydrolysable tannins, total sugar and proteins contents, antioxidant activity, tymol and carvacrol concentration). Results: Solid state fermentation of oregano wastes, with Aspergillus niger PSH, allowed the accumulation of a phenolic compound with catechin similar characteristics and could be responsible of the biotransformation of small amounts of carvacrol to thymol. Conclusion: These results could give an add value to Mexican oregano wastes and with more investigation the obtained products can be used in several industries.

Key words: Solid-State Fermentations (SSF), agroindustrial wastes, fungal biotransformation, phenolic compounds, hydrolysable tannins, support-substrates, Thin Layer Chromatography (TLC), results above described

INTRODUCTION

Lippia berlandieri Schauer or Lippia graveolens H.B.K. is the most distributed oregano specie in Mexico. Principal products obtained from the plant and marketing are the leaves and the essential oil; however the essential oil extraction generates large amounts of agro industrial wastes. These wastes have been used as animal feed, but the consumed amount is lower than the produced. To be friendlier with the environment, researchers had inverted efforts to reduce the amount of wastes by finding alternative uses (Orzua et al., 2009). One possibility is to use the agro industrial residues as support-substrates in Solid-State Fermentations (SSF).

SSF is defined as the cultivation of microorganisms on moist solid supports, either on inert carriers or on insoluble substrates that can, in addition, be used as carbon and energy source (Holker et al., 2004). The fermentation takes place in the absence or near absence of free water (Pandey et al., 2000); however, the substrate must possess enough moisture to support growth and metabolism of the microorganism (Singhania et al., 2009).

This activity has been done by other investigation groups and the molecules that have been released are enzymes, organic acids, polymers, flavors, antibiotics and aroma (Couto, 2008; Swe et al., 2009)) or bioconversions of crops and crop-residues (Holker et al., 2004; Medina-Morales et al., 2011). In this study we report a methodology development to find Mexican oregano wastes applications.

MATERIALS AND METHODS

The Mexican oregano waste was obtained from the rural industry “Oreganeros of central-south of Chihuahua” (in Chihuahua, Mexico). Was placed in black bags and transported to the Microbiology Laboratory of the Food Research Department, were the material was dried at 60°C during 48 h, pulverized in a...
blender until fine powder, and stored at room temperature in black bags.

Fungal strain tested were Aspergillus niger GH1, A. niger PSH, A. niger ESH, A. terricola PSS, Penicillium pinophilum EH2 and P. pinophilum EH3 (culture collection of the DIA-UADEC, Saltillo, Coahuita, Mexico) because of its high potential to degrade polyphenols (Cruz-Hernandez et al., 2005). Strains were propagated in Sabouraud broth at 30°C for 3 days. Wastes and Pontecorvo Medium with the following components (g L−1): C₆H₁₂O₆ (30), KH₂PO₄ (2.47), (NH₄)₂SO₄ (6.6), CaCl₂ (0.48), MgSO₄·7H₂O (0.38), NaCl (0.32), FeSO₄·7H₂O (0.124), yeast extract (0.05) and oligo-elements solution (ZnSO₄·7H₂O, H₂BO₃, MnCl·4H₂O, CoCl₂·6H₂O, CuSO₄·5H₂O)(1.0 m L−1) were sterilized, 15 Pa for 15 min. Wastes and medium were mixed and subsequently weighed out 10 g in Petri’s dishes (70% of humidity). 10^7 m L−1 of the samples were inoculated at the center of the plates. Mexican oregano plants were evaluated too as support-substrate for the fermentation process was A. niger PSH. The rest of the strains had little growth, from 11-2.4 mm. Based on the results showed that proteins, hydrolysable tannins and total sugar amount had similar behavior. The maximum value is at 20 h of fermentation time (1.05, 4.5 and 169.54 mg g−1 dry matter respectably). While hydrolysable tannins was at 40 h of fermentation time (0.73 and 72.04 mg g−1 dry matter). The rest of the strains had little growth, from 11-2.4 mm. Based on the adaptability to support-substrate (phase lag duration) and the invasion capacity of the wastes, selected strain for the fermentation process was A. niger PSH.

In the chemical partial-characterization of extracts the results showed that proteins, hydrolysable tannins and total sugar amount had similar behavior. The maximum value is at 20 h of fermentation time (1.05, 4.5 and 169.54 mg g−1 dry matter respectably). Minimum value for proteins and total sugar was at 60 h of fermentation time (0.73 and 72.04 mg g−1 dry matter respectably), while hydrolysable tannins was at 40 h of fermentation time (2.65 mg g−1 dry matter). However, after this, the values rise again. On the other hand, the antioxidant activity was higher at the beginning of the kinetics (73.2%) and it totally decreased at 80 h of fermentation time.
Fig. 1: Kinetical growth of six fungal strains above Mexican oregano wastes

Fig. 2: HPLC-Chromatographic profile of aqueous extract at 120 h solid state fermentation with A. niger PSH used as support-substrate Mexican oregano wastes

In HPLC profiles is possible to see a release compound since time 60-120 h, it has a singular peak with retention time of 14.5 min (Fig. 2). This compound agrees on time where proteins, total sugar and hydrolysable tannins had its lower values; however the retention time of the compound did not match with any standard (13.3 min for catechin, 7.15 min for gallic acid and 18.34 min for ellagic acid). As result in TLC, only one compound could be observed and its chemical properties seem to catechin; the Rf of the standards were 0.12 for gallic acid and 0.75 for catechin, while in the samples were 0.76.

Fig. 3: GC-Chromatogram profile of non polar extract at (A) 0 h and (B) 120 h solid-state fermentation with A. niger PSH used as support-substrate Mexican oregano wastes
In the no polar extract we found that main terpenes (thymol and carvacol) not were extracted totally in essential oil. Chromatograms showed that the carvacrol signal was lower with increasing fermentation time, while the thymol signal was higher (Fig. 3).

DISCUSSION

Oregano plants are not possible using as support-substrate in SSF because the main antimicrobial compounds (tymol and carvacrol) (Portillo-Ruiz et al., 2005) may not allow fungal growth and by consequence the bioprocess; instead, in the Mexican oregano wastes all the fungal strains grew because the thymol and carvacrol concentrations was minimal and culture media provide the necessary nutrients to the fungal metabolism.

Fungal strains, especially filamentous organisms, with a potential enzymatic machine to degrade chemical complex compounds are one of the most useful microorganisms in solid state fermentation (Banerjee et al., 2005, Medina et al., 2010). A. niger PSH had been used to produce tannases and release gallic and elagic acids from tar bush, creosote bush (Ventura et al., 2008) and pomegranate residues (Robledo et al., 2008) as others uses of agroindustrial wastes to obtain compounds with industrial applications; these reports support the strain selection to the bioprocess in Mexican oregano wastes.

Proteins, total sugar and hydrolyzed tannins consumption is major at the beginning of the fermentation because the microorganism needs them to start its reproduction phase. After several days, when the principal nutrients were consumed, the microorganism releases enzymes to degraded complex molecules, obtaining by this way sugars and other nutriments. If the metabolism of the microorganism is able, can be accumulated other molecules with industrial applications; these reports support the strain selection to the bioprocess in Mexican oregano wastes.

With the results of HPLC and TLC is able to think that the main compound present in the aqueous extract of fermented oregano waste is a phenolic compound with catechin in its molecule or a catechin derivate. This can be supported because the major amount groups of molecules that can be found in oregano plants and essential oil are monoterpenes and phenolics acids (Silva-Vazquez et al., 2008) but flavonoids (Arcila-Lozano et al., 2004) are present too. Bhat et al. (1998) mentioned that the distribution of tannins, based on their structure is hydrolysable and condensed; condensed tannins are composed by flavonoid units, however there is an intermediate position group formed by catechin tannins. We can propose that as consequence of fungal invasion, condensed or catechin tannins are formed with the components of the oregano wastes and this is the reason of the observed HPLC chromatogram profile.

On the other hand, thymol and carvacrol molecules are isomers, so we made the hypothesis that A. niger PSH makes a bioconversion (Bicas et al., 2009; Leutou et al., 2009; Ramachandran et al., 2008; Shimoda et al., 2006) during fermentation process and for these reason the thymol content was higher in later fermentation times than earlier. The isomerization from carvacrol to thymol can be a new method to purify this compound and then it can be used in pharmacy, food or perfumes industries (Carrera et al., 2005; Lambert et al., 2001; Tsimogiannis et al., 2006).

CONCLUSION

With the results above described, we propose that the compound released or/and accumulated at 120 h of SSF did not had antioxidant activity because it has tannins condensed structure, more similar to catechin than other molecules, as flavonoids. Also we have the hypothesis that A. niger PSH makes possible monoterpene isomerization of carvacrol to thymol. This possibilities gives an add value to Mexican oregano wastes, but is necessary to do more investigation about that.

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