LWT - Food Science and Technology 50 (2013) 575–580

Contents lists available at SciVerse ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Production of edible mushroom and degradation of antinutritional factors in jatropha biodiesel residues

José Maria Rodrigues da Luz, Sirlaine Albino Paes, Denise Pereira Torres, Mateus Dias Nunes, Juliana Soares da Silva, Hilário Cuquetto Mantovani, Maria Catarina Megumi Kasuya*

Departamento de Microbiologia, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, Campus UFV, Viçosa, Minas Gerais 36570-000, Brazil

ARTICLE INFO

Article history:
Received 6 June 2012
Received in revised form
1 August 2012
Accepted 7 August 2012

Keywords:
Jatropha curcas
Phytic acid
Tannins
Seed cake
Pleurotus ostreatus

ABSTRACT

The elimination of antinutritional factors of the *Jatropha curcas* L. seed cake is important for decreasing environmental damage and adding economic value to this residue of the biodiesel industry. In this study, we analyzed the ability of *Pleurotus ostreatus* to degrade antinutritional factors and produce edible mushrooms using different proportions of the *J. curcas* seed cake as substrate. After 60 d of incubation at 25 °C, we observed 95% phytic acid and 85% tannins reductions, and high mushrooms productivity. There was no evidence of tannins or phytic acid in these mushrooms. Furthermore, the phorbol ester concentration observed in these mushrooms was around 1000-fold lower than that found in the non-toxic variety of *J. curcas*. Thus, *P. ostreatus* can degrade the antinutritional factors found in *J. curcas* seed cake. The jatropha seed cake can potentially be used for mushroom production, with high nutritional value, and animal ration, after treated by *P. ostreatus*, adding economic value to the biodiesel residue and avoiding inadequate disposal in the environment.

1. Introduction

Phorbol ester, phytic acid and tannins are the main compounds that are found in the *Jatropha curcas* L. seed cake, that make this residue unusable as animal feed. The phorbol esters that are found in the seed and the oil are the major toxic compounds of *J. curcas* L. (Makkar, Becker, Sporer, & Wink, 1997). Due to the formation of an insoluble complex between the polyvalent cation and proteins, the phytic acid decrease the absorption of both mineral and protein in the gastrointestinal tract of animals (Liang, Han, Nout, & Hamer, 2009; Liu et al., 2008). Additionally, tannins also have a high capacity to form insoluble complex and precipitate protein, thereby inhibiting the digestion of proteins and amino acids (Rehman & Shah, 2005).

The elimination of these antinutritional factors (phytic acid and tannins) is important for increasing economic value and for making it possible to be used as animal feed. This elimination has been made by thermal (Deshpande & Damodaran, 1990; Rehman & Shah, 2005) or biological treatment (Batra & Saxena, 2005). The biological method besides being more specific and efficient than thermal treatment can result in products of economical interest (e.g. enzymes, mushrooms, animal feed).

Pleurotus ostreatus has been used in the bioremediation of pollutants and the degradation of lignocellulosic residue by the action of different enzymes (Dundar, Acay, & Yildiz, 2009; Haritash & Kaushik, 2009), including the lignocellulolytic enzymes, tannase and phytase (Batra & Saxena, 2005; Cavallazzi, Brito, Oliveira, Villas-Bôas, & Kasuya, 2004; Collopy & Royse, 2004). In addition, this fungus produces mushrooms using different lignocellulosic residues (Dundar et al., 2009; Fan, Soccol, Pandey, Vandenberghe, & Soccol, 2006; Nunes et al., 2012). The *P. ostreatus* mushrooms have high nutritional value and are sources of protein, carbohydrates, vitamins (e.g. B1, B2 and B3), calcium and iron (Dundar et al., 2009; Wang, Sakoda, & Suzuki, 2001).

Major agroindustrial residues have in its chemical composition higher fibers content with low availably than protein, minerals and vitamins (Villas-Bôas, Esposito, & Mitchell, 2002). Colonization and solid fermentation by fungi have been used to increase the availably and the nutritional value of these residues (Pereira, 2011; Sánchez, 2009; Villas-Bôas et al., 2002). This procedure has been used with success in cocoa (Alemawor, Dzogbefia, Oddoye, & Oldham, 2009), sawdust (Kwak, Jung, & Kim, 2008) and jatropha seed cake (Pereira, 2011).

* Corresponding author. Tel.: +55 31 3899 2970; fax: +55 31 3899 2573.
E-mail address: mkasuya@ufv.br (M.C.M. Kasuya).

0023-6438
http://dx.doi.org/10.1016/j.lwt.2012.08.006

Thus, in this study, we tested the ability of *P. ostreatus* to degrade antinutritional factors and produce edible mushrooms using different proportions of the *J. curcas* seed cake as substrate.

2. Materials and methods

2.1. Microorganism, fungal growth conditions and inoculum production (spawn)

The isolate Plo 6 of *P. ostreatus*, which were used in this study, belong to collection of the Department of Microbiology of Federal University of Viçosa, MG, Brazil. This isolate was grown in a Petri dish containing potato dextrose agar culture medium (Merck) at pH 5.8 and incubated at 25 °C. After 7 days, the mycelium was used for inoculum production (spawn) in a substrate made of rice grains with peel (de Assunção et al., 2012). The rice grains were cooked for 30 min in water at a 1:3 (rice grains:water, w/w). After cooking, the grains were drained and supplemented with 0.35 (g/100 g) $CaCO_3$ and 0.01 (g/100 g) $CaSO_4$. These grains (70 g) were packed into small glass jars and sterilized in an autoclave at 121 °C for 1 h. After cooling, each jar was inoculated with 4 agar discs (5 mm diameter) containing mycelium and incubated in the dark at 25 ± 2 °C for 15 d.

2.2. substrate and inoculation

The *J. curcas* seed cake used in this study was obtained from an industry of biodiesel (Fuserman Biocombustíveis, Barbacena, Minas Gerais State, Brazil).

The proper substrate composition for optimal growth and enzyme production by *P. ostreatus* was chosen based on previously experiments with jatropha seed cake and different agroindustrial residues (Da Luz, 2009). In these experiments, *P. ostreatus* was cultivated in substrates with seed cake added to different proportions of eucalypt sawdust, corncob, eucalypt bark and coffee husk. The purpose of adding these agroindustrial residues was to balance the carbon and nitrogen ratio, which may stimulate the mycelial growth (Nunes et al., 2012). The substrate compositions that were selected for this study were based on the results of these previous experiments were jatropha seed cake (Sc), Sc + 10 (g/100 g) of eucalypt sawdust (ScEs), Sc + 10 (g/100 g) of eucalypt bark (ScEb) and Sc + 30 (g/100 g) of coffee husk + 30 (g/100 g) of rice bran (ScCh). In these substrates, the isolate Plo 6 had better biomass production and greater degradation rate of lignocellulosic compounds when compared to other tested substrates (Da Luz, 2009).

The substrates were humidified with water at 75% of the retention capacity and 1.5 kg of each substrate was placed in polypropylene bags. Next, the bags containing the substrates were autoclaved at 121 °C for 2 h. After sterilization, the substrates were inoculated with 70 g of spawn and incubated at 25 °C for 60 d. Samples for analyses were taken at intervals of 15 d.

2.3. Enzymatic assays

Phytase activity (myo-inositol hexakisphosphate phosphohydrolase, EC 3.1.3.8) was determined using Taussky–Schoor reagent according to Harland and Harland (1980). To extract the enzyme, 3 g of the substrate was transferred to Erlenmeyer flasks (125 mL) containing 10 mL of sodium chloride (1 g/100 mL). The flasks were kept in a shaker for 1 h at 100 rpm, and the extracts were filtered through Millipore membranes (Whatman 1). The filtrate was centrifuged for 5 min at 2000 × *g*. The reaction to determine phytase activity contained 100 μL of the filtrate and 1 mL of sodium phytate solution (0.5 g/100 g, Sigma). This reaction was incubated in a water bath at 60 °C for 10 min, and then 1 mL of trichloroacetic acid (10 g/100 mL) and 5 mL of Taussky–Schoor reagent were added. The phosphorus content was determined with a spectrophotometer (Thermo, Evolution 60) at 500 nm. The standard curve for phosphorus quantification was made using dibasic potassium phosphate (Sigma) with concentrations ranging from 0.004 to 0.02 g/100 mL.

One unit of phytase was defined as the amount of enzyme required to release 1 μmol of inorganic phosphate per min from sodium phytate at 37 °C.

2.4. Determination of phytic acid concentration

To determine phytic acid content, 3 g of each substrate was transferred to Erlenmeyer flasks (125 mL) containing 25 mL hydrochloric acid (4 g/100 mL, Vetec). These flasks were kept in a shaker for 16 h at 220 rpm. The supernatants were transferred to centrifuge tubes (50 mL) containing 1 g of sodium chloride (Vetec), centrifuged at 1000 × *g* for 20 min and frozen at −20 °C for 30 min.

After thawing, the supernatants were centrifuged under the same conditions and filtered through Millipore membranes (Whatman GF/D, 4.7 cm). The filtrate (1 mL) was diluted in 24 mL of deionized water. Then, 3 mL of this solution was transferred to another centrifuge tube (50 mL) containing 1 mL of Wage reagent (0.03 g/100 g of ferric chloride hexahydrate, 0.3 g/100 mL of sulphosalicylic acid, 2.4 g/100 mL hydrochloric acid 0.65 mol/L). This mixture was again centrifuged (1000 × *g*) at 10 °C for 10 min, and the absorbance of the supernatant was detected at 500 nm using a spectrophotometer (Latta & Eskin, 1980). Sodium phytate (Sigma) concentrations ranging from 0.03 to 1.6 g/100 mL were used to make a standard curve.

2.5. Determination of tannins concentration

For extraction of the tannins in 3 g of substrate were added 10 mL methanol (Sigma) and 0.5 g of polyvinylpyrrolidone (Makkar, Bluemmel, & Becker, 1995). This material was homogenized in a shaker at 220 rpm for 1 h, and 5 mL barium hydroxide (0.1 mol/L) and 5 mL of zinc sulfate were added. The reaction to determine the tannins content (tannic acid equivalent) contained 2 mL of the supernatant, 5 mL of sodium carbonate (2 g/100 mL) in sodium hydroxide (0.1 mol/L) and 1 mL of Folin–Ciocalteu's reagent. This reaction was incubated in a water bath at 37 °C for 10 min, and the absorbance was detected at 765 nm using a spectrophotometer (Makkar et al., 1995). The standard curve was made using a solution of tannic acid (Sigma) with concentrations ranging from 0.01 to 1 g/100 mL.

2.6. Mushroom production

Polypropylene bags containing substrates with mycelial growth after 28, 43 and 58 d of incubation were transferred to a cold chamber at 10 °C for 48 h. This procedure was performed to induce the formation of the primordial of fruit bodies. Mushrooms fructification was performed in a chamber with temperature controlled at 18 ± 2 °C.

The biological efficiency (BE) was calculated according to Wang et al. (2001): BE = 100 × (fresh mass of mushroom (g)/dry mass of substrate (kg)).

2.6.1. Mushroom chemical analysis

The mushrooms were chemically analyzed to verify the concentrations of antinutritional factors, phosphorus, ergosterol, phorbol ester, soluble protein and reducing sugars. Mushrooms produced in each substrate were mixed, and from this mixture,