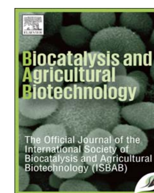
Biocatalysis and Agricultural Biotechnology 8 (2016) 204–208

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

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



Influence of cultivation *Rhizopus oryzae* on rice bran on lipid fraction: Fatty acids and phospholipids

Kelly Cristina Massarolo*, Taiana Denardi de Souza, Anelise Christ Ribeiro, Eliana Badiale Furlong, Leonor Almeida de Souza Soares

Laboratory for Mycotoxins and Food Science, Post Graduate Program in Engineering and Food Science, School of Chemistry and Food, Federal University of Rio Grande – FURG, Avenida Itália km 8, Campus Carreiros, 96203-900 Rio Grande, RS, Brazil

ARTICLE INFO

Keywords:
Biomass
Gas chromatography
Inorganic phosphorus
Lipids

ABSTRACT

The aim of this study was to evaluate changes in the lipid fraction of rice bran after cultivation with *Rhizopus oryzae*. The content of lipids, phospholipids and fatty acid profile, besides the three phosphorus forms were regularly analyzed during the cultivation time. The total lipids from cultivated rice bran increased from 16.2% to 19.0% in the range between 0 h and 60 h of cultivation and phospholipid contents were increased 29% after 12 h of cultivation. In the control sample, linolelaidic, oleic and palmitic acids prevailed, with a decrease in oleic (15.5%) and increase in linolelaidic (11.9%) at 84 h of cultivation. In the same range the essential fatty acid (ω6 and ω3) increased from 44.5% to 50.4%. The inorganic phosphorus increased from 12 h cultivation and it reached content more than the organic with 36 h. This study showed that the cultivation with *Rhizopus oryzae* on rice bran can be applied to change the essential fatty acids profile and phospholipids.

1. Introduction

Rice bran is a byproduct obtained from the beneficiation operation of rice, derived from the grain coating layers, germ and minor amounts of broken endosperm, that add up together 10% of total grain (Poulari et al., 2009). In the rice bran are concentrated lipids, between 19.4% and 25.5% (Liu et al., 2013), of which is obtained the oil that is considered one of the most valuable and healthy because contain vitamins of B complex, vitamins E (α-tocopherol and tocotrienol), vitamin K, γ-oryzanol and fatty acid (Khoei and Chekin, 2015; Lemos and Souza-Soares, 2000).

Rice bran also is employed as substrate to filamentous fungus cultivation, which can promote the available of nutrients or production of interest compounds to food industry, pharmaceutical, among others (Poulari et al., 2010). The commercial products obtained by fungal culture in rice bran that stands out are the lactic acid (Watanabe et al., 2013); proteic biomass (Oshoma and Ikenebomeh, 2005); proteolytic (Ali and Vidhale, 2013), cellulolytic (Kupski et al., 2015) and amylolitics enzymes (Grover et al., 2013).

Oliveira et al. (2011) and Schmidt et al. (2014) employed rice bran as substrate to *Rhizopus oryzae*, where they highlighted and found changes in the lipids availability, phospholipids, and phenolic acids. However, these authors did not explore the variation in the lipid fraction profile, which is a determinant aspect to the indication of applicability of cultivation process aiming the obtaining of industrial interest compounds.

To fill this gap, this study evaluated the influence of solid state cultivation of *Rhizopus oryzae* in rice bran on the change of lipids profile, as well as the phospholipids content and the phosphorus forms in the fungal biomass.

2. Material and methods

2.1. Material

Rice bran (RB) was provided by industries from Rio Grande do Sul, Brazil. The fungus *Rhizopus oryzae* CCT 7560 was obtained from the André Tosello Foundation, Campinas, Brazil. The standard methyl ester (Supelco® 37 Component FAME Mix) and Potassium Phosphate Monobasic P.A. ACS anhydrous (purity $>99\%$) were purchased from Sigma Aldrich, USA.

2.2. Rice bran

The granulometry profile of the RB was evaluated in sieves with openings of 1.67; 0.73; 0.50 and 0.39 mm, taking up 100 g of sample as initial quantity. The proximal composition was determined according to AOAC methods (2000).

* Corresponding author.
E-mail address: kelly_massa@hotmail.com (K.C. Massarolo).

http://dx.doi.org/10.1016/j.bcab.2016.10.002
Received 24 July 2016; Received in revised form 7 September 2016; Accepted 3 October 2016
Available online 03 October 2016
1878-8181/ © 2016 Elsevier Ltd. All rights reserved.

2.3. *Rhizopus oryzae cultivation*

The cultures were maintained at 4 °C in slants of potato-dextrose agar (PDA). The spores were scraped in medium PDA and incubated during 7 days at 30 °C until a whole new sporulation of the fungus. Spore suspension for cultivation was achieved by adding 10 mL of an aqueous emulsion of Tween 80 (0.2%) to each plate and the release of spores by scraping the plates with Drigalski handle. The spore's concentration was estimated by enumeration in a Neubauer chamber.

The generation of biomass was based on the method described by Oliveira et al. (2010), where the RB was used as substrate. Rice bran (100 g) was placed in bioreactors with dimensions of 12,5×12,5×5 cm^3, forming a fine layer of ~2 cm and autoclaved, after its homogenization with 45 mL nutrient solution (2 g/L of KH_2PO_4, 1 g/L of $MgSO_4$ and 1.8 g/L of NH_2CONH_2 in HCl 0.4 M), previously autoclaved. Bran initial spores concentration was 4×10^6 spores/g_{bran} and sterile water was added to the medium in order to adjust the humidity to 50%. The bioreactors were placed in a fermentation chamber at 30 °C. Upon expiry of the incubation time (0–120 h, with sampling every 12 h), the biomass was stored at −18 °C. The control (0 h) was RB sterilized and nutrient and spores solution addition after immediately stored at −18 °C.

2.4. *Characterization of biomass lipid fraction*

The biomass fungal collected during the cultivation were submitted a lipids extraction by the method Folch et al. (1957) following the determination of phospholipids and fatty acids profile.

2.4.1. *Determination of phospholipids*

Phospholipids were determined according Esteves et al. (1995) with adaptations, in the lipids extracted were added MgO and the dry matter was incinerated in muffle at 800 °C until constant weight. The gray was dissolved in water and sulfuric acid 1 M for colorimetric determination of phosphorus (phospholipids), using the redox reaction with molybdate.

The standard curve of phosphorus (PO_4) (0.1 a 1.25 µg/mL) was used to estimate the concentration of phospholipids.

2.4.2. *Fatty acids profile*

In the lipid fraction extracted from biomass and control also was verified the fatty acid composition by gas chromatography. Lipids were esterified by Metcalfe et al. (1966) adapted method, which consisted in lipid saponification with KOH 0.5 M in methanolic solution and catalyzed by boron trifluoride methanolic solution and solvent evaporation. The sample was solubilized by dichloromethane, from which 1 µL was injected for GC analyses.

To separate and quantify the esterified fatty acid mixture, a gas chromatograph was used (Shimadzu 2010 Plus), equipped with split/splitless injector, capillary column RTX®−1 (30 m×0.25 mmID×0.25 µm) and flame ionization detector (FID). Hydrogen was the carrier gas at a flow of 1.25 mL/min. The injector and detector temperatures were set to 260 °C. The chromatographic conditions for separation were column initial temperature of 50 °C, raising to 200 °C at a flow rate of 6 °C/min, holding during 4 min at this temperature. The second step consisted in increased at a heating rate of 2 °C/min to 240 °C, and held for 10 min

The comparation of retention times with methyl ester standards was used to identification of fatty acids profile of the samples and quantified by standardization of the areas.

2.5. *Determination of organic, inorganic and total phosphorus*

For total phosphorus determination were weighed 300 mg of samples and digestion was performed in a microwave oven (Multiwave 3000, Microwave Sample Preparation System, Anton Paar, Graz, Austria) in PTFQ bottles (HF 100) with 6 mL of concentrated HNO_3. The heating program was realized with power of 1400 W, ramp of 10 min, and temperature of 210 °C for 20 min permanence and in the second step the same parameters for more 20 min

After digestion, samples were diluted in HNO_3 5%, for subsequent quantification by optical emission spectrometer with inductively coupled plasma (ICP OES) using axial view of observation in the equipment (model Optima 4300 DV, Perkin Elmer, Shelton, USA). The introduction of the samples in the spectrometer were made employing a GemCone nebulizer and a cyclonic chamber fogging and the equipment operating conditions were: power 1400 W; main gas flow 15 L/min; auxiliary gas flow 0.2 L/min; nebulization gas flow 0.7 L/min and wave-length 214.914 nm. The plasma was formed from argon (White Martins, São Paulo, Brazil), with purity of 99.9%.

Inorganic phosphorus extraction (freeform) was realized from 1 g biomass, added 50 mL hydrogen chloride 1 M and orbital shaking in 220 rpm for 3 h. After extracts obtainment, phosphorus levels were determined by colorimetry with reagent vanadate molybdate and measure in spectrophotometry at wave-length 420 nm. For quantification was used phosphorus calibration curve (0–30 µg/mL). Organic phosphorus was determined by the difference between inorganic and total phosphorus.

2.6. *Statistical analysis*

All the determinations were carried out in triplicate. To evaluate the significant differences of dependent variables (lipids, phospholipids, phosphorus and fatty acids) during the cultivation time (independent variable) was used Analysis of Variance (ANOVA), using the Software *Statistica* 6.0. Differences with a probability value of < 0.05 were considered significant and the results were reported as mean ± standard deviation.

3. Results and discussion

3.1. *Rice bran characterization*

The rice bran analyzed showed 46.1% of the particles with sizes smaller than 0.39 mm (Table 1) and 14.4% of whole, broken grains and rice husk, being these particles sizes bigger than 0.73 mm. The sample showed only 85.6% of rice bran, and the other part of the sample showed rice husk and grain. This can cause low extraction yield if it will be used to extraction of interest compounds that are concentrated in the rice and not in the husk or grain, such as lipids (Marchezanll and De Avilall, 2008). This suggests that it would be interesting to review the separation process of bran during the rice beneficiation to become the byproduct more uniform and appropriate to different techniques of its valuation.

In each particle size were determined the lipids content (Fig. 1). The larger particles sizes from rice bran showed smaller lipids content, and the less content verified in particles sizes larger than 1.67 mm. The

Table 1
Particle sizes distribution in rice bran.

Particle sizes	Mean (g)*	Description	Yield (%)
Larger than 1.67 mm	0.59±0.06	Whole grains and rice husk	0.6
Between 1.67 and 0.73 mm	13.67±0.71	Broken grains and rice husk	13.8
Between 0.73 and 0.50 mm	13.22±2.00	Thin rice husk and bran	13.3
Between 0.50 and 0.39 mm	26.06±11.53	Bran	26.2
Smaller than 0.39 mm	45.68±13.57	Bran	46.1

* Values are expressed as means ± S.D (n=3). S.D= Standard deviation.

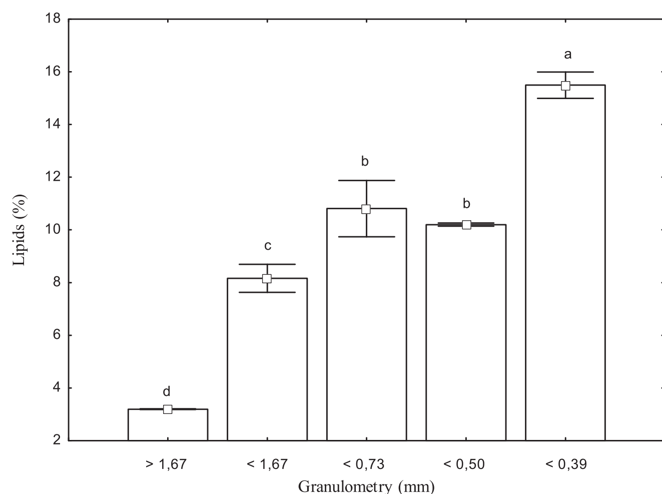


Fig. 1. Lipids content in different particle sizes. Same letters indicate no significant difference ($p > 0.05$).

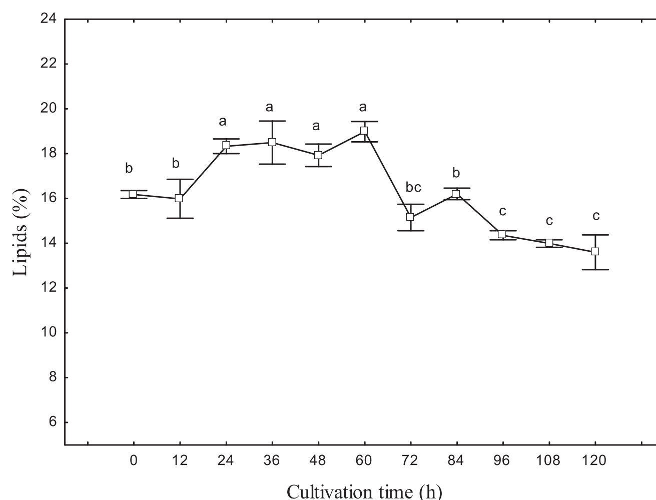


Fig. 2. Lipids content during *Rhizopus oryzae* cultivation. Same letters indicate no significant difference ($p > 0.05$).

increase of lipids in small particles (< 0.39 mm) can be explained by the physical effects on the surface of the particle, because with the reduction of particles size the accessible surface is increased and, consequently, the access for extractive solvent too, resulting more lipid extraction. The diffusion to the surface of solid is reported to be one of the major limiting steps in solid/liquid extraction (Vandenburg et al., 1997), being that the intraparticle diffusion resistance is generally lower for smaller particles due to their diffusion path is shorter..

For the cultivation, was standardized the rice bran containing 40% particles sizes smaller than 0.39 mm, 25% particles with sizes between 0.50 and 0.39 mm, 25% particles sizes between 0.73 and 0.50 mm and 10% particles sizes between 1.67 and 0.73 mm. The particles sizes used to the cultivation were defined taking into account the lipid content and studies that showed that particles with larger sizes result in a positive effect on biomass production and substrates with smaller particles sizes offer a larger contact area between the substrate and the fungus, favoring their growth, while only small particles can cause the compaction and formation of agglomerates, which results in decreased oxygen transfer, affecting respiration and fungal growth (Membrillo et al., 2011; Schmidt and Furlong, 2012). In view of this information, the use of different particles sizes portions can provide increased growth of *Rhizopus oryzae* in rice bran.

With the standardized particle size, it was determined macro constituents moisture 11.2% ± 0.29, ash 10.0% ± 0.52, fiber 6.5% ± 4.11, lipids 17.9% ± 5.75, protein 13.0% ± 4.29 and carbohydrates 41.3% ± 3.01. The moisture content and ash are similar to those reported by Oliveira et al. (2010). However, the fiber, lipids and proteins content were lower, this is due to the fact that rice bran is an agro-industrial byproduct and, therefore its chemical composition depends on factors associated with the variety and the agronomic aspects, such as soil type, climate, quality of raw material used and the beneficiation process, becoming ever need different studies involving bran from several origins to increasing knowledge of conditions that affect the fungal growth.

With this composition the resulting bran can be used as a substrate for *Rhizopus oryzae* cultivation, because the macronutrients are sufficient for generating fungal biomass after supplementation with mineral micronutrients.

3.2. Lipid fraction

The fungi synthesize lipids to attend theirs basic functions and formation of cell membranes, although some species can accumulate lipids generating biomass up to 20% of them (Cheirsilp and Kitcha, 2015; Dey et al., 2011; Lin et al., 2010).

The total lipid content in biomass was evaluated during cultivation (Fig. 2), it was verified that occurred increase in the level of extractable lipids by chloroform: methanol mixture, with more content (19.0%) at 60 h cultivation, which represented an increase 17.3% in relation the content lipid in 0 h. The lipid increased can occur through a straight path of conversion the lignocellulosic biomass, by a set of cellulolytic enzymes, in sugars and consequently lipids can be developed (Cheirsilp and Kitcha, 2015)..

3.2.1. Phospholipids

Due to their technological and nutritional properties, phospholipids were determined over the formation of biomass in experimental ranges (Table 2). In the first 12 h of cultivation there was a significant increase of 29% of phospholipids, and these was reducing from 24 h arriving in the end of the range cultivation with 0.13 mg/g biomass (45.8% reduction compared with the control). Different results to that seen by Oliveira et al. (2011), when the phospholipid content increased in the range between 0 h and 120 h of rice bran fermentation with *Rhizopus oryzae*. The particle size standardized by the author was different in our study and Oliveira et al. (2011) expressed the phospholipids in mg P g^{-1} l_{ipid} and the lipid content reduced with the fermentation. The reduction in phospholipids in biomass after 96 h followed the behavior of lipid levels (Fig. 2) as well as an increase of inorganic phosphorus (Fig. 3) which can be phosphate from phospholipids..

Phospholipids obtaining is interesting for industry, because to being amphiphilic and highly active in surfaces they can influence significantly in the physical properties of emulsions and foams, in

Table 2
Phospholipid in biomass during cultivation.

Time cultivation (h)	Phospholipid (mg/g biomass)	Phospholipid (mg/g lipid)
0	0.24 ± 0.01^{bc}	1.49 ± 0.08^{bcd}
12	0.31 ± 0.03^{a}	1.97 ± 0.05^{a}
24	0.20 ± 0.01^{cd}	1.09 ± 0.02^{ef}
36	0.22 ± 0.01^{bcd}	1.17 ± 0.07^{ef}
48	0.22 ± 0.02^{bcd}	1.20 ± 0.06^{f}
60	0.21 ± 0.01^{bcd}	1.11 ± 0.06^{ef}
72	0.26 ± 0.02^{ab}	1.72 ± 0.07^{b}
84	0.24 ± 0.02^{bc}	1.50 ± 0.12^{bc}
96	0.19 ± 0.01^{cd}	1.31 ± 0.07^{cdf}
108	0.18 ± 0.00^{de}	1.26 ± 0.01^{df}
120	0.13 ± 0.00^{e}	0.94 ± 0.05^{e}

Same superscript letters in the same column indicate no significant difference ($p > 0.05$).
Values are expressed in dry base and as means ± S.D (n=3). S.D= Standard deviation

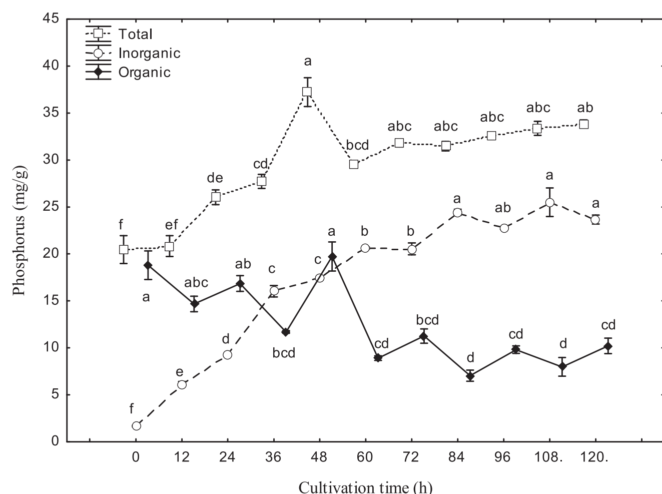


Fig. 3. Total, organic and inorganic phosphorus during *Rhizopus oryzae* cultivation. Same letters indicate no significant difference by Tukey test ($p > 0.05$).

addition to forming supramolecular structures of self-assembly, a key component in nanoscience to separating immiscible phases, vital to dispersions of food and cosmetic formulations (Patino et al., 2007).

3.2.2. Fatty acid profile

Of the 37 fatty acids (FAs) analyzed in the lipids from biomass (Table 3), in the control sample (0 h) the main FAs found were: 40.2% of linolelaidic (C18:2n6t), 33.6% oleic (C18:1n9c) and 20.2% palmitic acids (C16:0).

The palmitic acid percentage has not changed during the interval studied. However, oleic acid was reduced in 15.5% and linolelaidic increased in 11.9% in the range of 84 h. The reduction of oleic acid, a major fatty acid composing of *Rhizopus oryzae* (Oliveira et al., 2011), is related to fungal response to cultivation conditions and its metabolism. Silveira et al. (2010) observed that after 72 h of fermentation of rice and wheat bran with the same micro-organism, it occurred an increase in the levels of palmitic and linoleic acids.

In the Oliveira et al. (2011) study, the predominant FAs were the same identified in this study, however it has not been observed change in the content of oleic (C18:1) and palmitic (C16:0) acids during the bio cultivation. The authors verified also an increase of 10% of linoleic acid (C18:2), without differentiate the linoleic of linolelaidic acid, which are different for *cis* and *trans* isomers, though natural FAs predominantly exhibit *cis*-isomer, some unsaturated FAs from vegetables and micro-organisms can show *trans*-isomer. Some polyunsaturated FAs can be mixed, that is, show *cis* and *trans* isomers in the same molecule (De Souza et al., 2012).

An interesting aspect in the change on FA profile was the reduction of saturated FAs in 6.2% and increased of 13% of polyunsaturated at 84 h. Fact which becomes interesting to recommendation of the previous use of cultivation in preparing food formulations, because polyunsaturated FAs tend to lower blood cholesterol levels, while saturated raise (Scherr et al., 2015).

The ratio of polyunsaturated/saturated increased from 2.1 to 2.6, reinforcing the importance of cultivation to improve the nutrition intake of FA, because for the Department of Health in England recommends relations greater than 0.45 to prevent cardiovascular disease. Another important indicator to health benefits is the ratio of ω6/ω3 (recommended 4:1 to 10:1). With the cultivation process this index decreased by 34.9% reaching the ratio value of 12.3, still standing up recommending. This reduction in the ratio of ω6/ω3 is due to the increase of α-linolenic acid (C18:3n3) in 55.6%, eicosatrienoic (C20:3n3) in 40% and the production of eicosapentaenoic acid (EPA) (C20:5n3), which was identified only after 48 h.

The high production of α-linolenic acid is also important, because it is a precursor in the biosynthesis of EPA and docosahexaenoic acids (DHA), which are required for biological membranes, retina, cerebral cortex, neural tissue, testicles and platelets blood, which makes these essential FAs indispensable for the metabolic balance (De Souza et al., 2012).

To Oliveira et al. (2011) changes in FAs profile are related with *Rhizopus oryzae* in response the fermentation conditions, because the

Table 3
Fatty acids profile in each cultivation time.

Fatty acid	Cultivation time (h)										
	0	**12**	**24**	**36**	**48**	**60**	**72**	**84**	**96**	**108**	**120**
14:0	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{bc}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}
16:0	20.2 ± 0.5^{a}	20.3 ± 0.5^{a}	21.2 ± 1.2^{a}	20.1 ± 0.3^{a}	20.6 ± 0.2^{a}	23.0 ± 4.8^{a}	19.3 ± 0.7^{a}	18.4 ± 0.3^{a}	19.5 ± 0.3^{a}	19.3 ± 1.1^{a}	19.2 ± 0.3^{a}
16:1	0.1 ± 0.0^{ab}	0.1 ± 0.0^{ab}	0.1 ± 0.0^{bc}	0.1 ± 0.0^{cd}	0.1 ± 0.0^{cd}	0.1 ± 0.0^{cd}	0.1 ± 0.0^{d}	0.1 ± 0.0^{d}	0.1 ± 0.0^{cd}	0.2 ± 0.0^{a}	0.1 ± 0.0^{cd}
18:0	0.1 ± 0.0^{c}	0.1 ± 0.0^{c}	0.5 ± 0.0^{b}	0.7 ± 0.0^{ab}	0.7 ± 0.0^{ab}	0.8 ± 0.0^{a}	0.7 ± 0.0^{ab}	0.5 ± 0.0^{b}	0.8 ± 0.1^{ab}	0.7 ± 0.1^{ab}	0.7 ± 0.0^{ab}
18:1n9c	33.6 ± 0.1^{a}	32.8 ± 0.1^{a}	31.5 ± 0.1^{b}	30.7 ± 0.1^{bcd}	30.0 ± 0.4^{cde}	29.9 ± 0.2^{de}	29.5 ± 0.3^{e}	28.4 ± 0.1^{f}	31.1 ± 0.7^{bc}	29.7 ± 0.1^{de}	29.8 ± 0.3^{de}
18:1n9t	n.d.^{c}	n.d.^{c}	n.d.^{c}	0.3 ± 0.1^{a}	0.1 ± 0.0^{b}	0.2 ± 0.0^{ab}	0.2 ± 0.0^{ab}	0.2 ± 0.0^{ab}	0.2 ± 0.0^{b}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}
18:2n6c	0.7 ± 0.0^{a}	0.6 ± 0.0^{ab}	0.4 ± 0.0^{b}	n.d.^{d}	n.d.^{d}	0.1 ± 0.0^{c}	n.d.^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}	n.d.^{d}
18:2n6t	40.2 ± 0.2^{e}	40.3 ± 0.2^{de}	40.8 ± 0.8^{de}	41.8 ± 0.6^{cde}	42.0 ± 0.2^{cde}	44.0 ± 0.8^{ab}	43.0 ± 0.0^{bc}	45.0 ± 0.5^{a}	42.2 ± 0.8^{bcd}	43.6 ± 0.8^{abc}	43.3 ± 0.2^{abc}
18:3n6	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.4 ± 0.0^{a}	0.5 ± 0.1^{a}	0.6 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.0^{a}	0.5 ± 0.1^{a}	0.5 ± 0.1^{a}
18:3n3	1.8 ± 0.0^{e}	1.8 ± 0.0^{e}	2.2 ± 0.1^{cd}	2.3 ± 0.0^{bc}	2.2 ± 0.0^{cd}	2.5 ± 0.1^{b}	2.4 ± 0.0^{bc}	2.8 ± 0.1^{a}	2.1 ± 0.0^{d}	2.2 ± 0.1^{cd}	2.1 ± 0.0^{d}
20:3n6	n.d.^{c}	n.d.^{c}	$< 0.1 \pm 0.0^{b}$	n.d.^{c}	$< 0.1 \pm 0.0^{ab}$	$< 0.1 \pm 0.0^{ab}$	$< 0.1 \pm 0.0^{ab}$	0.1 ± 0.0^{a}	$< 0.1 \pm 0.0^{ab}$	$< 0.1 \pm 0.0^{ab}$	$< 0.1 \pm 0.0^{ab}$
20:3n3	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.4 ± 0.0^{b}	0.6 ± 0.0^{ab}	0.6 ± 0.0^{ab}	0.7 ± 0.1^{a}	0.6 ± 0.0^{a}	0.7 ± 0.0^{a}	0.6 ± 0.0^{ab}	0.6 ± 0.0^{ab}	0.6 ± 0.0^{ab}
20:4n6	0.9 ± 0.2^{a}	0.9 ± 0.2^{a}	0.7 ± 0.1^{a}	0.9 ± 0.0^{a}	0.9 ± 0.0^{a}	1.0 ± 0.2^{a}	0.9 ± 0.0^{a}	1.0 ± 0.0^{a}	0.8 ± 0.0^{a}	0.9 ± 0.1^{a}	0.9 ± 0.0^{a}
20:5n3	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.5 ± 0.1^{a}	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.6 ± 0.2^{a}
23:0	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.4 ± 0.1^{ab}	0.6 ± 0.0^{ab}	0.5 ± 0.0^{ab}	0.6 ± 0.1^{ab}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	0.5 ± 0.0^{ab}	0.6 ± 0.1^{ab}	0.6 ± 0.0^{a}
24:0	n.d.^{b}	n.d.^{b}	n.d.^{b}	n.d.^{b}	$< 0.1 \pm 0.0^{a}$	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	$< 0.1 \pm 0.0^{a}$	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}
24:1n9	0.7 ± 0.1^{b}	0.7 ± 0.1^{b}	0.8 ± 0.2^{ab}	1.2 ± 0.2^{ab}	1.2 ± 0.0^{ab}	1.2 ± 0.3^{ab}	1.4 ± 0.0^{a}	1.2 ± 0.0^{ab}	1.2 ± 0.1^{ab}	1.3 ± 0.3^{ab}	1.3 ± 0.1^{ab}
SFA	21.0 ± 0.1^{ab}	21.0 ± 0.2^{ab}	22.4 ± 0.3^{ab}	21.6 ± 0.3^{ab}	22.0 ± 0.3^{ab}	24.5 ± 0.3^{ab}	20.8 ± 0.2^{b}	19.7 ± 0.1^{b}	21.0 ± 0.2^{ab}	20.7 ± 0.4^{ab}	20.6 ± 0.2^{ab}
MFA	34.5 ± 0.1^{a}	33.7 ± 0.2^{ab}	32.4 ± 0.2^{bc}	32.3 ± 0.2^{cd}	31.4 ± 0.4^{cd}	31.3 ± 0.5^{cd}	31.1 ± 0.3^{d}	29.9 ± 0.1^{e}	32.5 ± 0.8^{bc}	31.3 ± 0.2^{cd}	31.3 ± 0.2^{cd}
PUFA	44.6 ± 0.3^{d}	44.6 ± 0.2^{d}	45.2 ± 1.0^{d}	46.1 ± 0.5^{cd}	46.6 ± 0.4^{cd}	49.3 ± 1.2^{ab}	48.1 ± 0.1^{bc}	50.4 ± 0.6^{a}	46.5 ± 0.7^{cd}	48.2 ± 1.0^{abc}	48.1 ± 0.1^{abc}
PUFA/SFA	2.1 ± 0.1^{ab}	2.1 ± 0.1^{ab}	2.0 ± 0.2^{b}	2.1 ± 0.1^{ab}	2.1 ± 0.0^{ab}	2.1 ± 0.4^{ab}	2.3 ± 0.1^{ab}	2.6 ± 0.1^{a}	2.2 ± 0.0^{ab}	2.3 ± 0.2^{ab}	2.3 ± 0.0^{ab}
w6	42.3 ± 0.3^{d}	42.3 ± 0.1^{d}	42.5 ± 0.9^{cd}	43.2 ± 0.5^{bcd}	43.3 ± 0.3^{bcd}	45.6 ± 0.9^{a}	44.5 ± 0.1^{abc}	46.6 ± 0.5^{a}	43.5 ± 0.8^{bcd}	45.1 ± 0.9^{ab}	44.8 ± 0.2^{ab}
w3	2.2 ± 0.1^{f}	2.2 ± 0.1^{f}	2.6 ± 0.1^{ef}	2.9 ± 0.1^{de}	3.2 ± 0.2^{bcd}	3.7 ± 0.3^{ab}	3.5 ± 0.1^{abc}	3.8 ± 0.1^{a}	3.0 ± 0.1^{cde}	3.1 ± 0.1^{cde}	3.3 ± 0.2^{abcd}
w6/w3	18.9 ± 0.4^{a}	18.9 ± 0.5^{a}	16.1 ± 0.3^{b}	14.8 ± 0.5^{bc}	13.5 ± 0.8^{cde}	12.5 ± 0.7^{de}	12.7 ± 0.3^{de}	12.3 ± 0.3^{e}	14.4 ± 0.8^{bcd}	14.4 ± 0.2^{bcd}	13.6 ± 0.8^{cde}

Values are expressed as means ± sd. The values in each line with the same superscript letter are not significantly different by Tukey test ($p < 0.05$), where each fatty acid was compared for different cultivation times. n.d.=not detected, SFA= saturated fatty acid, MFA=monounsaturated fatty acid, PUFA=polyunsaturated fatty acid.

rice bran contains your lipids and these act inducing fungal metabolism during production biomass. The microbial cells usually express physiological changes in response to changes in the environment, it has been shown by Oda et al. (2002) when two groups of 15 strains of *Rhizopus oryzae* were cultured on a liquid medium to analyze the metabolic products and there was increased of unsaturated FA promoted by calcium carbonate addition to neutralize the acid produced.

3.3. Total, inorganic and organic phosphorus

In addition to the total phosphorus in the lipid fraction (phospholipids) was also evaluated the total and inorganic phosphorus (free) during bio cultivation time in the biomass (Fig. 3).

Rice bran has naturally inorganic and organic forms of compounds of phosphorus, and the phytic acid (phytate) which is particularly known as a major constituent of organic phosphates in rice bran, what it was demonstrated by the organic phosphorus content (unavailable) at 0 h (control) 18.8 mg/g (Fig. 3), corresponding with 92% of the total phosphorus present in rice bran.

In cereals and their derivatives, the increase of inorganic phosphorus is important to improve the bioavailability of phosphorus to monogastric animals (Conte et al., 2002). From the 12 h of cultivation the biomass showed a statistically significant increase ($p < 0.05$), and in 36 h of cultivation the inorganic phosphorus content were larger than organic phosphorus. In the end of the cultivation the inorganic phosphorus represented 70% of total phosphorus. In this study, the fungus *Rhizopus oryzae* showed promise to increase the available phosphorus content, probably due to the production of phytase enzyme that acts hydrolyzing phytates present in the bran and releasing phosphorus (Bohn et al., 2008).

Oliveira et al. (2010) in a study of rice bran cultivation with the fungus *Rhizopus oryzae,* verified that phytic acid content after 24 h of cultivation reduced significantly (55% to 66%) compared to the beginning of the fermentation, due to enzyme production phytase by the medium.

4. Conclusion

The cultivation process in solid state increased total lipids and phospholipids and it can be applied for decreasing saturated FAs in 6.2% and increase of 13% in unsaturated FAs, as well as for reducing organic phosphorus in 45.7% and increase inorganic from 8% to 70% in the end of cultivation.

Acknowledgment

The authors acknowledge the support of Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) (CNPq 14/2012- faixa A- NÚMERO 472562/2012), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Brazil) and Dr. Fábio Andrei Duarte for his assistance in total phosphorus analysis.

References

Ali, S.S., Vidhale, N.N., 2013. Protease production by *Fusarium oxysporum* in solid-state fermentation using rice bran. Am. J. Microbiol. Res 1, 45–47.

AOAC - Association of Official Analytical Chemists, 2000. Official Methods of Analysis 17th ed.. AOAC (CDROM), Washington, DC.

Bohn, L., Meyer, A.S., Rasmussen, S.K., 2008. Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J. Zhejiang Univ. Sci. B 9, 165–191.

Cheirsilp, B., Kitcha, S., 2015. Solid state fermentation by cellulolytic oleaginous fungi for direct conversion of lignocellulosic biomass into lipids: fed-batch and repeated-batch fermentations. Ind. Crops Prod. 66, 73–80.

Conte, A.J., Teixeira, A.S., Figueirado, A.V.D., Vitti, D., Silva Filho, J.C.D., 2002. Effect of phytase on phosphorus bioavailability of rice bran in brooler chickens. Braz. Agric. Res. 37, 547–552.

De Souza, N.E., Matsushita, M., Visentainer, J.V., 2012. Fatty acids: structure, classification, nutrition and health. Dyn. Mus. Arch. Interdiscip. 2, 102–107.

Dey, P., Banerjee, J., Maiti, M.K., 2011. Comparative lipid profiling of two endophytic fungal isolates – Colletotrichum sp. and Alternaria sp. having potential utilities as biodiesel feedstock. Bioresour. Technol. 102, 5815–5823.

Esteves, W., Gonçalves, L.A.G., Barrera-Arellano, D., 1995. German Standard Methodology for Analysis of Fats and Other Lipids. UNICAMP/FEA/Laboratory of Oils and Fats, Campinas.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 22, 497–509.

Grover, A., Maninder, A., Sarao, L.K., 2013. Production of fungal amylase and cellulase enzymes via solid state fermentation using *Aspergillus oryzae* and *Trichoderma reesei*. Int. J. Adv. Res. Technol. 2, 108–124.

Khoei, M., Chekin, F., 2015. The ultrasound-assisted aqueous extraction of rice bran oil. Food Chem. 194, 503–507.

Kupski, L., Carvalho Silvello, M.A., Fontes, M.R.V., Lima, T.S., Treichel, H., Badiale Furlong, E., 2015. *R. oryzae* Cellulases: a new approach to degrading lignocellulosic material. J. Food Biochem. 39, 129–138.

Lemos, M.R.B., Souza-Soares, L.A., 2000. Rice and its byproducts in southern Brazil. Vetor-Rev. De. Ciências Exatas e Eng. 10, 21–36.

Lin, H., Chen, W., Ding, H.T., Chen, X.J., Zhou, Q.F., Zhao, Y.H., 2010. Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of Aspergillus oryzae A-4 in solid-state fermentation. Bioresour. Technol. 101, 7556–7562.

Liu, L., Waters, D.L.E., Rose, T.J., Bao, J., King, G.J., 2013. Phospholipids in rice: significance in grain quality and health benefits: a review. Food Chem. 139, 1133–1145.

MarchezanII, M.W.E., De AvilaII, L.A., 2008. Rice: composition and nutritional characteristicsRural Science, 38.

Membrillo, I., Sánchez, C., Meneses, M., Favela, E., Loera, O., 2011. Particle geometry affects differentially substrate composition and enzyme profiles by *Pleurotus ostreatus* growing on sugar cane bagasse. Bioresour. Technol. 102, 1581–1586.

Metcalfe, L.D., Schimtz, A.A., Pelke, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas liquid chromatography. Anal. Chem. 38, 514–515.

Oda, Y., Yajima, Y., Kinoshita, M., Ohnishi, M., 2002. Differences of *Rhizopus oryzae* strains in organic acid synthesis and fatty acid composition. Food Microbiol. 20, 371–375.

Oliveira, M., Feddern, V., Kupski, L., Cipolatti, E.P., Badiale-Furlong, E., Souza-Soares, L.A., 2011. Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid-state fungal fermentation. Bioresour. Technol. 102, 8335–8338.

Oliveira, M.D.S., Feddern, V., Kupski, L., Cipolatti, E.P., Badiale-Furlong, E., Souza-Soares, L.A., 2010. Physico-chemical characterization of fermented rice bran biomass. Cyta – J. Food 8, 229–236.

Oshoma, C.E., Ikenebomeh, M.J., 2005. Production of *Aspergillus niger* biomass from rice bran. Pak. J. Nutr. 4, 32–36.

Patino, J.M.R., Caro, A.L., Niño, M.R.R., Mackie, A.R., Gunning, A.P., Morris, V.J., 2007. Some implications of nanoscience in food dispersion formulations containing phospholipids as emulsifiers. Food Chem. 102, 532–541.

Poulari, O., Asghari, F.S., Yoshida, H., 2009. Simultaneous rice bran oil stabilization and extraction using sub-critical water medium. J. Food Eng. 95, 510–516.

Poulari, O., Asghari, F.S., Yoshida, H., 2010. Production of phenolic compounds from rice bran biomass under subcritical water conditions. Chem. Eng. J. 160, 259–266.

Scherr, C., Gagliardi, A.C.M., Miname, M.H., Santos, R.D., 2015. Fatty acid and cholesterol concentrations in usually consumed fish in Brazil. Arq. Bras. De. Cardiol. 104, 152–158.

Schmidt, C.G., Furlong, E.B., 2012. Effect of particle size and ammonium sulfate concentration on rice bran fermentation with the fungus *Rhizopus oryzae*. Bioresour. Technol. 123, 36–41.

Schmidt, C.G., Gonçalves, L.M., Prietto, L., Hackbart, H.S., Furlong, E.B., 2014. Antioxidant activity and enzyme inhibition of phenolic acids from fermented rice bran with fungus *Rhizopus oryzae*. Food Chem. 146, 371–377.

Silveira, C.M., Oliveira, M.S., Badiale-Furlong, E., 2010. Lipid content and fatty acid profile of defatted rice bran and wheat bran submitted to solid state fermentation by *Aspergillus oryzae*. Bol. CEPPA 28, 133–140.

Vandenburg, H., Clifford, A., Bartle, K., Garden, L., Dean, J., Costley, C., 1997. Critical review: analytical extraction of additives from polymers. Analyst 122, 101R–116R.

Watanabe, M., Makino, M., Kaku, N., Koyama, M., Nakamura, K., Sasano, K., 2013. Fermentative L-(+)-lactic acid production from non-sterilized rice washing drainage containing rice bran by a newly isolated lactic acid bacteria without any additions of nutrients. J. Biosci. Bioeng. 115, 449–452.