Bioresource Technology 102 (2011) 8335–8338

Contents lists available at ScienceDirect

Bioresource Technology

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

Short Communication

Changes in lipid, fatty acids and phospholipids composition of whole rice bran after solid-state fungal fermentation

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ARTICLE INFO

Article history:
Received 25 March 2011
Received in revised form 3 June 2011
Accepted 6 June 2011
Available online 13 June 2011

Keywords:
Rhizopus oryzae
Lipid fraction
Fermented bran

ABSTRACT

The aim of this study was to evaluate fermented rice bran phospholipids, lipids and fatty acid content in a fermentation solid system with *Rhizopus oryzae* fungus. For this, aliquots were withdrawn every 24 h over 120 h. The content of phospholipids was determined by colorimetric method. Esterified fatty acids were separated by gas chromatography, then identified and quantified. The total lipids from fermented rice bran (FB) decreased from 20.4% to 11.2% in the range between 0 h and 120 h of fermentation while phospholipid contents were increased up to 2.4 mg P g^{-1}_{lipid}. In fermented bran, oleic, palmitic and linoleic acids prevailed, with a decrease in saturated fatty acids (20%) and increase in the unsaturated ones (5%). This study showed that rice bran fermentation with *R. oryzae* can be applied to the production of phospholipids altering the saturated to unsaturated fatty acid ratio.

1. Introduction

Rice bran is obtained from grain milling process, representing 5–8% of the total grain. Among the nutrients, bran contains minerals such as iron, phosphorus and magnesium, between 11% and 13% crude protein, approximately 11.5% of fibers and it is considered a very good source of oil because it may contain 20% of its weight in oil (Silva et al., 2006; Lemos and Souza-Soares, 2000).

Based on cellular distribution and its association, rice lipids are usually classified as starch lipids, which are associated with starch granules and non-starch lipids which are distributed throughout the grain, but are concentrated in the bran (Zhou et al., 2002). In crude rice bran oil, between 90% and 96% of lipid components are saponifiable (mono-, di- and triacylglycerols, free fatty acids and waxes) while 3–5% are unsaponifiable (sterols, tocopherols, tocotrienols, triterpene alcohols). Among fatty acids (FA), palmitic (21–26%), linoleic (31–33%) and oleic (37–42%) are predominant and due to the high content of mono- and polyunsaturated fatty acids (PUFA), rice bran oil is considered healthy (Lemos and Souza-Soares, 2000).

Rice bran is being studied as a substrate for solid-state fermentation (SSF) processes, in order to increase nutrient availability through changes arising from microorganisms' metabolic activity which results in interesting compounds such as enzymes, antioxidants, biosurfactants, organic acids, PUFA and others (Jang and Yang, 2008; Silveira and Badiale-Furlong, 2007). Bran is also used as a substrate for microscopic and macroscopic (mushrooms) fungal biomass development for animal feed enrichment (Jang and Yang, 2008).

From these considerations the aim of this study was to investigate the levels of phospholipids and FA profile in rice bran through solid-state fermentation by *Rhizopus oryzae* over 120 h to provide knowledge to apply this process in obtaining lipid compounds for special purposes. The FA profile of both the non-fermented rice bran and the fungus was also the purpose.

2. Experimental methodology

2.1. Rice bran

Whole rice bran was supplied by a rice institute named IRGA (Instituto Rio Grandense de Arroz), located in Rio Grande do Sul, Brazil, being kept at −10 °C, until fermentative process. Rice bran preparation as a substrate for SSF process consisted of standardizing its granulometry between 0.35 and 0.70 mm.

2.2. Inoculum preparation

Fungus strain (*R. oryzae* CCT 7560) used as fermentative agent was isolated from rice bran and identified in the Laboratory of Microbiology at the Food Processing Center of Passo Fundo

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doi:10.1016/j.biortech.2011.06.025

University (UPF), RS, Brazil. The cultures were kept at 4 °C in potato-dextrose agar (PDA) medium and the spores were scraped from the slopes into Tween 80 (0.2%) aqueous emulsion. The same medium was used for spores incubation during 7 d at 30 °C until new and complete fungi sporulation in the culture. Spores were enumerated in Neubauer chamber.

2.3. Solid-state fermentation (SSF)

Fermentation was carried out in tray bioreactors, with dimensions of $29 \times 17 \times 5.5$ cm^3. Rice bran substrate (100 g) was placed in bioreactors, forming a fine layer of ~2 cm and autoclaved, after its homogenization with 45 mL saline solution (KH_2PO_4 2 g L^{-1}, $MgSO_4$ 1 g L^{-1}, NH_2CONH_2 1.8 g L^{-1} in HCl 0.4 M). Bran initial spores concentration was 4.0×10^6 spores g^{-1} (Badiale-Furlong et al., 2007). Moisture was adjusted to 50% with sterilized water addition and trays were covered with sterilized gauze to allow aeration, before being incubated at 30 °C for 120 h. Fermentation chamber was previously sterilized with formaldehyde. To carry out physic-chemical characterization, samples were withdrawn at the beginning and each 24 h of SSF, being stored at −18 °C.

2.4. Fungal biomass

The fungal biomass was produced in Petri dishes containing PDA, sterilized and placed on plates in a sterile environment. Each dish was added 1 mL aliquot of *R. oryzae* spore suspension and incubated for 120 h in an oven at 30 °C. Fungal mycelium was recovered from PDA by scraping with the aid of a spatula, excluding PDA. FA profile as well as phospholipids were determined in the biomass in order to know the constitution of the fermenting agent.

2.5. Lipid extraction

Rice bran sample (particle size between 0.35 and 0.7 mm) was subjected to Soxhlet (AOAC, 2000) and Folch et al. (1957) extraction to verify their yield.

2.6. Phospholipids assessment

First of all, a calibration curve to determine phospholipids was prepared using potassium dihydrogen phosphate, according to Badiale-Furlong et al. (2006). To determine phospholipids in the samples, lipids extracted from fermented and not fermented rice bran by Folch et al. (1957) method were weighed between 0.3 and 1.0 g, dried, incinerated and dissolved, being measured spectrophotometrically at 800 nm. Further details are described in the Supplementary file.

2.7. Fatty acid profile

Bran lipid fraction extracted by Folch et al. (1957) method was subjected to gas chromatography (GC) for FAs separation. Lipids were esterified with 0.5 N KOH methanolic solution catalyzed by BF_3 solution (15 mL of 20% BF_3 diluted in methanol 1:1). The solvent was evaporated under nitrogen stream and the residue solubilized by 10 mL hexane, from which 1 μL was injected for GC analyses.

2.8. Statistical analysis

The data generated from this study were subjected to one-way analysis of variance (ANOVA) at 5% level of significance. Means were compared by Tukey test. All determinations were carried out in triplicates.

3. Results and discussion

Table S1 shows the total lipid content of fermented and unfermented bran extracted by Soxhlet (AOAC, 2000) and Folch et al. (1957) method. Details can be verified in the Supplementary file.

The extraction by Soxhlet method is officially recommended for lipid determination in solid samples and was used in this work only to establish extraction yield. This method was not adopted to characterize the lipid profile, because high extraction temperatures could favor FA configuration change, impairing the distinction between the effect of fermentation and extraction method. On the other hand, Folch method was developed to extract lipids from samples with high moisture content, such as fermented biomass because it is a cold extraction, which preserves the original composition of FAs, thus it is chosen for FA profile study of fermented rice bran. Other authors also extracted lipids to analyze the FA composition of these materials from fungal biomass by Folch et al. (1957) method (Abu et al., 2000; Stredansky et al., 2000). Thus, this method has been chosen for lipid extraction for further FA profile analysis due to its greater accuracy.

Not fermented rice bran (NF) showed a lipid content of 18.9% by Soxhlet method, which is in agreement with values already reported by other authors (Amissah et al., 2003; Feddern et al., 2007; Silva et al., 2006). This method shows the possibility of obtaining close to 20% lipid extraction, already expected for rice bran (Zhou et al., 2002). Although, lipid content decreased after 24 h fermentation, it was significant after 48 h, and may be the result of lipid use by fungus, possibly in the synthesis of phospholipids constituents of the cell membrane of fungal tissue. Oduguwa et al. (2008) also reported a 40% reduction in lipid content in fermented rice bran with *Rhizopus oligosporus* and *Saccharomyces cerevisiae*. Moreover, Abu et al. (2000) fermented sweet potato with *Aspergillus niger* and *Aspergillus oryzae*, observing an increase in total lipid content during fermentation, while the action of *P. ostreatus* decreased lipids from 1.9% to 0.5%. Silveira et al. (2010) investigated rice and wheat bran SSF by *R. oryzae* and observed no significant change ($p < 0.05$) over 72 h fermentation in total lipid content for the remaining fermented biomass with an average of 3% and 4.9% for rice and wheat bran, respectively.

Table S2 illustrates the fatty acid profile of rice bran and fermented bran for 120 h (FB 120 h), as well as the lipid profile according to legislation and other literature.

In general, fungi in fermentation process synthesize their own lipids, with no need to add them to culture media, however, their addition improves fungal growth. The determination of fatty acids and phospholipids in fungal biomass produced in solid systems is still uncommon due to the fact that fungal species are considered non-oleaginous (Abu et al., 2000; Stredansky et al., 2000). Moreover, there are few studies employing crude rice bran as a substrate for fungal biomass production in order to increase the extraction process yield and/or alter the lipid profile for specific purposes.

R. oryzae fungus grown on PDA, as well as fermented and unfermented rice bran FA composition are shown in Table 1. Phospholipids in fermented and not fermented rice bran are also demonstrated.

Rice bran showed phospholipid content of 0.14 mg P g_{bran}^{-1}, below from that reported by Amissah et al. (2003) when analyzing 16 varieties of rice bran 0.27–0.51 mg P g_{bran}^{-1}. However, the authors did not quantitate the phosphorus content only in the lipid fraction but also in the bran.

Research on phospholipid content during fungal growth is rarely studied, especially when dealing with non-oleaginous fungi and development on solid substrate. However, it is evident in this work the increment of phospholipids in FB 24 h. This content is

Table 1
Fatty acid profile and phospholipid content of *R. oryzae* strain, fermented and unfermented bran.

Fatty acids	Fatty acid profile (%)							
	R. oryzae[*]	Bran	FB 0 h	FB 24 h	FB 48 h	FB 72 h	FB 96 h	FB 120 h
C14:0	0.63 ± 0.1^{a}	0.3 ± 0.0^{b}	0.3 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
C16:0	29.3 ± 2.6^{a}	17.0 ± 0.3^{b}	20.4 ± 0.8^{b}	19.7 ± 0.2^{b}	18.5 ± 0.8^{b}	17.6 ± 0.8^{b}	17.2 ± 0.4^{b}	15.7 ± 0.7^{b}
C16:1	0.8 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.15 ± 0.0^{b}	0.15 ± 0.0^{b}	0.16 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
C18:0	8.3 ± 0.9^{a}	2.1 ± 0.0^{b}	2.5 ± 0.0^{b}	3.2 ± 0.1^{b}	2.5 ± 0.0^{b}	2.8 ± 0.1^{b}	2.3 ± 0.0^{b}	2.3 ± 0.2^{b}
C18:1n9 *c* and *t*	33.6 ± 1.8^{a}	38.8 ± 0.3^{b}	36.5 ± 0.1^{ab}	37.9 ± 0.3^{b}	38.8 ± 0.2^{b}	38.8 ± 0.5^{b}	37.8 ± 0.2^{b}	37.3 ± 0.5^{ab}
C18:2 n6 *c* and *t*	14.6 ± 0.0^{a}	32.0 ± 0.3^{b}	34.9 ± 0.2^{ef}	32.8 ± 0.0^{bc}	33.4 ± 0.1^{cd}	33.8 ± 0.5^{cde}	34.7 ± 0.4^{def}	35.8 ± 0.5^{f}
C18:3n6	4.6 ± 0.1^{a}	n.d.^{b}	n.d.^{b}	1.1 ± 0.0^{c}	1.4 ± 0.0^{e}	1.6 ± 0.0^{e}	1.9 ± 0.0^{f}	2.3 ± 0.0^{g}
C18:3n3	0.3 ± 0.0^{a}	1.3 ± 0.0^{bc}	1.9 ± 0.0^{e}	1.4 ± 0.0^{c}	1.2 ± 0.0^{b}	1.2 ± 0.0^{b}	1.2 ± 0.0^{b}	1.2 ± 0.0^{b}
C20:0	1.12 ± 0.3^{a}	0.5 ± 0.0^{b}	0.75 ± 0.0^{ab}	0.8 ± 0.0^{ab}	0.7 ± 0.0^{ab}	0.8 ± 0.0^{ab}	0.7 ± 0.0^{ab}	0.7 ± 0.0^{ab}
C20:1	0.9 ± 0.0^{a}	0.5 ± 0.0^{b}	0.56 ± 0.0^{b}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}
C22:0	0.9 ± 0.2^{a}	0.3 ± 0.0^{b}	0.46 ± 0.0^{ab}	0.5 ± 0.0^{ab}	0.6 ± 0.0^{ab}	0.6 ± 0.0^{ab}	0.5 ± 0.1^{ab}	0.7 ± 0.0^{ab}
C22:6n3	1.2 ± 0.0^{a}	0.30 ± 0.0^{b}	0.11 ± 0.0^{c}	0.7 ± 0.1^{d}	0.8 ± 0.0^{e}	0.9 ± 0.0^{ef}	0.9 ± 0.0^{ef}	1.0 ± 0.0^{f}
n.i.	5.8 ± 0.5	0.6 ± 0.0	1.6 ± 0.0	1.0 ± 0.0	1.3 ± 0.2	0.9 ± 0.0	1.9 ± 0.4	2.0 ± 0.7
SFA	40.3 ± 1.3^{a}	20.2 ± 0.4^{bd}	24.5 ± 0.8^{c}	24.5 ± 0.1^{c}	22.2 ± 0.3^{cd}	22.0 ± 0.8^{cd}	21.0 ± 0.2^{d}	19.6 ± 0.5^{d}
PUFA	56.3 ± 1.9^{a}	73.1 ± 0.5^{c}	74.7 ± 0.3^{b}	74.9 ± 0.2^{b}	76.8 ± 0.2^{bc}	77.4 ± 0.9^{bc}	77.7 ± 0.6^{bc}	78.8 ± 1.1^{bc}
S/U	0.5 ± 0.0^{a}	1.7 ± 0.0^{bc}	1.5 ± 0.1^{bc}	1.5 ± 0.1^{b}	1.7 ± 0.0^{cd}	1.7 ± 0.1^{cd}	1.8 ± 0.0^{d}	2.1 ± 0.1^{e}
ω6	19.2 ± 0.2^{a}	32.0 ± 0.2^{b}	35.0 ± 0.5^{cd}	33.9 ± 0.5^{c}	34.7 ± 0.2^{cd}	35.4 ± 0.5^{de}	36.6 ± 0.4^{ef}	38.0 ± 0.6^{f}
ω3	1.5 ± 0.0^{a}	1.6 ± 0.0^{b}	2.0 ± 0.04^{c}	2.1 ± 0.1^{c}	2.0 ± 0.2^{c}	2.1 ± 0.1^{c}	2.1 ± 0.0^{c}	2.2 ± 0.0^{c}
ω6/ω3	13.0 ± 0.0^{a}	19.4 ± 0.8^{b}	17.5 ± 0.4^{c}	16.1 ± 1.0^{c}	17.3 ± 1.3^{c}	16.8 ± 0.7^{c}	17.4 ± 0.5^{c}	17.3 ± 0.4^{c}
Phospholipid content (mg P g_{bran}^{-1})		0.14 ± 0.0^{a}	0.18 ± 0.0^{a}	0.37 ± 0.03^{b}	0.36 ± 0.0^{b}	0.29 ± 0.0^{ab}	0.37 ± 0.0^{b}	0.29 ± 0.0^{ab}
Phospholipid content (mg P g_{lipid}^{-1})		0.73 ± 0.0^{a}	1.8 ± 0.0^{b}	2.4 ± 0.04^{c}	2.8 ± 0.06^{d}	3.1 ± 0.07^{e}	3.7 ± 0.07^{f}	4.2 ± 0.14^{g}

SFA = C14:0 + C16:0 + C18:0 + C20:0 + C22:0; AGI = C16:1 + C18:1n9*tc* + C18:2n6*tc* + C18:3n6 + C18:3n3 + C20:1 + C20:2 + C20:3n3 + C20:5n3 + C22:1n9 + C22:6n3; S/U = (C18:2n6t + C18:2n6c + C18:3n6 + C18:3n3 + C20:2 + C20:3n3 + C20:5n3 + C22:6n3)/(C14:0 + C16:0 + C18:0 + C20:0 + C22:0); n.i. = not identified peaks (absent in the standard); ω6/ω3 = (C18:2n6t + C18:2n6c + C18:3n6)/(C18:3n3 + C20:3n3 + C20:5n3 + C22:6n3); n.d. = not detected; Same letters in the same row mean no significant difference between the means at 95% confidence, where each fatty acid was compared for different fermentation times. Values are expressed as means ± SD (n = 2). FB = fermented bran for 0 h, 24 h, 48 h, 72 h, 96 h and 120 h.

[*] *Rhizopus oryzae* biomass grown in 24 h, 48 h, 72 h, 96 h and 120 h.

related to fungal growth, as can be seen in the results of this compound in *R. oryzae* biomass in PDA. On 24 h growth, fungus showed 3.03 mg P g_{lipid}^{-1} and after this period this content has tripled (48 h – 11.8 ± 0.01; 72 h – 9.8 ± 0.02; 96 h – 11.4 ± 0.36; 120 h – 10.0 ± 0.4 mg P g_{lipid}^{-1} and was stable until 120 h, showing an increase in polar lipids during fungal growth as observed by Fakas et al. (2009).

The method used for determining rice bran phospholipids showed satisfactory performance (89% recovery and 2.0% coefficient of variation). The same method used to analyze fish skin presented 91% recovery, detection limit of 0.0546 μg/mL and quantitation limit of 26.1 mg/100 g (Badiale-Furlong et al., 2006). The production of phospholipids reveals a possible source of these bioemulsifying compounds which can be applied in the food industry and/or in the formulation of material for bioremediation.

According to Table 1, *R. oryzae* lipids were composed mainly by 33.6% oleic (C18:1n9) 29.3% palmitic (C16:0) 14.6% linoleic (C18:2n6) 8.3% stearic (C18:0) and 4.6% linolenic FAs. Oleic, palmitic and linoleic FAs have also been reported as prevalent in several fungi species (Silva et al., 1998). The authors raised the possibility of identifying fungal species by lipid profile, although fungi have less difference among FAs than bacteria.

Rice bran showed predominantly oleic (45%), linoleic (32%) and palmitic FAs (17%), as mentioned in the literature (Lemos and Souza-Soares, 2000), in addition to surveys that have been performed at the Laboratory of Food Science in rice varieties grown in southern Brazil. Silveira et al. (2010) observed that over 72 h of rice and wheat bran fermentation with *R. oryzae*, a significant increase in palmitic and linoleic contents was achieved as well as a significant reduction of stearic and linolenic acid when compared to unfermented bran.

The relations of polyunsaturated/saturated FAs (PUFA/SFA) and ω6/ω3 for rice bran were 1.7 and 19.4 respectively, while for *R. oryzae*, the values were 0.5 and 13.0. The Department of Health in England mentions that PUFA/SFA ratio lower than 0.45 is not advisable for health and may cause heart disease (Department of Health, 1994).

The recommendations for ω6/ω3 ratio range from 4:1 to 10:1, but some authors reported countries whose diets contain up to 50:1. Thus, it can be concluded that PUFA/SFA ratio of rice bran is beneficial to health, but ω6/ω3 ratio does not favor the provision of α-linolenic acid, a fact that affects the synthesis of long chain FAs, being a condition that contributes to the development of allergic, inflammatory and cardiovascular diseases (Martin et al., 2006).

The predominant FAs (oleic, linoleic and palmitic acids) in fermented rice bran remained the same as before fermentation. However, there was a significant ($p < 0.05$) change in linoleic acid (C18:2) profile after 48 h. Comparing unfermented bran and FB 120 h, 10% increase occurred. Along fermentation process no significant change in C18:1 and C16:0 was observed. From FB 24 h on, it was noted an interesting fact for γ-linolenic acid, which was present in fungi lipid but absent in rice bran in concentrations of 1.1–2.3% in 120 h. The appearance of γ-linolenic acid resulted in a significant increase in PUFA/SFA and ω6. In addition, caused a trend to increase ω6/ω3 ratio from 0 h to 120 h. Silveira et al. (2010) also observed an increase of approximately 10% in the incidence of FAs from ω6 series in defatted rice bran and wheat bran, both solid-state fermented with *A. oryzae* for 72 h. This change in ω6/ω3 ratio could be better assessed in order to search environment conditions that favor the production of α-linolenic acid, a precursor in the biosynthesis of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These two ω3 unsaturated FAs are important in the composition of brain and retina cell membranes.

Fermentation for 120 h decreased SFAs while unsaturated FAs increased. This fact also suggests the necessity in studying the oxidative stability of fermented bran lipids to evaluate fermentation efficiency on unsaturated FAs production or as a way to stabilize the co-product.

The changes are related to *R. oryzae* response to fermentation conditions, i.e., whole rice bran (not defatted) own lipids may have

functioned as inducers for the metabolism of lipids. In other words, microorganisms had excess lipids and could use them to produced phospholipids to act as an emulsifier and thus fatty acid profile changes towards the unsaturated fatty acids, which are more abundant in phospholipids.

4. Conclusion

Rice bran solid-state fermentation with *R. oryzae* can be applied for producing phospholipids (130% increase) as well as for decreasing total fat and saturated FAs in 45% and 20% respectively, with an increase of 5% in unsaturated FAs and change in saturated to unsaturated FAs ratio.

Acknowledgements

CAPES, FAPERGS and CNPq are thanked for providing research support.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.biortech.2011.06.025.

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