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Sperathe Effects of Solid-state Fermentation in the Functional Properties of Defatted Rice Bran and Wheat Bran

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ABSTRACT

Functional properties of fermented bran produced by Aspergillus oryzae *and* Rhizopus *sp. in a solid-state fermentation system were determined, with an aim to evaluate their application in food formulation. The defatted rice bran and wheat bran were inoculated with the spores of the cultures and incubated at 30℃ for 72 h. Samples were withdrawn at 0, 24, 48 and 72 h. Protein content, protein solubility,* in-vitro *digestibility, gelation and water holding capacity were determined in bran with or without fermentation.* Rhizopus *sp. increased significantly the protein content (69.0 and 56.0%, respectively, for defatted rice bran and wheat bran); protein solubility (28.5 and 36.2) and water holding capacity (11.4% for wheat bran). When A. oryzae was used all these properties were modified significantly after fermentation.*

Key words: functional properties, solid-state fermentation, defatted rice bran, wheat bran

INTRODUCTION

Defatted rice and wheat bran are agro-industrial residues that are good sources for proteins, lipids, vitamins and minerals. They are rich in indigestible carbohydrates that decrease their biological value and make the sensory characteristics less acceptable. During the processing of bran, not much care is taken for the hygienic quality, which results in low commercial value, consequently, restricting their use in food formulation for human consumption. Solid-state fermentation of bran can increase the nutrients availability and improve their sensory characteristics, thus, adding value to these materials and creating new opportunities for their utilization. This also helps in reducing the environmental pollution concern that their disposal would cause.

Fermentative process, especially solid-state fermentation had been used to add value to raw materials (Kang et al., 2004; Laufenberg and Nystroem, 2003; Thanh and Nout, 2002; Anupama and Ravindra, 2001; Pandey and Soccol, 1998; Huerta et al., 1994). Microbial activity (especially for fungi) in absence or near absence of free water offers many advantages for the production of metabolites, protein enrichment and disposable

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nutrients from wastes (Costa et al., 1998; Pandey and Soccol, 1998; Pitt and Hocking, 1997; Huerta et al., 1994). The lack of information in the literature on the functional properties of fungal biomass produced by solid-state systems makes it difficult to use the fermented bran in food formulation (Paraskevopoulou et al., 2003; Anupama and Ravindra, 2001; Anupama and Ravindra, 2000; Sgarbieri, 1998; Finley, 1989).

The knowledge of functional properties is very interesting for the industrial and nutritional evaluation of fermented products, because a number of different chemical or physical alterations can occur during the process. These alterations can cause positive or negative effects on the final product quality (Finley, 1989). Protein solubility and *in-vitro* digestibility contribute with the estimation of the nutritional food value as source of essential amino acids. Water holding capacity and gelation have a significant impact on the quality of the product because they influence the sensory characteristics and consequently the functional performance of a food product. Production of many foods such as dehydrated gelatins, soups and others is based on these properties (Chavan and Shahidi, 2001; Chevalier et al, 2001; Sze-Tao and Sathe, 2000; Sgarbieri, 1998; Nakai and Li-Chan, 1989).

A limitation for the study of fermented products is the rare specific methodology for the evaluation of their final quality. There are some reports describing the functional properties of biomass produced by the more diverse microorganisms, although not always they are considered as GRAS (generally recognized as safe). Ramachandran et al. (2004) determined the protein solubility of biomass produced by *Rhizopus* and Guil-Guerrero et al. (2004) evaluated the water absorption capacity of biomass produced by three microalgal species.

The present work studied the influence of solid-state fermentation by *Rhizopus* sp. and *Aspergillus oryzae* on the functional properties of defatted rice bran and wheat bran intending to provide information about their use in food products formulation.

MATERIALS AND METHODS

Bran characterization

Defatted rice bran and wheat bran were collected from cereal agro-industries, homogenized and grounded to obtain from 0.35 to 0.59mm particles. Their composition were determined by AOAC methods (2000). Fiber levels were determined by the chemical methods using acid and alkaline hydrolysis. Samples were also submitted to mycotoxin evaluation by thin layer chromatography multimethod as described by Soares and Rodriguez-Amaya (1989) to determine the presence of aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone.

Fermented bran production

A. oryzae and *Rhizopus* sp. were grown on potato-dextrose agar (PDA) containing 1% bacteriological agar and incubated at 30ºC for 7 days until complete sporulation. Solid substrate used was defatted rice bran and wheat bran. Mineral salts and urea were added as nutrient (2.0 g/L KH_2PO_4, 1.0 g/L $MgSO_4$ and 1.8 g/L urea in 800mM HCl). Media were adjusted to 50% moisture and pH 5 by addition of HCl (400mM). Substrates were inoculated using 4 x 10^6 spores/g of medium (Silveira et al., 2003; Costa et al., 1998). Fermentation was carried out in trays in a 1 cm thickness layer. Trays were incubated at 30^0C for 72 h. Samples, in duplicate, were collected at 0, 24, 48 and 72 h of fermentation.

Determination of fermented bran protein content, solubility and *in-vitro* digestibility

Total protein level of the fermented defatted rice and wheat bran were determined by the method of micro-Kjeldahl (AOAC, 2000) and converted into protein percentage using a conversion factor of 6.25. The protein solubility of fermented bran was determined by the method of Morr et al. (1985). A saline solution (NaCl, 0.9%) was added to the sample and shaken for 40 minutes at 50°C. The mixture was centrifuged, filtered and soluble protein were determined in the supernatant by the Lowry method (1951), having bovine serum albumin as standard (0.1 to 1.0 mg/mL).

In-vitro protein digestibility of fermented bran was determined the method of Sgarbieri (1996). Dry biomass (1 g) was hydrolyzed with 10 mL of pepsin suspension (1.5mg/mL in 100mM HCl with a specific activity 0.8 mg tyrosine.min^{-1}.mg^{-1} protein) for three hours in a bath at 37°C. The pH was raised to 7.0 and the samples were centrifuged. To the precipitate, 10mL of a pancreatin suspension (22.5mg/mL with specific activity 23.8 mg tyrosine.$min^{-}1$.mg^{-1} protein) was added and the samples were hydrolyzed for 24 h in

a bath at 37°C. Then the samples were boiled at 100ºC for 5 minutes, cooled and centrifuged at 4000g. The products of proteolytic hydrolysis were quantified by the Folin-Ciocalteau spectophotometric method, having tyrosine as standard (0.01 to 0.1 mg/mL) (Plumer, 1978).

Determination of fermented bran gelation and water holding capacity

Gelation was determined by the method described by Huang and Kinsella (1987). Samples of fermented bran were diluted with water (1:8, w/v), heated in a bath at 80°C during 30 minutes, centrifuged at 4000g for 15 minutes and, after keeping static for 45 minutes, drained with an hypodermic syringe and weighed.

Water holding capacity of fermented bran was carried out according to the method of Huang and Kinsella (1987) adapted by Silveira et al. (2005). Samples were diluted at 1:8 (w/v), centrifuged at 4000g during 15 minutes and, after keeping static 45 minutes, drained with an hypodermic syringe and weighed.

Statistical analysis

Each experiment was carried out four times. Analysis of variance (ANOVA) was performed for the properties in study, considering fermentation time, microorganism and kind of bran like factors of variation. The difference between the averages was determined using Tukey's Test at the significant level of 5%. The software was STATISTICA v. 6.0.

RESULTS AND DISCUSSION

Bran characterization

The composition of wheat bran and defatted rice bran are shown in Table 1. These were in agreement with Silva et al. (2001), keeping in mind the variability of the cereal cultivation and the milling conditions.

Table 1 - Chemical composition of defatted rice bran and wheat bran.

	Deffated rice bran	Wheat bran
Moisture (%)	9.2	9.4
Proteins (%)	19.2	13.8
Lipids (%)	5.7	5.2
Carbohydrates (%)	47.1	60.1
Ash (%)	11.7	6.3
Fibers (%)	7.1	5.2

The presence of mycotoxins was tested because they influenced the fermentation process. The methods employed for mycotoxin determination had detection limits of 2.5 ppb for aflatoxin B_1, 6.7 ppb for ochratoxin and 47.0 ppb for zearalenone and median recovery of 87% for each mycotoxin. No mycotoxin was detected in the samples evaluated.

Protein content, solubility and *in-vitro* digestibility of fermented bran

Laufenberg et al. (2003); Anupama and Ravindra (2000) and Moraes (1999) demonstrated that some fungal species were able to increase the protein level in agro-industries wastes. The same was observed in this work, after the fermentation of defatted rice and wheat bran by *A. oryzae* and *Rhizopus* sp. Table 2 shows the average of the protein concentration in fermented bran at different intervals of fermentation time.

An increase was observed in the protein content during the fermentation. This was due to supplementation of nitrogen during the biomass production (where nutrients were provided). The highest protein level was reached after 72 h of fermentation with *Rhizopus* sp., approximately 69 and 56%, respectively, for defatted rice bran and wheat bran, in relation to un-fermentation bran.

A. oryzae produced the highest protein content after 48 h with defatted rice bran (about 11% in relation to un-fermented bran). The maximum protein in the fermented wheat bran was observed after 72 h and the increase was around 14%, but this was not significant ($p = 0.84$ and $p = 0.15$, for wheat and defatted rice bran, respectively).

Table 2 - Protein content of defatted rice bran and wheat bran after different fermentation times.

Time (hours)	DRB *RHI* (%)	WB *RHI* (%)	DRB *ASP* (%)	WB *ASP* (%)
NF	19.2	13.8	19.2	13.8
0	21.0	15.6	17.8	12.7
24	22.8	16.8	18.4	14.9
48	25.8	18.9	21.3	14.3
72	32.4	21.6	20.6	15.8

NF = un-fermented; DRB = defatted rice bran; WB = wheat bran; RHI = *Rhizopus* sp.; ASP = *A. oryzae.*

The fermentation carried out by *Rhizopus* sp. increased 69% in the protein content of defatted rice bran. This was different from the result reported by Moraes (40%). However, the 11% increase was lower than the one observed in the biomass produced by *A. oryzae* mentioned by the same author. The fermentation system and granulometry of the substrate might have caused the difference.

It is important to point out that conversion factor of 6.25 was also used for the estimation of protein level in un-fermented bran, intending to get similar comparisons.

The protein solubility index of fermented bran was carried out with saline solution (NaCl, 0.9%) because it reflected the most frequent condition in food processing. The results can be observed in Figure 1 as percentage of the total protein level.

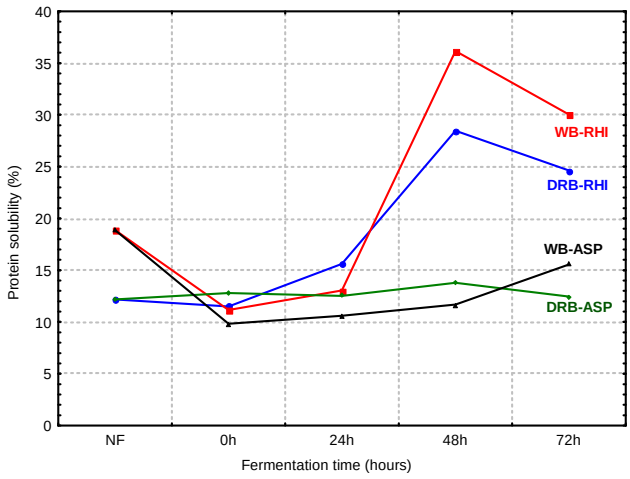

Figure 1 - Protein Solubility in NaCl where NF = un-fermented, DRB = defatted rice bran; WB = wheat bran; ASP = *A oryzae*; RHI = *Rhizopus* sp.

The observed behavior in the protein solubility during the fermentation process reflected the protein alteration caused by the fungal biomass development. When *Rhizopus* sp. was used, there was a significant increase in the protein solubility after 48 h of fermentation ($p = 0.0001$ for defatted rice bran and $p = 0.004$ for wheat bran). However, after 72 h, the protein solubility decreased but not significantly ($p = 0.12$ for defatted rice bran and $p = 0.33$ for wheat bran). The protein solubility was higher when wheat bran was used, reaching an average of 36.2% of soluble protein after 48 h, while for the defatted rice bran, the value was 28.5%. The protein solubility of biomass produced by *Rhizopus* sp. was similar as reported by Ramachandran et al. (2004) who added different vegetable oils in the wheat bran before fermentation.

When the bran was fermented by *A. oryzae*, the protein solubility of the wheat bran diminished significantly, except after 72 h of fermentation. The statistical evaluation confirmed that the solubility of the defatted rice bran was not influenced by the fermentation time ($p = 0.88$). There are reports describing this fungus as a good hydrolytic enzymes producer (Kang et al., 2004;

Moraes, 1999). These results suggested the better degradation of raw material and more homogeneous biomass distribution by *Rhizopus* sp. than by *A. oryzae*, as reported by Walsh et al. (2003).

Digestibility coefficient was evaluated in the fermented bran as an indicative of biological availability of the protein (Fig. 2).

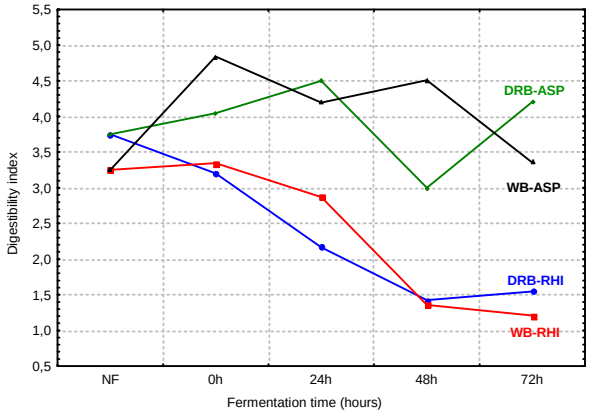

Figure 2 - Variation of *in-vitro* digestibility where NF = un-fermented, DRB = defatted rice bran; WB = wheat bran; ASP = *A. oryzae*; RHI = *Rhizopus* sp.

When *Rhizopus* sp. was used, a significant reduction in digestibility of both bran was observed. The reduction was around 49% in the defatted rice bran and 64% in wheat bran. Such alteration was not expected, considering that their solubility was increased. Using similar fermentation conditions, Silveira et al. (2003) found a reduction of 59% in the fermented wheat bran digestibility when *Rhizopus* sp. was used, which could be explained by the high chitin contents in the fungal biomass (Than and Nout, 2002).

A. oryzae showed no significant increase in the digestibility of the fermented defatted rice bran ($p = 0.37$) but significantly increase for wheat bran ($p = 0.99$). According to Sze-Tao and Sathe (2000), there was an increasing linear relation between the digestibility and the solubility in aqueous systems, but this was not confirmed in this work.

Gelation

Gelation of the fermented and un-fermented bran is presented in Figure 3, expressed as variation in weigth in relation to the initial sample weight. The results indicated that gelation property did not change after fermentation.

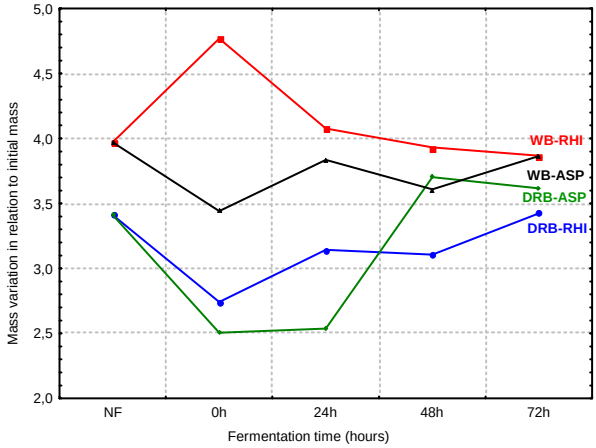

Figure 3 - Gelation of the fermented and un- fermented bran, where NF = un-fermented, DRB = defatted rice bran; WB = wheat bran; ASP = *A. oryzae*; RHI = *Rhizopus* sp.

A similar behavior was observed in the defatted rice bran fermented by *Rhizopus* sp. and the wheat bran fermented by *A. oryzae*. This showed that the fermentation process modified the gelation in the bran by the modification in initial protein levels or others biomolecules during the fungal development. Possibly in the case of the *A. oryzae* biomass, the main alteration arose from the amylases and cellulases released to the medium (Costa, 1998; Sanzo, 1998).

Water holding capacity

Bran fermented by *Rhizopus* sp. Presented significant difference in relation to un-fermented bran. Rice bran presented significant reduction ($p = 0.05$), while the wheat bran showed a significant increase ($p = 0.02$). There was no significant difference between the bran fermented or un-fermented by *A. oryzae* ($p = 0.94$ for defatted rice bran and $p = 0.99$ for wheat bran). Figure 4 shows the water holding capacity variation during the fermentation process and suggested that the substrate influenced the functional property more than fungi.

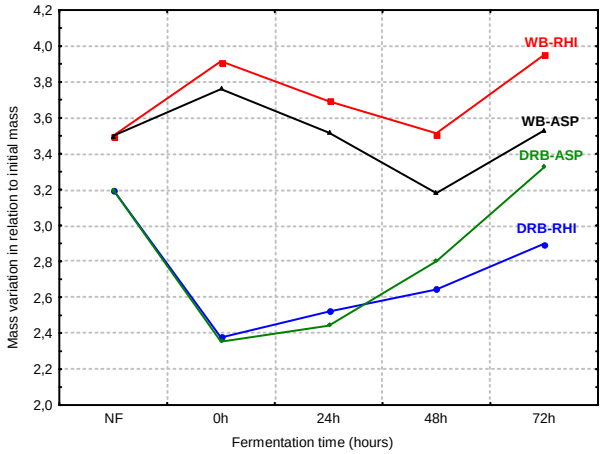

Figure 4 - Water holding capacity of un-fermented and fermented bran, where NF = un-fermented, DRB = defatted rice bran; WB = wheat bran; ASP = *A. oryzae*; RHI = *Rhizopus* sp.

CONCLUSIONS

The fermentative process increased the protein content of the bran fermented by *Rhizopus* sp. significantly, while the increase was not significant for the bran fermented by *A. oryzae*. *Rhizopus* sp. increased the protein solubility of the wheat bran by 36.2% and for defatted rice bran 28.5%; however, there were no increase in the bran digestibility. The gelation was not modified by the fermentative process; however, *Rhizopus* sp. influenced the water holding capacity, increasing for the wheat bran and reducing for the defatted rice bran. The fermentative process using *Rhizopus* sp. showed the best possibility to be used to modify the functional properties of the cereal bran, as it increased their protein solubility and the water holding capacity.

RESUMO

O objetivo do presente trabalho foi investigar as propriedades funcionais de farelo de arroz desengordurado e farelo de trigo, submetidos à fermentação em estado sólido pelos fungos *Aspergillus oryzae* e *Rhizopus* sp., para avaliar seu potencial de aplicação em formulações alimentícias. O farelo de arroz desengordurado e o farelo de trigo foram inoculados com esporos de *Rhizopus* sp. e *Aspergillus oryzae* ($4x10^6$ esporos/grama de meio) *e* incubados durante 72 horas a 30°C. Amostras foram coletadas em 0, 24, 48 e 72 horas de fermentação. Foram determinados o conteúdo protéico, a solubilidade protéica, a digestibilidade *in-vitro*, a capacidade de formação de gel e a capacidade de retenção de água nos farelos fermentados. Quando *Rhizopus*

sp. foi utilizado, as seguintes propriedades aumentaram significativamente: o conteúdo protéico 69,0 e 56,0%, respectivamente, para farelo de arroz e farelo de trigo), a solubilidade protéica (28,5 e 36,2%) e a capacidade de retenção de água (11,4% para farelo de trigo). Quando *Aspergillus oryzae* foi empregado, as propriedades não foram modificadas de maneira significativa pelo processo fermentativo.

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