World Applied Sciences Journal 22 (1): 53-56, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.22.01.2967

Comparative Study in the Bioconversion of Brown Rice and Polished Rice to Rhizopus Oligosporus Fermented Products

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Abstract: Solid-state fermentation was carried out using brown rice (BR) and polished rice (PR) as substrates for the production of tempeh using a fungal culture of *Rhizopus oligosporus*. The rice based tempeh was further analyzed for its nutrient composition like carbohydrate, protein, fat, fiber and ash content. The prepared BR and PR tempeh samples were baked and its sensory qualities were evaluated. On the sensory evaluation of the tempeh, the flavor, color, taste and texture were found to be acceptable, for both of the tempeh samples.

Key words: Brown rice · Polished rice · *R. oligosporus* · Solid state fermentation · Nutrient analysis · Sensory evaluation

INTRODUCTION

Tempeh, an Indonesian food is made from fermenting soybeans with the *Rhizopus* fungus. Besides the academic interest in the fermentation process, the study of tempeh has been stimulated because of its low-cost protein diet [1-5]. Tempeh is a mycelia-knitted compact cake fermented by the genus *Rhizopus* [6]. The total food production majorly consists of plant sources which are nutritious, readily available and cheaper than animal sources. Solid state fermentation of plant materials results in high quality products. Soybeans are the common substrate for tempeh production [7]. In recent years, cereals like barley [7-10], wheat [8, 9, 11], oats [12], maize [6], sunflower seeds [13], coconut residue [14], groundnut [15], chick pea, horse bean [16]. The main ingredient in tempeh is the plant material, suitable for human consumption after fermentation.

The basic fermentation process is similar for all substrates. It includes soaking, dehulling (when cereals are used it is sometimes necessary to modify the surface of the grain by cutting, cracking or pearling to obtain good growth of the mold), boiling and fermenting. *Rhizopus* fermentation on soybeans is responsible for the production of aglycone isoflavones. There are evidences reporting the adverse effects of soy isoflavones that are not bioavailable. During digestion, these aglycone isoflavones can be assimilated [17]. These disadvantages can be sorted out by rice, the staple food of Asian countries. Brown rice grains contain more nutritional bioactive components, such as ã-oryzanol, dietary fibers, phytic acids, vitamin E, vitamin B and ã-aminobutyric acid (GABA), exist in the germ and bran layers which are removed during grain polishing or milling. Research indicates that the brown rice exhibited highest level of phenolic compounds like ferulic acid esters of steryl ferulates have antioxidant, antimutagenic, anticancer and other positive effects as well as play an important role in maintaining health [18, 19]. The purpose of this work was to study the effect of brown rice and polished rice for the growth of *R. oligosporus* which could be used as an alternative for soybean.

MATERIALS AND METHODS

Preparation of Substrates: White Ponni Paddy was procured from Soil and Water Management Research Institute, TNAU, Kattuthotam. The paddy was dehulled as brown rice (BR) and they were polished to get polished rice (PR). The rice grains were soaked in water for 4 h at room temperature and cooked in a pressure cooker.

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Preparation of Inoculum: The fungal culture *Rhizopus oligosporus* MTCC-556 was obtained from IMTECH, Chandigarh and maintained in potato dextrose agar slants at 37°C. Before inoculation, the organism was transferred to fresh potato dextrose broth. The inoculated slants are incubated at 25°C for 2 days. One ml of fungal spore suspension was used as inoculum, for fermenting the substrates.

Preparation of Rice Tempeh: One ml of the spore suspension was mixed with 100 g cooked rice. The inoculated rice samples BR and PR were loosely spread approximately 1 cm thick in an aluminum foil and sealed tightly. The foil was perforated and incubated at 37°C for 5 days.

Proximal Nutrient Analysis

Determination of Total Carbohydrate Following Anthrone Method: Briefly, 100 mg of samples containing carbohydrates were taken and hydrolyzed into simple sugars with 2.5 N HCl. The mixture was heated for 3 h in boiling water bath where glucose is dehydrated to hydroxymethyl furfural that forms green color with anthrone reagent having an absorption maximum at 630nm. The spectral data was recorded using a double beam spectrophotometer (Shimadzu UV 1601) [20].

Estimation of Protein Following Lowry's Method: About 100 mg of sample was taken and alkaline copper solution was added. The protein binds to copper in alkaline medium and produces Cu^{++}. In the second step, Folin-Ceocaliteau reagent was added and incubated in the dark for 30 mins, where Cu^{++} catalyses oxidation of aromatic amino acid by reducing phosphomolybdotungstate to heteropolymolybdanum blue. This reaction produces strong blue color. The readings were taken at 660 nm using a double beam spectrophotometer (Shimadzu UV 1601) [21].

Determination of Crude Fat Content Following Soxhlet Method: About 10g of sample was taken in a porous thimble and placed in the apparatus. The crude fat was extracted with hexane which was recycled again. This extends the contact time between the solvent and the sample and allows it time to dissolve all of the fat contained in the sample. The percentage oil content of the sample was calculated using the formula (B-A/W) ×100 where W is Weight of sample taken, A is Weight of empty flask and B is Weight of flask + oil [22].

Determination of Fiber and Ash Content: Sulphuric acid of concentration1.25% was added to 2g of defatted sample (residue after extraction of oil) and filtered. The residue was treated with sodium hydroxide 1.25% and filtered. The filtrate was kept at 105°C for 2 h and weighed to obtain fiber content of the sample. It was then burned to yield ash content. The fiber and ash content were calculated as follows [23]:

Percentage of crude fiber alone = (B-A)-(C-A)x100
Percentage of Ash= C/Wx100

Where:

W = Weight of sample taken
A = Weight of empty crucible
B = Weight of crucible + insoluble residue
C = Weight of crucible + ash

Sensory Evaluation: Tempeh was baked in microwave oven and was subjected to sensory evaluation by a panel of ten members. Appearance, color, taste, flavor and overall acceptability were evaluated using score card.

RESULTS AND DISCUSSION

Growth of *R. Oligosporus* in BR and PR Samples: The growth of the fungus was monitored at intervals of 1, 2 and 4 days of incubation. Fungal mycelia could be observed from second day in the PR sample whereas BR sample showed growth only after 2 days. After 4 days of incubation, dense growth and mycelia filaments were noted in PR sample when compared with the BR sample. The reason for the enhanced growth in polished rice was attributed to the fact that fermentation acidifies the substrate, which activates the phytase (phytic acid-degrading enzyme) present in the rice and it also promotes the growth of microorganisms, where brown rice does not contain much phytase [24]. The results were tabulated in Table 1.

Nutritional Evaluation Brown Rice vs. Polished Rice: Chemical analysis showed in Figure 1 presented significant increase in levels of protein and fibre in brown rice [25, 26], where carbohydrate content was approximately equal in brown rice and polished rice, i.e. 75.2% and 74.9, respectively.

In this study, brown rice and polished rice were used as substrates for *R. oligosporus* fermentation. The BR tempeh was light brown in color partially covered with

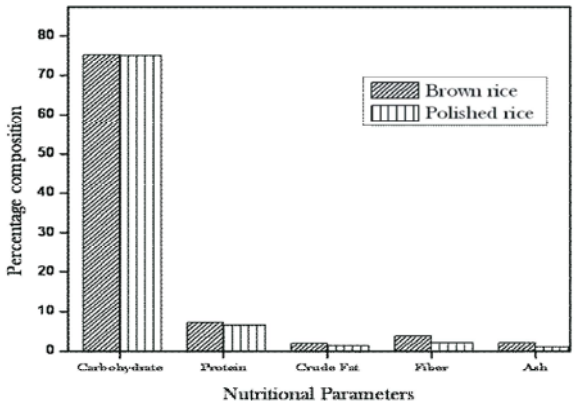

Fig. 1: Nutritive evaluation of BR and PR

Fig. 2: Growth of R. oligosporus in BR and PR tempeh after fermentation

Table 1: Growth of R. oligosporus in BR and PR samples

Incubation days	BR	PR
0th day	-	-
1th day	-	+
2th day	+	++
4th day	++	+++

"-" No growth: "+" Moderate growth: "++" Dense growth: "+++" Dense growth with mycelial filaments

Table 2: Sensory evaluation of flavored BR and PR tempeh

		Average Grade	
S.No	Parameters	BR	PR
1	Appearance	8.1	9.3
2	Color	7	8.4
3	Taste	8.9	8.5
4	Flavor	9.9	7.1
5	Overall acceptability	8.4	8.3

white cottony layer of *Rhizopus* but PR tempeh was white in color completely covered by spongy layer of *Rhizopus* (Figure 2).

Sensory Evaluation: Both the BR tempeh and PR tempeh showed appealing color, appearance and was overall acceptable (Table 2). Though, the samples received overall acceptability, the panel members preferred brown rice tempeh over white rice tempeh. They were aware of the nutritional benefits of the brown rice and they chose to consume brown rice tempeh.

CONCLUSION

Cereals are considered a vital source of nutrients for human and animal nourishment. In this paper nutritional value of brown rice is compared to that of white rice. In recent years, much attention has been on the health benefits of unpolished rice as a source of important bioactive compounds and nutrients. Results showed higher nutrients contents of brown rice compared to white rice. Earlier studies by Sie-Cheong Kiing and Shahin Roohineja [27, 28] have also reported increased fiber, protein and micronutrients content in brown rice. As, the sensory evaluation showed people preferred to consume brown rice tempeh than polished rice tempeh, brown rice can also be considered for making tempeh which is rich in GABA, protein and fiber for antioxidant properties as well as anticancer properties.

ACKNOWLEDGEMENT

R. S. Subhasree thanks DRDO for the fellowship. The authors are thankful to Mr. A. Alagusundaram, Director IICPT and Mr. R. Bhakyaraj for their immense help in our study. We thank D. Selvakumar, Group Head, BTG, DEBEL for his stimulating discussion and support during paper writing.

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