

ORIGINAL RESEARCH PAPER

IMPACT OF BIO-PROCESSING ON RICE

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Received 16 September 2010

Revised 30 January 2011

The usual way of preparing rice is boiling, thermal process that gives it a lower digestibility as compared to instantiation, extrusion or expansion. Having in view this fact, the possibility to biotechnologically improve the boiled rice digestibility was investigated in a laboratory study. In this respect, boiled rice was solid state fermented using a strain of *Saccharomycopsis fibuligera*, an amylase producing yeast originating from *ragi*. Fermented rice was then analyzed from the point of view of its content in easily assimilable sugars, protein, amino-acids, phosphorus and vitamins from B group.

Biochemical analyses revealed that the fermented rice has a ten times higher content of reducing sugars than boiled rice, due to starch hydrolysis, while chromatographic studies proved that the fermented rice contains glucose, maltose, maltotriose and maltotetrose that are easily assimilable carbohydrates.

Fermented rice has a protein content that is two times higher than that of boiled rice because it contains the yeast biomass, and is enriched in vitamins from B group (B1, B2, and B6) that are synthesized by the yeast. Inorganic phosphorus present in rice doubles its concentration in fermented rice, which means that phosphorus bioavailability is increased.

The sensorial profile of boiled rice is also improved by fermentation.

This study proves the possibility to have a processing method which is relatively cheap, practical and of which the resulting product has good nutritive qualities and does not pose safety problems due to pure culture utilization as starter.

Keywords: rice, *ragi*, *Saccharomycopsis fibuligera*, B vitamins, glucose, phosphorus.

Introduction

Rice is the most economically important food crop in many developing countries, and has also become a major crop in many developed countries where its consumption has increased considerably, particularly in North America and the European Union (EU) due to food diversification and immigration (Tran, 1998). Since a large portion of maize crops are grown for other purposes than human

consumption, rice is probably the most important grain with regards to human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans (Smith, 1998).

Rice grain average content is 80% starch, 7.5% protein, 0.5% ash and 12% water. The proportion of amylose and amylopectin in starch determines the cooking and eating qualities of the rice. In spite of the fact that rice is a primary source of carbohydrate, it is also a good source of protein, but it is not a complete protein, which means that it does not contain all of the essential amino acids in sufficient amounts for good health, and should be combined with other sources of protein, such as nuts, seeds, beans, fish, or meat (Wu *et al.*, 2003) in order to provide a balanced nutrient intake.

While in developed countries rice is boiled or submitted to instantiation, extrusion or expansion (puffing, popping) before consumption, in many developing countries the fermentation of rice grains to prepare a variety of foods has a long history.

Fermented rice food products were classified into three categories. These include solid, paste, and liquid. The solid-state fermented products include starter types such as *pekka*, *anka* (China), *ragi* (Indonesia), *koji* (Japan), predigested “yellow rice” (Ecuador), and bread like foods. Paste products include *miso* (Japan), *mochi* (Japan) and *chiang* (China). The liquid products are *shao-hsing* wine (China), *sake* (Japan), and rice vinegar (Panmei *et al.*, 2003).

According to Parveen and Hafiz (2003), the traditional fermented foods have high nutritive value and a diversity of flavours, aromas and textures. Food fermentations are important in developing countries where the lack of resources limits the use of techniques such as vitamin enrichment of foods, and the use of energy and capital intensive processes for food preservation.

The vitamin and essential amino acid content of rice products significantly increases during fermentation and remains at a superior level to the one existing in rice, even if fermented rice is used as raw material for producing rice crackers, chips, snacks (Tongnual and Fields, 2006) or ready-to-eat breakfast cereals.

Despite the fact that traditionally fermented products are appreciated for their nutritional quality, being considered functional foods, it is always possible for them to represent a health hazard from the microbiological point of view.

The aim of this study was to prove the possibility to have a new processing method which is relatively cheap, practical and of which the resulting product to be preferred both for its nutritive qualities and safety.

Materials and Methods

The experiments were carried on polished rice originating from Romania.

The fermentative process was developed by the strain *Saccharomycopsis fibuligera* MIUG 3.12, an yeast isolated from *ragi*. According to Nout (2004), *ragi* is prepared from fermented rice flour and contains mixed populations of yeasts, moulds and bacteria. Tablets of *ragi* can be stored up to six months and constitute a

conventional starter material for application in home and in small-scale industrial fermentations of rice or cassava.

Stock cultures of the yeast were maintained on Sabouraud dextrose broth. All cultures were grown at 25°C and stored at 4°C between transfers. A *Saccharomycopsis fibuligera* inoculum was prepared by cultivating the yeast in Petri dishes, on Sabouraud medium overlaid with sterile cellophane membranes. The biomass developed within 5 days at 25°C, was harvested with the cellophane membrane, introduced in an Erlenmayer flask with sterile water, and gently shaken on an orbital shaker. The inoculum concentration was determined using a Thoma cytometer.

Petri dishes (Ø10 cm) were used as substrate containers for fermenting the rice. Forty grams of rice and 60 ml of water were added to each dish (proportion 1:1.5). The rice was autoclaved for 15 minutes at 121°C, cooled at room temperature and inoculated in order to contain 10⁶ yeast cells per gram. Rice inoculated with *Saccharomycopsis fibuligera* as starter was incubated at 30°C during 3 days.

A sample consisted of the entire contents of a dish. Samples were frozen at -20°C, until the analysis could be performed. Prior to analysis, samples were thawed at room temperature, aseptically introduced in sterile plastic bags and then homogenized using a Bagmixer.

Carbohydrates were quantified as reducing sugars with DNS reagent. Sugars range was chromatographically assayed using a RP-HPLC Jasco PU 980 equipped with a refractive index detector and a Coregel 87H column. Working conditions were: isocratic system, 20 µl injection loop, mobile phase 100% water, flow rate 0,6 ml/min, temperature 85°C. Chromatographic standards consisted of a mixture of carbohydrates (glucose, maltose, isomaltose and maltotriose from Merck).

Protein content in rice samples was estimated by the Bradford method at λ=595 nm in a UV-VIS Jasco 530 spectrophotometer. Bovine serum albumine (BSA, Thermoscientific) was used as standard.

Aminoacids were determined by Sørensen method and were expressed as glycochol.

The B group vitamins were chromatographically assayed using a RP-HPLC Jasco PU 980 equipped with a UV-VIS detector (Jasco UV -1570) at λ=256 nm. Chromatographic conditions: isocratic system, 20 µl injection loop, 30°C controlled temperature, rate flow 1 µmol/min. Vitamin standards of chromatographic quality were used. Vitamins were separated in a Vydac column (C 18). The eluents used were the following:

- Sodium hydrogen phosphate pH 4.5 (Rt=14,73 min), for vitamin B1
- Methanol-phosphate pH 6 (Rt=4,5 min), for vitamin B2
- Sodium hydrogen phosphate pH 4.5 (Rt=5,16 min) for vitamin B6

Phosphorus was determined according to Mahadevaiah and co-workers (2007).

Ethanol was spectrophotometrically assayed at λ= 340nm (spectrophotometer UV-VIS Jasco 530) by the enzymatic kit Boeringer Mannheim.

Results and discussion

Amylolytic activity of the yeast *Saccharomycopsis fibuligera* MIUG 3.12 (Figure 1) was used in an attempt to obtain fermented rice.

The fermentation process applied to boiled rice imitated at laboratory scale the process of obtaining *tape ketan*, an Indonesian rice product. The flow chart used to obtain fermented rice is shown in Figure 2. In practice, rice sterilization could be replaced by boiling.

The result of fermenting sterilized rice with *Saccharomycopsis fibuligera* MIUG 3.12 was a new food product consisting of soft rice grains with fruity pleasant smell and sweet-sour taste. Fermented rice grains are softer than those of boiled rice, but they still have a nice texture.

The chemical composition of fermented rice was compared to that of boiled rice in order to point out the major differences existing between them (Table 1).

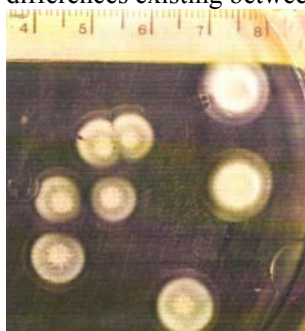


Figure 1. Colonies of *Saccharomycopsis fibuligera* MIUG 3.12 developed on Sabouraud agar

Compared to boiled rice, fermented rice has the reducing sugars content ten times higher, the protein content two times higher and the amino acids content almost unchanged.

Table 1. Important chemical compounds in boiled and fermented rice

Chemical compound	Boiled	Fermented rice
Reducing sugars, mg glucose per g d.m	19.3±1	208.6±1
Soluble protein, mg BSA per g d.m.	35.3±0.5	69.64±0.5
Aminoacids, mg glyocol per g d.m	18.25±0.5	19.52±0.5
Inorganic phosphorus, mg per 100 g d.m.	45±1	92.5±1
Ethanol, g per 100 g	-	0.3±0.1

Saccharomycopsis fibuligera is an amylolytic yeast and its action on starch explains the high content of reducing sugar found in the fermented product. Chromatographic analysis performed on fermented rice revealed that glucose concentration is 5.33 g/100 g rice (Figure 3). Due to very close retention times, the maltotriose and maltotetrose contents of fermented rice were quantified all

together. Two compounds, which appear in the chromatogram as unknown, were not identified and suppositions regarding their identity are not correct to be made due to the fact that the column could also be used for organic acids separation. It is possible for the *Saccharomyces fibuligera* strain to generate isomalto-oligosaccharides, which are prebiotic compounds, but the column did not put them into evidence.

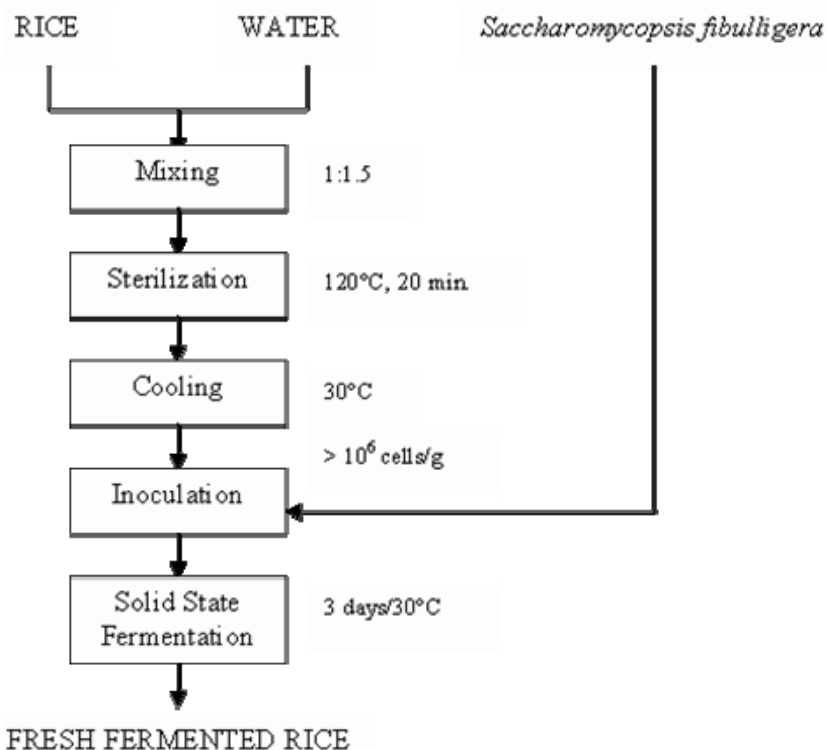


Figure 2. Flow chart for obtaining fermented rice with *Saccharomycopsis fibuligera*

The proportion between sugars, which is represented in Figure 4, shows that glucose is present in a significant amount (40%) as well as maltotriose and maltotetraose (55%), while maltose is present in a low amount (5%).

The protein content doubles in the fermented product because the yeast grows on the grains' surface and its biomass becomes part of the final product. The soluble protein content increases as result of yeast action.

On one hand the yeast releases aminoacids from the soluble protein, while on the other hand it grows and utilizes a part of the amino acids content. This explains why the aminoacid content of fermented rice does not significantly differ from the one of boiled rice. The aminoacid profile of fermented rice was not determined in this study, but it is expected to be improved by the yeast presence. This affirmation is sustained by the fact that yeast biomass is rich in lysine (Charatian and Wolnova, 2004) an amino acid that lacks in rice.

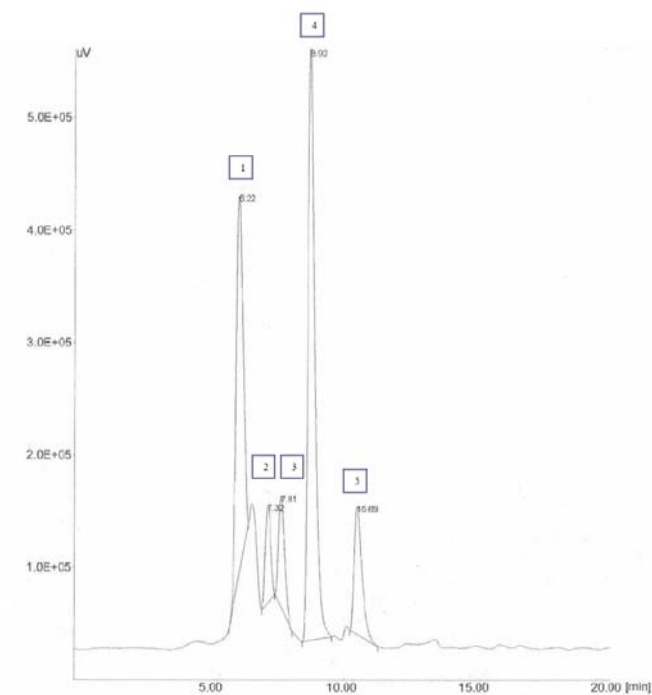


Figure 3. Chromatogram showing carbohydrates presence in rice fermented with *Saccharomyces fibuligera* MIUG 3.12 (1 – maltotriose and maltotetrose (Rt = 6.22) – 5.92 g/100 g rice; 2 – maltose (Rt = 7.32) – 0.87 g/100 g rice; 3 – unknown (Rt = 7.81); 4 – glucose (Rt = 8.92) – 5.71 g/100 g rice; 5 – unknown (Rt = 10.69))

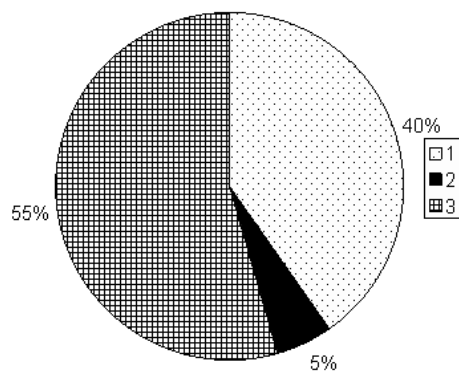


Figure 4. Proportion between chromatographically identified carbohydrates in rice fermented with *Saccharomyces fibuligera* MIUG 3.12 (1 – Glucose; 2 – Maltose; 3 – Maltotriose and maltotetrose)

Saccharomycopsis fibuligera makes its contribution to double the rice content of inorganic phosphorous, which means that this mineral bioavailability is increased in fermented rice as compared to boiled rice.

A small quantity of ethanol (0.3%) was detected in fermented rice.

The vitamin B (B1, B2, B6) content in boiled and fermented rice is shown in Figure 5. Compared to boiled rice, vitamin B1 is 4 times higher, vitamin B2 2 times higher and vitamin B6 3 times higher. Yeast presence on rice surface explains the spectacular increase of vitamin B.

From the microbiological point of view, the rice fermented with *Saccharomycopsis fibuligera* as starter culture is a safe product. Even if *Bacillus* spore forming bacteria survive to the thermal treatment of rice grains, they are not able to develop in the alcoholic-acidic media resulted after fermentation.

This product can be preserved a couple of weeks under refrigeration and eaten as a delicacy or as ingredient in creams and sauces.

If dried and ground, fermented rice could become flour and used as food ingredient able to improve the nutritional value and sensorial quality of other food products.

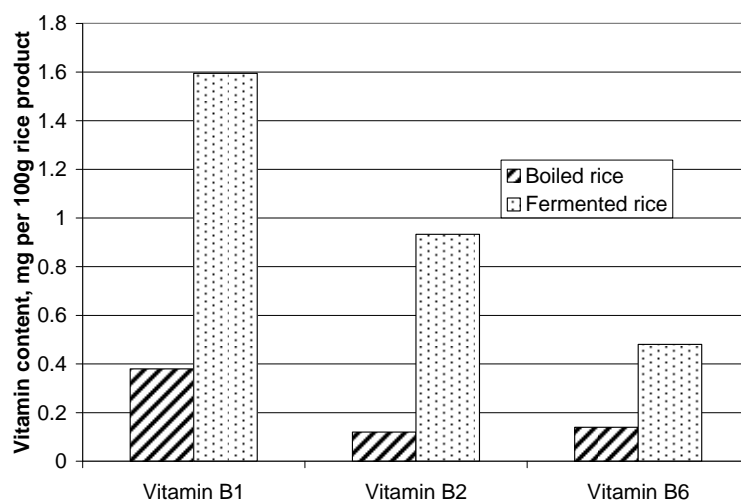


Figure 5. Vitamin B content in boiled rice and rice fermented with *Saccharomycopsis fibuligera* MIUG 3.12

Conclusions

Similar to tape ketan production, boiled rice was fermented using a strain of *Saccharomycopsis fibuligera* as starter culture.

Yeast development on rice grains improved the rice protein, inorganic phosphorus and vitamin B content.

The yeast generated oligosaccharides that have good digestibility and are useful in children nutrition.

Further studies are necessary in order to prove the health benefits of the fermented rice and the possibility of using it as food ingredient.

Acknowledgements

This research was performed in the laboratories of the Research Center for Food Biotechnology and of the Bioaliment Platform, both established at the Faculty of Food Science and Engineering from the University *Dunarea de Jos* Galati, Romania.

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List of abbreviations

BSA – Bovine Serum Albumine

DNS – 3,5-dinitrosalicylic acid

MIUG – Acronym for the Collection of Industrial Microorganisms from the University *Dunarea de Jos* Galati, Romania

RP-HPLC – Reverse Phase High Pressure Liquid Chromatography