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# Food Fermentation Products Induced by Mucorales

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Fermentation processes are developed with the help of omnipresent moulds viz., *Rhizopus, Actinomucor elegans* and *Mucor indicus* by utilizing the natural starch of *Colocasia esculenta, Ipomoea batatus, Manihot esculeuta* and *Solanum tuberosum* for the single cell proteins. The flavour and taste of *Solanum tuberosum* gave the best results. The rate of conversion of starch to proteins was not uniform in all the substrates studied. The differential behaviour exhibited by these substances were explained on the basis of the texture of the tubers used. From the study it was revealed that the best alternative for single cell proteins from potato is cassava in India.

The good quality tempeh was produced by *Rhizopus microsporus* var. *oligosporus.* Biochemical estimate of the above tempeh revealed a major increase in the soluble protein contents. Similarly a cheese like product "Sofu" was also developed by fermentation with *Actinomucor elegans.*

A fermented beverage "Bhakar" from seven different cereals viz., bajra, barley, jowar, maize, oat, rice and wheat was prepared by simultaneous fermentation with *Rhizopus oryzae* and *Mucor indicus* at 30°C. Organoleptic tests indicated that the drink was liked by the judges and it has a refreshing flavour and butter milk like taste.

**Key words :** Food Fermentation, Products, Mucorales

## INTRODUCTION

The order Mucorales comprises of an omnipresent group of fungi, which are known to act as adversaries and allies of mankind. As allies, these moulds are being commonly used in different industries for synthesizing organic acids and alcohols. These are also employed in the dairy industry for cheese production

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as well as other sources of protein for human consumption. This has resulted into the oriential fermented food products flooding the market as delicacies of the present times. Therefore, it was considered imperative that, some more works on traditional fermented food as well as enrichment of proteins in starch rich food be undertaken in the present study.

## MATERIALS AND METHODS

The fermentation was undertaken using three common species of *Rhizopus* and their varieties, a species of *Actinomucor elegans* and *Mucor indicus* by utilizing the starch of *Colocasia esculenta*, *Ipomoea batatus*, *Manihot esculenta* and *Solanum tub rosum*. While for traditional asian food like Tempeh, Sufu and Bhakar some cereals eg. *Glycine max*, *Penniseteum typhoides*, *Hordeum vulgare*, *Sorghum vulgare*, *Zea mays*, *Avena sativa*, *Oryza sativa*, and *Triticum aestivum* were selected.

The methodology used in the present study was of Brook *et al* (1969). Starchy tubers were boiled, peeled and mashed so as to achieve uniform consistency. Additional nutrients in the form of urea 10 g, $KH_2PO_4$ 2 g, $MgSO_4$, 7H O 0.5 g, per kg were added. These were packed into petriplates (10 cm) and sterilized. Six different *Rhizopus* strains were grown on PDA slants. Sterile water (1.5 ml) was added to each of the four days old slant and vigorously shaken to form a spore suspension. This was used as inoculum for the petriplate.

The inoculated plates were incubated at 30°C for 48 h, which was sufficient enough to form a solid "cake". This cake was boiled for 5 min in 10% brine solution and dried in an oven at 100°C for 10 min. These cakes, when, stored in the deep freezer in polythene bags for 10 days showed no deterioration in quality.

Tempeh, one of the most popular Indonesian food was prepared from different soybean varieties viz., Clark-63, Bragg and Pusa 24. One hundred grams of seeds of each variety were soaked in 300 ml of tap water for 24 h. These were naturally dehulled and boiled for 30 min. The excess of water was drained off and the soybean seeds were packed into 10 cm diameter petriplates. The inoculation and further processing was similar to that followed in case of tubers.

Sufu, also a soybean based Indonesian food, was prepared by soaking 100 g of soybean seeds overnight. These seeds were ground in a blender, till it was converted into a milk like consistency. The milk was coagulated with one tea spoonful of calcium sulphate. The coagulated milk was pressed into a small box ( size 4" x 4" x 2" ) with a perforated bottom, to remove the excess water. This formed a tightly packed solid "cake" commonly known as Tofu. Tofu was sliced into cubes, carefully removed from the box and placed in sterilized petriplates. The surface of these cubes was aseptically inoculated at the four corners with *Actinomucor elegans*. These were incubated at 20°C for 3 days. Subsequently, the 'Sufu' cubes were treated with 10% brine solution and dried at 100°C in a hot air oven and finally stored in a deepfreezer.

Seven different cereals mentioned earlier were used in the preparation of "Bhakar". Twenty grams each of the cereals were soaked overnight in water, coarsely ground with a mortar and pestle and mixed with 250 ml of distilled water. To one set of the slurry 10 g of sucrose was added. The slurry was dispensed into 500 ml conical corning flasks and steamed for 30 min. Before storing at room temperature, sodium meta-bisulphite ( @ 12.5 g/l ) was added as a preservative. The above fermented foods were analysed by standard method for their moisture, fat, carbohydrate, protein and enzyme. The fat content was estimated by soxhlet apparatus, carbohydrate by Dobois *et al.* (1956) and protein by micro Kjehldahl method ( AOAC 47021, 1980 ). Lowry's method was used for soluble proteins.

## RESULTS AND DISCUSSIONS

The following five *Rhizopus* species were used for the production of single cell proteins viz., *R. microsporus* var. *microsporus*, *R. microsporus* var. *oligosporus*, *R.*

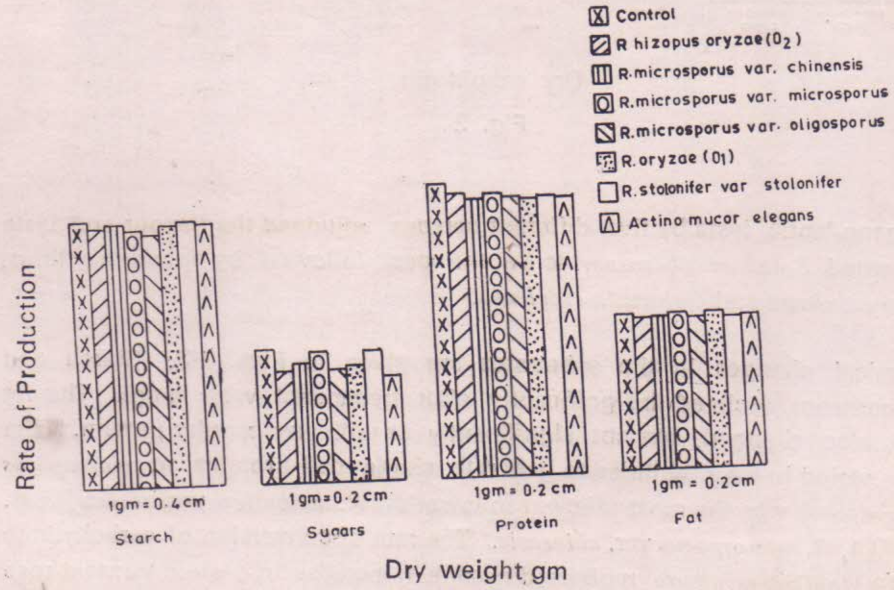



Fig. 1

*microsporus* var. *chinensis*, *R oryzae* ( two isolates $0_1$ and $0_2$ ) and *R. stolonifer* var. *stolonifer*, four different continuously consumed food products which are rich in carbohydrate in, *Colocasia esculenta* (arbi), *Ipomoea batatus* (sweet potato), *Manihot esculenta* ( cassava/tapioca ) and *Solanum tuberosum* ( potato ) were sliced in the present study. Each of the four substrate was enriched by the addition of urea minerals in order to provide a nitrogen source for protein synthesis. These were inoculated with the five *Rhizopus* species. After an incubation period of 48 h at 30°C the fermentation was brought to a halt by boiling in 10 per cent brine solution for 5 min and then dried at 100°C for 10 min.

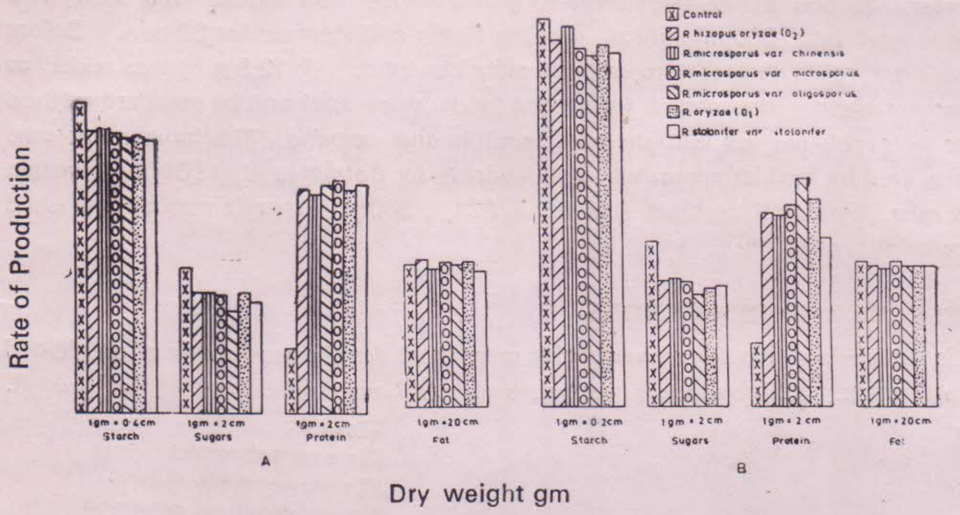
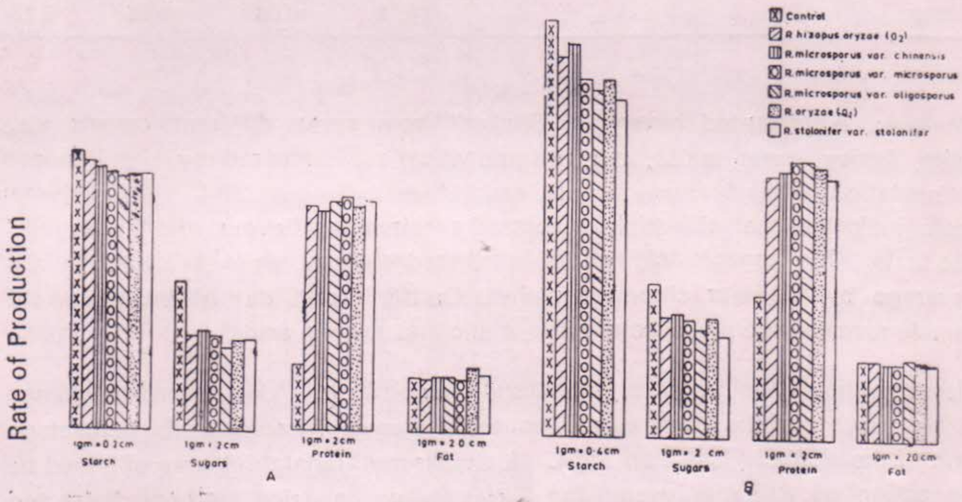



Fig. 2

The organo-leptic tests by five different persons adjudged the flavour and taste of fermented *Solanum tuberosum* to be the best followed by *Ipomoea batatus*, *Manihot esculenta* and *Colocasia esculenta*.

Biochemical changes in the substrates are given in Figs. 1-3. Starch and sugar contents declined insignificantly after treatmeant with fungi. The fat content also changed, but not significantly or with any regular pattern. The species varied in their abilities to generate single cell proteins, *R. microsporus* var. *oligosporus* was the most efficient followed by *R. stolonifer* var. *stolonifer*, *R. oryzae* ($0_1$), *R. microsporus* var. *chinensis*. The rate of conversion of carbohydrate studied, *Manihot esculenta* registered maximum increase in protein content rose to nearly twice the amount observed in the tubers normally. This was followed by *Ipomoea batatus*, *Solanum tuberosum* and finally *Colocasia esculenta*. In colocasia the protein content increased to a maximum ninety percent.

The differential behaviour exhibited by these substrates may probably be explained by the varied texture of the tubers used. Cassava has a fibre content ( Wealth of India : VI : 288 ) which lends porosity to it, therefore helps in penetration of the fungal mycelia leading to better growth of the *Rhizopus* species. On the other hand colocasia contains several gummy substances ( *Wealth in India* II ; 310 ) which render compactness to the substrate and probably inhibit mycelial penetration. Potato and sweet potato gave intermediate reaction as compared to cassava and colocasia. The organoleptic trials with these four kinds of single cell proteins indicate that potato is the most palatable product as compared to cassava, sweet potato and colocasia. Therefore, the present study has brought out an acceptable alternative to single cell proteins from potato to cassava in India.




Dry weight gm

Fig. 3

*Tempeh* The tempeh produced from *Glycine max* required an incubation period of 24 h at 30°C, followed inoculation with *Rhizopus* species. The tempeh was best in terms of appearance, taste and flavour when produced by *R. microsporus* var. *oligosporus* followed by *R. stol nifer* var. *stolonifer*, *R. oryzae* ( $O_1$ ), *R. microsporus* var. *microsporus*, *R. oryzae* ( $O_2$ ) and *R. microsporus* var. *chinensis*. A biochemical analysis of the tempeh revealed a major increase in the soluble protein contents.

*Sufu* . A cheese like product was produced by fermentation with *Actinomucor elegans* for five days at 20°C and tasted like "cottage cheese" in organoleptic tests. The biochemical changes were similar to those brought by the *Rhizopus* sp. in tempeh ( Table 1 ).

**Table 1.** Effect of *Rhizopus* species and *Actinomucor elagans* on soybean

| Fungi | Moisture | Starch | Sugar | Protein | Fat |
|---|---|---|---|---|---|
| | ( g/100 g of dry weight of sample ) | | | | |
| Control (Soybean) | 8.50 | 18.20 | 1.80 | 39.35 | 21.00 |
| *R. microsporus* var. *chinensis* | 9.50 | 17.81 | 1.62 | 37.35 | 21.55 |
| *R microsporus* var. *microsporus* | 9.54 | 17.25 | 1.75 | 38.01 | 21.20 |
| *R. microsporus* var. *oligosporus* | 10.10 | 17.11 | 1.52 | 37.54 | 20.68 |
| *R. or zae* ($0_1$) | 9.68 | 17.65 | 1 55 | 37.25 | 21.01 |
| *R. oryzae* ($0_2$) | 9.98 | 17.54 | 1.51 | 38.25 | 20.80 |
| *R. stolonifer* var. *stolonifer* | 10.50 | 17.92 | 1.78 | 37.85 | 20.59 |
| *Actinomucor elegans* | 11.20 | 17.95 | 1.44 | 37.35 | 20.65 |
| SEM | | 0.85 | 0.01 | 0.30 | 0.01 |
| @ 5% | | 4.15 | 0.05 | 0.95 | 0.20 |

*Bhakar* : A fermented beverage "Bhakar" from seven different cereals viz., bajra, barley, jowar, maize, oat, rice and wheat was prepared by simultaneous fermentation with *Rhizopus oryzae* and *Mucur indicus* at 30 C. Organoleptic trials indicated that the drink possessed a refreshing flavour and 'butter milk' like taste. The organic acid usually attributed to taste were analysed in the beverage by thin layer chromatography. On the fourth day of incubation the sample revealed the presence of citric, malic and tartaric acids.

Although the extension of the fermentation period by one day caused no change in the flavour of the drink as well as there was no increase in the percentage limit of the alcohol ( less than 3% ). A similar traditional drink was obtained by the action of *Rhizopus oryzae* and *Mucor indicus* on rice slurry by Batra and Milliner (1974). The present study revealed the possible raw material like bajra, barley, jowar, maize, oat and wheat can also be utilised in the production of "Bhakar", with the exception of oat. Bajra and wheat both were considered feasible alternative substrate for bhakar production although the rice was the best source for preparing this drink as reported by Batra (1981).

The diagnostic tests for the production of enzyme by moulds revealed that the five species of *Rhizopus* and *Actinomucor elegans* could synthesize amylase, protein and lipids. These findings are in accordance with the work of Hesseltine (1965). However, *Mucor indicus* was deficient in lipase activity, a similar instance has been observed by Sasno and Baruki (1977) while working with the rabi moulds *Chlaydomucor oryzae* and *Rhizopus oryzae*. The nature of the enzyme synthesized governs the nature of reaction catalysed. Since all the enzyme assayed were lyases it is possible that these mould will help in degrading the complex molecules and rendering major chemical changes in the substrate, according to Van Veen and Schafer (1950). These changes make the food product more platab!e besides adding flavour to bland substrates like cereals and soybean.

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