Heat stabilized defatted rice bran (HDRB) fermented with Saccharomyces cerevisiae var MTCC 3813 to enhance the protein content with bio activity

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Abstract- The solid state fermentation effect of *Saccharomyces cerevisiae* on Heat stabilized defatted rice bran, was done at 30°C for 3 days. Nutritional and antinutritional factors were determined using standard procedures. The fermented HDRB (FHDRB) showed enhanced levels of dietary fiber (18.40g%), (11.23g%) and (38.90g%) compared to HDRB. Fermentation also significantly increased the invitro protein digestibility soluble and insoluble for HDRB (3.7%) & FHDRB (6.4%).

Index Terms- *Saccharomyces cerevisiae*, Solid State Fermentation, Heat Stabilized Defatted Rice Bran (HDRB), protein, antinutrition

I. INTRODUCTION

Heat stabilized defatted rice bran (HDRB) is a whole food that contains proteins, vitamins, minerals, complex carbohydrates, phytonutrients, phospholipids, essential fatty acids and more than 120 antioxidants. The HDRB is mainly used as animal and pet feed. Heat treatment of the rice bran at the time of oil extraction results in denaturation and aggregation (Hettiarachchy Navam, 2009) and reduces bioavailability of the protein and other bioactive compounds in digestive tract. The HDRB contains 10.5 – 12.0% moisture, 12.5 – 14.5% protein, 1.5-2.5% lipids, 15.3-16.8% starch (Hettiarachchy Navam, 2009), 2.8-3.4% of total fiber, 2.0-2.5% of total phenolics and 7-12.5% of ash content.

The HDRB is fermented with yeast to afford a food product having superior prebiotic for probiotic composition enrichment. Fermentation of HDRB with *Saccharomyces cerevisiae* (probiotic) yeids a prebiotic composition that can promote the growth of beneficial intestinal bacteria (probiotic).

The prebiotic and probiotic compositions release substances that have desirable health effects upon consumption. The fermented and dried HDRB with reduced particle size has desirable health benefits when consumed (Hettiarachchy Navam, 2009). The fermented HDRB contains several bioactive compounds including proteins, phenolics, phytic acid, arabinose, bioactive isoflavones, dietary fibers and several other beneficial compounds (Ryan, 2011). The protein production, especially the single cell protein is more in HDRB due to the bioconversion of cellulose in HDRB (Ravinder *et al.,* 2003). Solid state fermentation comes from its simplicity and closeness to the natural way of life for many micro organisms (Laufenberg, et al 2003) as larger quantity of water is not required in the substrates, fermentor volume remains small, necessary manipulations become less expensive and cost of water removal in post fermentative process is minimized (Pandey 2002; Suryanarayana, 2002).

S.cerevisiae is an advantage for experimentation and application because of much higher metabolic rate, rapid growth rate and the ability to bring very quick chemical reaction. The objectives of the present study is to release protein bound phenolics and other phyto chemicals from rice bran using the enzymes produced from *cerevisiae* at the time of fermentation, to enhance prebiotic and probiotic utilities of rice bran, and producing symbiotic value added food products by incorporating fermented HDRB.

II. MATERIALS AND METHOD

Raw material

Fresh Heat stabilized defatted Rice Bran (HDRB) was procured from M/s. Ramalingam & Co, Ponnmeni, Madurai, Tamil Nadu, India.

Pretreatment of HDRB

The HDRB was sieved using 50 mesh size and autoclaved at 121°C for 15 minutes. Then the HDRB was dried at 60°C in a cabinet drier and milled.

Microorganism

Six cultures of *Saccharomyces cerevisiae* MTCC 170, 171, 249, 846, 2636 and 3813 were purchased from IMTECH, Chandigar, India. Culture MTCC 2636 was grown under aerobic condition using yeast malt medium (Ryan, 2011), MTCC 249 and 3813 using malt yeast agar medium (Ryan, 2011) and MTCC 171, 170 and 846 using yeast peptone dextrose medium (Ryan, 2011) and were maintained in the respective slants.

Inoculum preparation

About one ml of sterile water was added to the vial containing the lyophilized culture. The loopfil of the suspension was streaked over malt year medium. The plates were inoculated at 37oC for 2 days the colonies formed were transferred to malt yeast broth. This served as starter inoculums. From the starter inoculums 2 per cent was inoculated over rice bran for fermentation.

Solid state fermentation

Twenty five gram of HDRB, was taken in 500 ml Erlenmeyer flask and for each culture (MTCC 170, 171, 249, 846, 2636 and 3813) three treatments viz., 70ml, 100ml and 130ml of water was added. Then the substrates were sterilized at 121^{o}C for 30 minutes and allowed to cool overnight at RT. The medium was inoculated at the rate of 2 per cent . These flasks were incubated for three days in stationery conditions at 30^{o}C for 3 d. After completion of prescribed incubation period, the fermented HDRB were subjected for quality analysis to screen the best *S. cerevisiae*.

***S. cerevisiae* growth on rice bran**

The screened *S. cerevisiae var MTCC 3813* culture was incubated at 37^{o}C and sampled at 24, 48 and 72 hours. Yeast cells were enumerated by drop plating serial dilution on YNB plates to determine total Colony Forming Units (CFU) as per Ryan *et al.* (2011).

Estimation of ethanol

Ethanol concentration was estimated calorimetrically as described by Captui *et al.* (1968). Three ml of fermented HDRB samples from each treatment was transferred to 250 ml round bottom flask connected to the condenser and was diluted with 30 ml distilled water. The samples were distilled at 74 to 75ºC. The distillate was collected in 25 ml of 0.23 N Potassium dichromate (K_2CrO_7) reagents, which was kept at the receiving end. The distillate containing ethanol was collected till total volume of 45 ml was obtained. Similarly, standards (20 to 100 mg ethanol) were mixed with 25 ml of Potassium dichromate **(**K_2CrO_7) separately. The distillate containing ethanol was collected till total volume of 45 ml was obtained. These samples and standards were kept in water bath at 60ºC for 20 min and were cooled. The volume was made up to 50 ml with distilled water and optical density was measured at 600 nm using spectrophotometer (Double beam UV- VIS Spectrophotometer 2201, M/S. Systronics, India). The standard curve was plotted considering the concentration against absorbance.

Estimation of protein

The BSA working standard 0.2 ml in 5 test tubes was taken and made to 1ml using distilled water. The test tube with 1 ml distilled water serve as blank. Then 4.5 ml of reagent I was added and incubated for 10 minutes. After incubation 0.5 ml of reagent II was added and incubated for 30 minutes. Then measured the absorbance at 660 nm and plotted the standard graph. Estimated the amount of protein present in the given sample from the standard graph. **(**Lowry et al., 1951).

Water and oil absorption capacities

Water absorption of unfermented and fermented HDRB was determined with little modification to the method reported by Anderson *et al.* (1969). Five g of each unfermented and fermented HDRB was weighted into a centrifuge tube and 30 ml of distilled water was added and mixed thoroughly. This was allowed to stand for 30 minutes and centrifuged at 3,000 rpm for 15 min. The supernatant was then decanted and the sample was weighed again. The amount of water retained in the sample was recorded as weight gain and was taken as water absorbed.

Nutrient analysis of unfermented and fermented HDRB

Iron was estimated by Spectrophotometry method (Lindsay and Norvell, 1978). Phosphorus was estimated by Colorimetry method (Piper, 1966). Potassium was estimated by Flame photometry method (Sumner, 1944).

Estimation of anti-nutritional compounds

The anti-nutritional compounds such as polyphenols and phytic acid were estimated by Folin-Cio calteu method (Wheeler and Ferrel., 1971). Benzoic acid, Oxalate and Cinnamic acid were estimated by Titrimetry method. Tannin was estimated by Colorimetry method (Schandert, 1970).

Estimation of *Invirto* protein digestability

Invitro protein digestibility of FHRDB was determined by the method of Sgarbieri (Silveira and Badiale-Furlong. 2009). Dry biomass (1g) 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 water bath at 37^{o}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 of 23.8 mg tyrosine, min^{-1}. mg^{-1} protein) was added and the samples were hydrolyzed for 24 h in a water bath at 37^{o}C. Then, the samples were boiled at 100^{o}C 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).

Estimation of dietary fibre, soluble and insoluble fibre

Soluble and insoluble fibre estimation was determined by the method of Hellodoorn Technique (James and Theander (1981) A quantity of 0.8g of sample with 50 ml of water was kept for 15 mins in a water bath at 100^{o}C. Then 50ml of 0.2m HCL was added, with 100mg of pepsin and incubated for 18 hrs at 40^{o}C. Sample was neutralized with 50ml of 0.1M Sodium thiophosphate. The pH was adjusted to 6.8 and then 100mg pancreatin was added and kept in agitator for 1 hour at 40^{o}C. Acidification to pH 4.5 then centrifuged at 3000 rpm for 30 min. Then filtered in a crucible and the sediment was kept with 150ml water. Supernatant was collected and washed with 4 volumes of 95% ethanol and centrifuged it. The sediment was washed with 150ml 80% ethanol and 100 ml acetone. Dried under nitrogen steam and then at 50-60^{o}C at 1 hour (Water soluble fibre) as dry weight of material. Residue was collected washed with 100ml of acetone and transferred with acetone to crucible dried at 105^{o}C overnight (Water insoluble fibre) Dry weight –tare.

III. Statistical analysis

All determinations were carried out in triplicate. All data are expressed as mean±SD. Data were analyzed by an analysis of variance ($P<0.05$) and the means separated.

IV. Results and Discussion

Effect of protein and ethanol content on screening of yeast culture *S. cerevisiae*

The 4% ethanol content is suitable for processing the fermented HDRB incorporated value added products. The protein content was high in *Saccharomyces cerevisiae var* MTCC 3813. Hence *var* MTCC 3813 is highly suitable for the production of fermented rice bran.

Effect of water and oil absorption

Water absorbed by fermented HDRB in model tests is close to 200g water / 100g (Barber and Barber, 1980). In baked produce, the high water-binding capacity of rice bran helps to maintain moisture and freshness. High fat absorption capacity in fermented HDRB would be desirable in products such as meat extenders to help maintain juiciness and improve mouth feel. The water and oil absorption is also increased when compared to the unfermented HDRB. (James and Sloan, 1984).

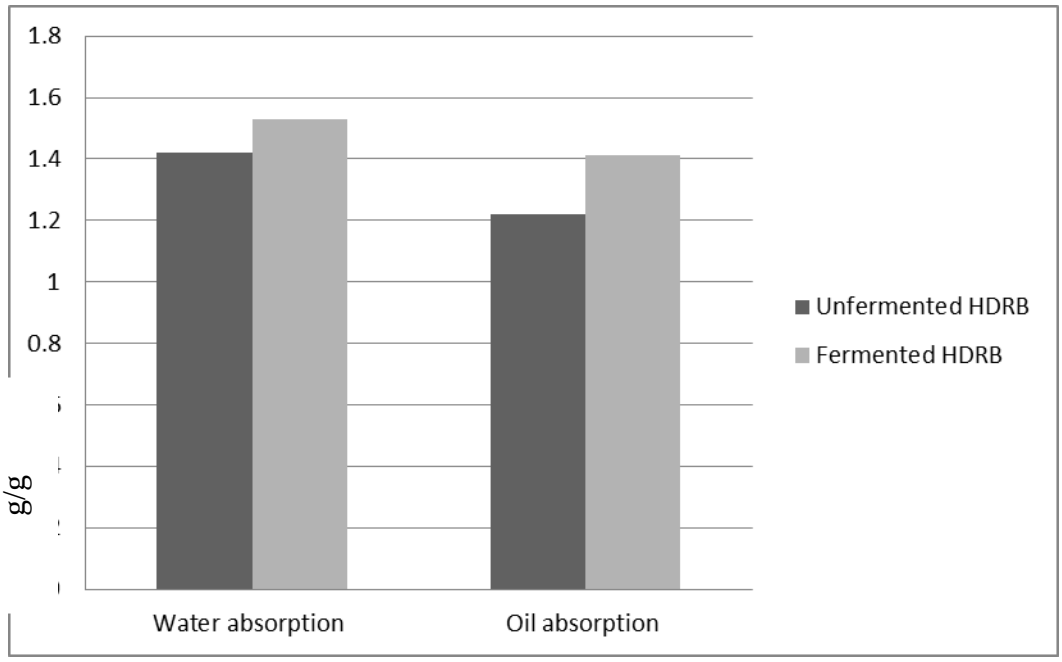

Fig.1.Effect of water and oil absorption of unfermented HDRB and FHDRB

Particle size

When *S. cerevisiae* MTCC 3813 was grown on HDRB of various particles size ranging from 30-70 mesh size protein yield increased with increase in particle size. Minimum protein yield was found in 30 mesh size (12 -18 mg%) while maximum protein yield was observed in 50 mesh size (18 – 20 mg%). Beyond 50 mesh size, the protein yield decreased, (Kupski *et al.*, 2012).

Effect of nutrient composition of HDRB and FHDRB

Table 3. Estimated nutrients composition of HDRB and FHDRB

S.No	Nutrients (g%)	UnFermented HDRB	Fermented HDRB
1	Moisture	6.88 ± 0.015	7.12 ± 0.020
2	Total Protein	17.60 ± 0.100	18.40 ± 0.025
3	Fat	0.09 ± 0.006	0.58 ± 0.020
4	Dietary Fibre	28.20 ± 0.151	38.90 ± 0.519
5	Calcium	0.241 ± 0.008	0.301 ± 0.003
6	Ash	9.76 ±0.070	11.23 ± 0.072
7	Iron	0.063 ± 0.001	0.101 ± 0.002
8	Phosphorus	0.078 ± 0.003	0.092 ± 0.001
9	Potassium	0.087 ± 0.003	0.1385 ± 0.0003

An increase in protein content during fermentation of HDRB was observed (Table3). This was due to supplementation of nitrogen during the biomass production by S.cerevisiae from HDRB. The high protein was noticed at 3 days of fermentation with *S. cerevisiae var* MTCC 3813. The 4% ethanol content is suitable for processing the fermented HDRB incorporated value added products. The protein content was high in *Saccharomyces cerevisiae var* MTCC 3813 is highly suitable for the production of fermented rice bran, approximately 18.40g% and 17.60g% for FHDRB relation to un-fermented HDRB. The fat content of HDRB was ranged between 0.09 to 0.58. FHDRB which exhibited the most concentrated source of dietary fibre of about 38.90 %. The ash content was about 11.23g%. Significant

difference was observed in HDRB and FHDRB (P=0.2431, Mean = 8.5402).

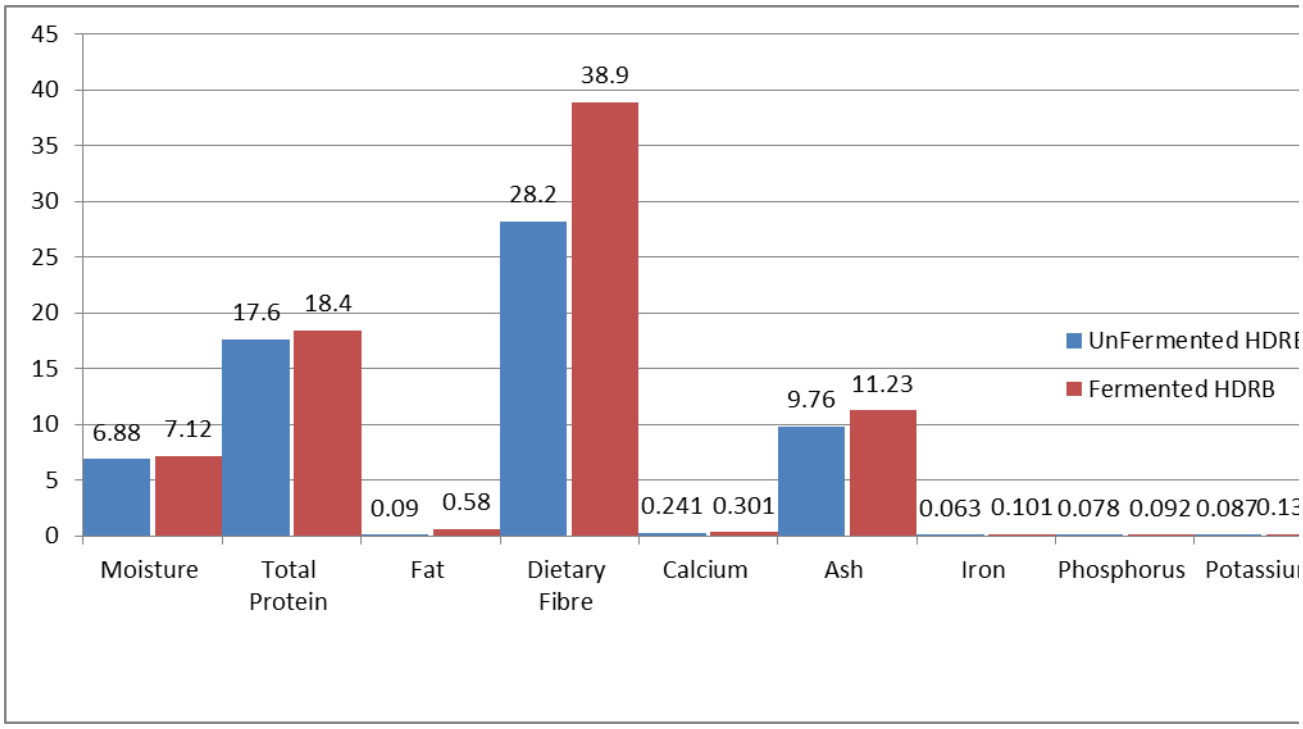

Fig. 2. Effect of nutrient composition of unfermented HDRB and Fermented HDRB

Soluble and Insoluble fibres

The Table 6 shows that the soluble fibre significantly decreased during fermentation (SEd: 0.3094, CD(.05) = 0.8590 CD(.01) =1.4246 CV% = 16.26). Soluble fibre was lower compared to UFDRB, reaching an average of 2.22% of soluble fibre after fermentation.

Insoluble fibre during the fermentation significantly increased (SEd = 1.0045, CD (.05) = 2.7890). The Fibre was higher compared to UFHDRB and reaching to 78.60% of insoluble fibre after fermentation.

Table 4.Estimation of Soluble and Insoluble fibre

[1] **Particulars**	[2] **Unfermented HDRB (%)**	[3] **FHDRB (%)**
[4] Soluble fibre	[5] 2.85	[6] 2.22
[7] Insoluble fibre	[8] 71.10	[9] 78.60

***Invitro* protein digestibility**

The Table 5 shows that the protein digestibility significantly increased during fermentation (SEd=0.3839; CD=0.5=1.0658). The protein digestibility was higher when UFHDRB was used reaching an average of 6.37% of protein digestibility after fermentation using *Saccharomyces cerevisiae* var MTCC 3813 culture.

Table.5. Estimation of Invitro Protein digestibility

[10] **Particulars**	[11] **Invitro Protein digestibility (%)**
[12] UFHDRB	[13] 3.70
[14] FHDRB	[15] 6.37

Table 6. Effect of Anti-nutritional factors of unfermented HDRB and fermented HDRB

S.No	Anti-nutrients (%)	Unfermented HDRB	Fermented HDRB
1	Polyphenols	1.54 ± 0.015	1.36 ± 0.010
2	Benzoic acid	0.64 ± 0.010	0.56 ± 0.015
3	Phytic acid	5.107 ± 0.009	4.819 ± 0.003
4	Cinnamic acid	0.54 ± 0.015	0.51 ± 0.020
5	Tannins	2.8 ± 0.125	2.5 ± 0.010
6	Oxalate	0.61 ± 0.020	0.55 ± 0.015

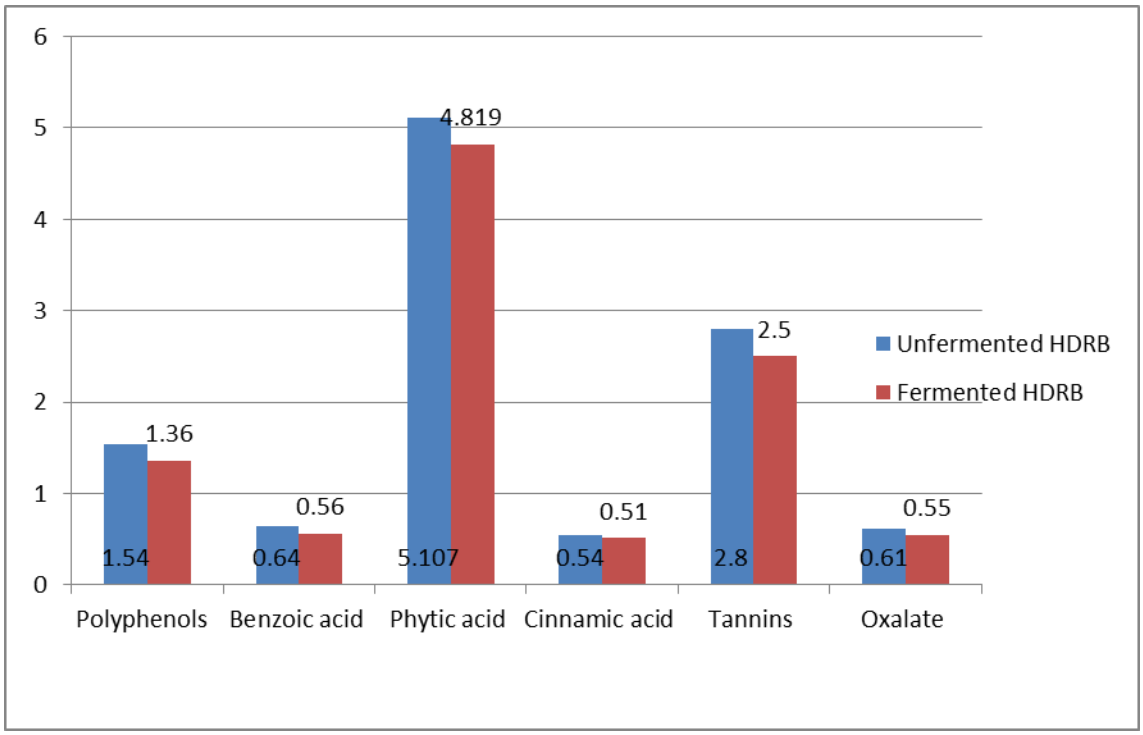

Fig.3. Effect of Anti-nutritional factors of unfermented HDRB and fermented HDRB

V. Conclusion

The Fermented Heat Stabilized Defatted Rice Bran (FHDRB) is an excellent source of dietary fibre for addition to food and it offers all the nutritional and neutraceutical benefits of whole grain. The antinutrient content of Fermented Heat Stabilized Defatted Rice Bran was reduced during the solid state fermentation compared to unfermented HDRB and an increase of 18.40g%, 11.23g% and 38.90g% respectively in the content of protein, ash and dietary fibre were observed. When yeast grown in Heat Stabilized Defatted Rice Bran in solid state fermentation substrate, extracellular enzymes including amylases, cellulases, xylanaze, esterases and proteases, secreted by the yeast can be converted to complex components of the rice bran into simpler molecules. This fermented rice bran contains indesgitable oligosaccharide, resistant starch, oligo peptides etc. that can be effectively used by the intestinal probiotics. This probiotic release phenolics and other health benefits rich phytochemicals from yeast like antimutagenic, antioxidant and antimicrobial activity (Hettiarachchy Navam, 2009). Hence, HDRB is a good source of prebiotic and probiotic food. This beneficial symbiotic food can be incorporated in human food. In solid state fermentation of rice bran, the substrate are utilized very slowly and steadily. So the same substrate can be used for long fermentation periods. Hence, this technique supports controlled release of nutrients, solid state fermentation is best suited for fermentation and to transfer of technology to food industry people as it require less a_w. Fermented HDRB powder and extracts from fermented HDRB is highly suitable for development of value added products.

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Table.1. Effect of solid state fermentation by S. *cerevisiae* on ethanol content of FHDRB

S. No	Particulars	Control			MTCC 170			MTCC 171			MTCC 249			MTCC 846			MTCC 2636			MTCC 3813		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1	Ethanol g%	0.8	0.9	1.0	1.5	2.2	1.9	1.3	1.8	1.8	1.2	1.8	1.9	3.4	3.4	3.4	3.8	3.8	3.8	3.8	4.0	4.0

	SED	CD(0.05)
t	0.12067	0.24389**
f	0.18433	0.37255**
tf	0.31927	0.64528**

T_1 – 70 ml water + 2%
T_2 – 100 ml water + 2%
T_3 – 130 ml water + 2%

* t- treatment (3), f-factors (7)

Table 2. Effect of solid state fermentation by S. *cerevisiae* on protein content of FHDRB

S. No	Particulars	Control			MTCC 170			MTCC 171			MTCC 249			MTCC 846			MTCC 2636			MTCC 3813		
		T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3	T_1	T_2	T_3
1	Protein g%	11.2	12.6	12.0	13.3	16.4	12.9	12.8	17.8	12.9	13.2	17.9	13.9	12.3	16.8	13.8	12.6	18.3	13.8	12.5	18.4	14.2

	SED	CD(0.05)
t	0.02722	0.05494**
f	0.04157	0.08392**
tf	0.07201	0.14535**

T_1 – 70 ml water + 2%
T_2 – 100 ml water + 2%
T_3 – 130 ml water + 2%

* t- treatment(3), f-factors (7)