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Heat-Stabilized Defatted Rice Bran (HDRB) as an Alternative Growth Medium for *Saccharomyces cerevisiae*

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Abstract

Heat-stabilized defatted rice bran (HDRB) is an inexpensive and underutilized coproduct of rice milling. The yeast *Saccharomyces cerevisiae (S. cerevisiae)* is commonly used in bread production and fermentation, and it has a variety of application within the food and pharmaceutical industries. The objective of the current study is to evaluate HDRB as a complex media for the growth of *S. cerevisiae.* Analysis determined that HDRB was composed of 35.9% total dietary fiber, 19.1% crude protein, 14.9% starch, 16.4% ash, 9.4% moisture, 1.9% soluble sugars, 1.8% crude lipid, and 8.9 mg/g total phenolics. *S. cerevisiae* cultured in HDRB grew from an initial inoculum of 1.5 Log CFU/mL to 7.2 Log CFU/mL within 42 hr. In comparison, yeast cultured in yeast media grew from an initial inoculum of 1.5 to 7.8 Log CFU/mL within 24 hr. The results from this study indicate that HDRB can serve as a low cost alternative growth medium for *S. cerevisiae*.

Keywords: Rice bran; *Saccharomyces cerevisiae*; Fermentation; Yeast; Medium

Introduction

Rice (*Oryza sativa* L) is a staple food for over half the world's population and supplies as much as 70% of the dietary energy and protein in certain regions of the world [1]. During rice milling, the outer brown layer is separated from the inner rice kernel, yielding white rice and rice bran. Over 63 million tons of rice bran is produced world-wide each year, and more than 90% is sold cheaply as animal feed [2]. Rice bran is often defatted and heat treated to prevent lipid oxidation. This milling coproduct is termed heat-stabilized defatted rice bran (HDRB). HDRB is widely available and inexpensive, costing about $125 to $195 per metric ton [3]. Although HDRB is widely used as an animal feed, it has been increasingly utilized by the food industry in recent decades [2]. The micronutrient and macronutrient profile of HDRB makes it well-suited for health foods, breads, cereals, crackers, and pasta. Its nutrient profile also makes HDRB a potential media for yeast propagation.

Yeast is vital to leavening of bread and the fermentation of alcoholic beverages. *Saccharomyces cerevisiae* (*S. cerevisiae*) is the yeast most commonly used in production of bread, beer, and wine [4]. In brewing, *S. cerevisiae* is propagated in wort, composed of malt grain and water. Wort consists of proteins, free amino acids, fermentable sugars (fructose, sucrose, glucose, maltose), dextrins, vitamins, and minerals [5]. This complex media supplies yeast with the nutrients necessary for aerobic growth. In the production of baker's yeast, *S. cerevisiae* is most often cultivated in a high sugar medium composed of beet or cane molasses supplemented with inorganic ammonia, vitamins, and trace minerals [6].

S. cerevisiae is also used as an alternative source of proteins, enzymes, vitamins, and yeast extracts for the food and pharmaceutical industries. Molasses is the most common substrate for growth of food grade yeast; however, approximately 40% of molasses is non-fermentable. This unused fraction increases the cost of yeast produc-

tion and leads to industrial waste. Therefore, recent efforts have focused on using food processing coproducts as substrates for food grade yeast production [7]. To date, *S. cerevisiae* has been grown on processing coproducts, including fruit and vegetable extract, date byproducts, hydrolyzed cassava waste, shrimp shell waste [8-11].

HDRB has been shown to support the growth of *Lactobacillus acidophilus* as well as several *Bacillus* species [12]. However, previous research has not fully evaluated HDRB as a growth media for food grade yeasts, such as *S. cerevisiae*.

Therefore, the objectives of this study were to determine the composition of heat-stabilized defatted rice bran on the basis of percent moisture, crude protein, starch, lipid, total dietary fiber, ash, soluble sugars, and total phenolics (mg/g) and evaluate the growth of *S. cerevisiae* ATCC 26603 in heat-stabilized defatted rice bran at high and low initial inoculum levels.

Materials and Methods

Materials

HDRB from Riceland Foods, Inc (Stuttgart, AR, USA) was ground with IKA M20 (IKA Works Inc. Wilmington, NC, USA) and passed through a 60 mesh sieve to obtain a fine homogenous powder. A food-grade yeast strain, *S. cerevisiae* ATCC 26603, was purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). The substances for preparing yeast media (YM) agar and phosphate buffer solution (PBS) were purchased from Difco Laboratories (Detroit, MI, USA). The reagents and enzymes used for starch determination were purchased from Megazyme International Ireland, Ltd. (Bray Business Park, Bray, Co. Wicklow, Ireland). Other analytical-grade chemicals were purchased from Fisher Scientific (Pittsburgh, PA, USA) and Sigma Chemical Co. (St. Louis, MO, USA).

Determination of moisture content

The moisture content of HDRB was determined by drying 2 g of each sample at 104 °C for 3 hr [13]. After drying, samples were covered, transferred to desiccator, cooled for 2.5 min, and weighed. Values are expressed as a mean of three determinations on dry weight basis.

Determination of protein content

The protein content of HDRB before and after heat sterilization (121 °C, 30 min) was determined by the automated Kjel-Foss method 46-08 [13]. Samples were digested with Kjeldahl Digestion System 6 for 1 hr at 420 °C, and nitrogen content of the samples was determined with a KjelTech Analyzer 2000 (Tecator Co., Hoganas, Sweden). Percentage total protein content in HDRB was calculated with a conversion factor of 5.95 [14]. Values are expressed as a mean of three determinations on dry weight basis.

Determination of starch content

The starch content of HDRB before and after heat sterilization (121 °C, 30 min) was determined by Megazyme total starch assay procedure AACC 76.13 [13]. For this procedure, 5 mL of aqueous ethanol (80% v/v) was added to 100 mg HDRB. The mixture was stirred by vortexing then was incubated at 85 °C for 5 min before mixing in an additional 5 mL of aqueous ethanol. The mixture was centrifuged for 10 min at 1000 x *g*. The supernatant was discarded, and the pellet was resuspended in 10 mL of aqueous ethanol before centrifuging a second time at 1000 x *g*. The supernatant was discarded, and the precipitate was immediately mixed with 3 mL of thermostable α-amylase in MOPS buffer (50 mM, pH 7.0). Samples were incubated in a boiling water bath for 6 min with vigorous stirring after 2 min and 4 min. Samples were transferred to a 50 °C water bath. Sodium acetate buffer (4.0 mL, 200 mM, pH 4.5) was added, followed by amyloglucosidase (0.1 mL, 20 U). Samples were vortexed and incubated at 50 °C for 30 min. An aliquot (1.0 mL) of each sample was diluted to 10 mL with distilled water and was centrifuged at 3,000 RCF for 10 min. Three milliliters of glucose determination reagent (GOPOD) was added to each sample, including glucose controls. Glucose controls consisted of 0.1 mL of glucose standard solution (1 mg/mL) and 3 mL of GOPOD Reagent. A reagent blank was used, consisting of 0.1 mL of water and 3 mL of GOPOD Reagent. Absorbance was determined at 510 nm. Values are expressed as a mean of three determinations.

Determination of soluble sugars

Soluble sugars were extracted in triplicate at 50 °C for 15 min using a 10:1 ratio of ethanol-to-sample [15]. Extracted sugars were quantified by the phenol-sulfuric acid colorimetric method of Dubois et al. [16] using a Shimadzu Model UV-1601 spectrophotometer (Kyoto, Japan). A standard curve was prepared using sucrose at concentrations from 3 to 50 μg/mL. Soluble sugars from triplicate determinations are expressed on a dry weight basis.

Determination of crude lipid content

The crude lipid content of HDRB before and after heat sterilization (121 °C, 30 min) was determined by the crude fat in soy flours method 30-26 [13]. In brief, 5 g of HDRB was weighed onto filter paper and enclosed in second filter. Samples were placed in butt-type extraction apparatus as described in AACC method 30-26 [13]. Approximately 250 mL of petroleum ether was added to each flask. Ether was evaporated from the samples for 3 hr using a water bath. Samples were then dried overnight. Values are expressed as a mean of three determinations on a dry weight basis.

Determination of total dietary fiber content

The total dietary fiber content was determined by enzymatic-gravimetric analysis following AACC method 32-07 [13]. For the analysis, one gram of HDRB sample from before and after heat sterilization (121 °C, 30 min) was individually subjected to sequential enzy-

matic digestion by heat stable α-amylase, protease, and amyloglucosidase. The mixture was precipitated with 4 volumes 95% ethanol. Precipitate was filtered and dried. The residues were corrected for protein and ash content. Values are expressed as a mean of three determinations on a dry weight basis.

Determination of ash content

The ash content was determined by the AACC method 08-03 for feedstuffs [13]. In brief, triplicate 2 g samples of HDRB were weighed into crucible and were heated in an electric furnace at 600 °C for 2 hr. Crucibles were then transferred to desiccator and cooled before weighing. Values are expressed as a mean of three determinations on a dry weight basis.

Determination of total phenolic contents

The total phenolic content of HDRB before and after heat sterilization (121 °C, 30 min) was determined using Folin-Ciocalteu's reagent according to the method of Swain and Hillis [17] and Joslyn [18]. Fifty milligrams of HDRB samples from before and after heat sterilization were weighed into screw-cap test tubes and were vortexed with 10 mL of methanol. The mixtures were heated in a water bath at 65 °C for 2 hr, then removed and allowed to cool to room temperature. An aliquot (0.5 mL) of the methanol extract was diluted to 7 mL with deionized water and was vortexed for 5 s. A volume of 0.5 mL of Folin-Ciocalteu's reagent was added. Samples were then vortexed for 5 s and were allowed to stand for 3 min. One milliliter of saturated sodium carbonate was added to the solution, and the total volume was brought to 10 mL with water before vortexing for 5 s. After 2 hr, absorbance was read at 725 nm using a spectrophotometer (Shimadzu Model UV-1601, Kyoto, Japan). Protocatechuic acid was serially diluted and used as a standard curve. Values are expressed as a mean of three determinations on dry weight basis. Results are expressed as protocatechuic acid equivalents (PAE) in milligram per gram dry material.

Growth of *Saccharomyces cerevisiae* with high initial inoculum level

In order to optimize growth of *S. cerevisiae* in HDRB, the proportion of HDRB-to-water was evaluated in duplicate at five levels: 2.5, 5, 7.5, 10 and 15% (w/v). Rice bran slurries were prepared by mixing 1.25, 2.5, 3.75, 5, and 7.5 g HDRB with 50 mL of DI water in a 250 mL flask. A control sample was also included; it consisted of 50 mL of DI water in a 250 mL flask. HDRB slurries were not completely sterilized by standard autoclaving at 121 °C, 20 min; however, 121 °C for 30 min was sufficient to kill all organisms. Therefore, the water control and HDRB slurries were autoclaved for 30 min at 121 °C. Yeast media (50 mL) was prepared in a 250 mL flask and was autoclaved for 20 min at 121 °C.

After cooling to room temperature, HDRB slurries, YM media, and water control were inoculated with 5 mL of activated (12 hr, 30 °C, YM) *S. cerevisiae* ATCC 26603 at a high initial inoculum level (6.5 Log CFU/mL). Samples were incubated with orbital shaking (100 rpm) at 30 °C for a total of 2 days. Yeast growth was measured at 4, 8, 12 24, 36, 48 hr by serial dilution in PBS buffer and plating on YM agar. Plates were incubated at 30 °C for 48 hr to enumerate colonies (CFU/mL).

Growth of *Saccharomyces cerevisiae* ATCC 26603 in HDRB at low initial inoculum level

S. cerevisiae growth was not significantly affected by the proportion of bran-to-water used in high inoculum experiments. Because industrial production costs increase with increasing substrate utilization, a 2.5% HDRB-water slurry was selected for subsequent study. Next, growth of *S. cerevisiae* was measured after a low-level inoculation (1.5 Log CFU/mL) into HDRB slurry and YM media. Duplicate rice bran samples were prepared for inoculation by mixing 1.25 g HDRB with 50 mL DI water then sterilizing at 121 °C for 30 min. A volume of 50 mL of YM was prepared as described earlier. To prepare the inoculum, ten milliliters of activated (12 hr, 30 °C) *S. cerevisiae* ATCC 26603 in YM broth was centrifuged (5,000 x *g*, 10 min), and the cell pellet was washed twice with PBS buffer solution. The pellet was transferred into the HDRB slurry (2.5% HDRB), YM, and water control (0 g HDRB) at the low initial inoculation level of 1.5 Log CFU/mL. Each of these samples was incubated with orbital shaking (100 rpm) at 30 °C for 3 days. Samples were taken at regular intervals, serially diluted (PBS, pH 7.0), and plated on YM agar. Plates were incubated (48 hr, 30 °C) and counted (Log CFU/mL). These procedures were repeated and results are reported as an average of duplicate procedures.

Statistical analyses

Student's t test was used to determine significant difference among the before and after autoclaved HDRB for each component tested using the fit y by x platform in JMP version 5.0.1 (SAS Institute Inc., Cary, NC). Student's t test was used to detect significant difference among the five concentrations of HDRB, water control, and YM at high initial inoculum level for *S. cerevisiae* ATCC 26603 using the fit y by x platform in JMP. The significant difference among the growth population at the time of maximum cell population for the low initial inoculum level in the 2.5% HDRB, 0% HDRB (water control) and YM were also evaluated and analyzed in comparison to the high initial inoculum level (for *S. cerevisiae* ATCC 26603) at 12 hr (time of maximum cell population) using the previously mentioned statistical analysis.

Results and Discussion

Composition analysis

HDRB after heat sterilization was composed of 35.9% total dietary fiber, 19.1% crude protein, 14.9% starch, 16.4% ash, 9.4% moisture, 1.9% soluble sugars,

1.8% crude lipid, and 8.9 mg/g total phenolics (Table 1). Autoclaving resulted in statistically significant decrease in extractable crude lipids from 3.1% before autoclaving to 1.8% after autoclaving. None of the other measured components were significantly affected by heat sterilization (Table 1).

[a]Results expressed on dry basis are the means and standard deviations of three replications. Mean values followed by same letter within columns are not significantly different ($p > 0.05$)

rice bran sample	moisture (%)	crude protein (%)	starch (%)	crude lipid (%)	total dietary fiber (%)	ash (%)	soluble sugars (%)	total phenolics (mg/g)
before	9.4 (± 0.2)	18.1a (± 0.4)	13.9a (± 0.9)	3.1a (± 0.0)	36.1a (± 0.1)	15.9a (± 0.2)	1.7a (± 0.1)	8.4a (± 0.0)
after	Same	19.1a (± 0.7)	14.9a (± 0.8)	1.8b (± 0.4)	35.9a (± 0.9)	16.4a (± 0.2)	1.9a (± 0.2)	8.9a (± 0.1)

Table 1: Moisture, crude protein, starch, lipid, total dietary fiber, ash, soluble sugars, and total phenolic content of heat-stabilized defatted rice bran before and after heat sterilization (121 °C, 30 min)[a]

The modest reduction in extractable crude lipids that accompanied autoclaving may have been caused by formation of amylose-lipid complexes, which are less amenable to ether extraction. This explanation is consistent with work by Szezodrak and Pomeranz [19], which demonstrated that autoclave heating of starch for 1 hr in the presence of lipids led to the formation of amylose-lipid complexes.

The composition of HDRB in this study was similar to the composition of defatted rice bran given in literature. Newman et al. [20] report a much lower level of total dietary fiber (24.1%) in defeated rice bran; however, the level of protein in their samples (16.2%) was only slightly lower than the protein level (19.1%) in the present study. Results from Pan and Cathcart [21] were similar to those reported here. They found defatted rice bran to contain 16.6% protein, 11.28% ash, 7.0% moisture, and 3.7% fat. Moreover, data from Claye et al. [22] is quite similar to our own work. Their analysis showed defatted rice bran to contain 19.3% protein, 11.9% ash, 5.1% moisture, and 1.3% crude fat.

Growth of *Saccharomyces cerevisiae* at high initial inoculum level

Inoculation with a high level of *S. cerevisiae* ATCC 26603 produced a very similar growth pattern among the five concentrations of HDRB and YM (Figure 1). After 12 hr. of incubation, there was no significant difference ($p > .05$) in growth among HDRB conditions or between HDRB media and YM (Table 2). The data suggests that differences in the carbon source and available nutrients between YM and HDRB did not significantly affect the yeast growth. Marginal growth was also observed in the water control (0.7 Log CFU/mL); however, this can be attributed to the high inoculum [23]. *S. cerevisiae*, ATCC 26603 was selected for study because its ability to grow on hydrolyzed bagasse pith, which is a byproduct from sugar cane processing [24]. The strain appears to be well adapted to growth in complex substrates, and may be a good candidate for industrial applications.

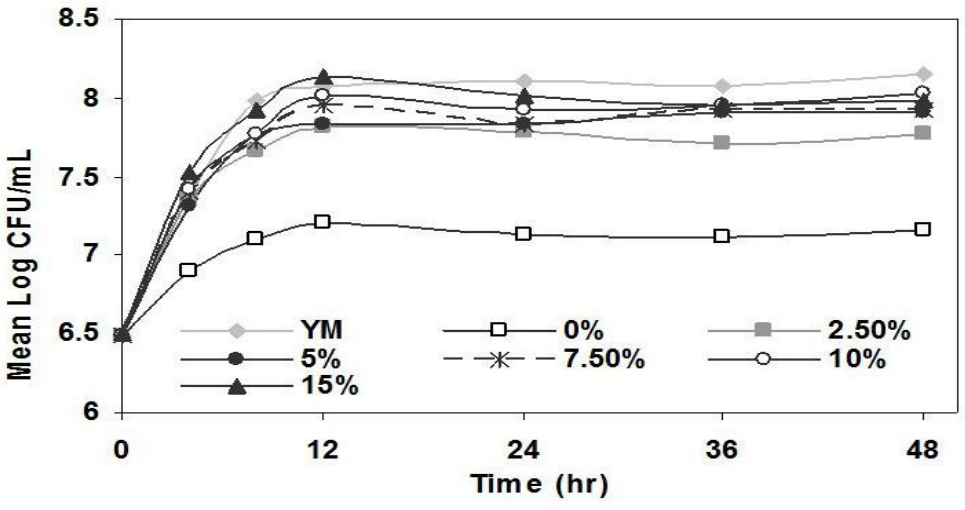

Figure 1: Mean growth of *Saccharomyces cerevisiae* ATCC 26603 in heat-stabilized defatted rice bran at high initial inoculation

[a]Results are the means and standard deviations of two replications. Mean values followed by same letter within columns are not significantly different ($p > 0.05$)

growth medium	growth increase 12 h incubation
YM (control)	1.6 ± 0.2a
0% HDRB	0.7 ± 0.3b
2.5% HDRB	1.3 ± 0.1a
5% HDRB	1.3 ± 0.1a
7.5% HDRB	1.5 ± 0.3a
10% HDRB	1.5 ± 0.3a
15% HDRB	1.6 ± 0.3a

Table 2: Growth increase (mean + stdev) Log CFU/mL of *S. cerevisiae* ATCC 26603 with high initial inoculum level[a]

Growth of *Saccharomyces cerevisiae* at low initial inoculum level

Inoculation of YM with a low level of *S. cerevisiae* produced a growth curve with a brief lag phase followed by typical exponential and stationary phases (Figure 2). When grown in YM, *S. cerevisiae* reached a stationary phase after 24 hr, and a maximum cell population of 7.8 CFU was observed at this time point (Table 3). Inoculation of 2.5% HDRB with a low-level of *S. cerevisiae* led to population growth that extended for 42 hr before reaching a maximum of 7.2 CFU after 42 hr. Yeast grew more slowly in HDRB media compared to YM; however, there was no significant difference in maximum cell population (Table 3 between YM and 2.5% HDRB ($p > 0.05$). These results demonstrate that when a low initial inoculum level (1.5 Log CFU/mL) and minimal amounts of HDRB (2.5%) are used *S. cerevisiae* ATCC 26603 can achieve maximum cell populations comparable to that of standard medium (YM), and thus, can sufficiently utilize the nutrients available in HDRB for its growth.

Conclusion

Heat-stabilized defatted rice bran was composed of 9.4 ± 0.2% moisture, 19.1 ± 0.7% crude protein, 1.8 ± 0.4% crude lipid, 14.9 ± 0.8% starch, 1.9 ± 0.2% soluble sugars, 16.4 ± 0.2% ash, 35.9 ± 0.9% total dietary fiber, and 8.9 ± 0.1 mg/g total phenolics. The data from this study showed that when compared to YM, HDRB was a comparable growth medium for *S. cerevisiae* ATCC 26603. Therefore, HDRB may serve as a low-cost alternative for industrial propagation of *S. cerevisiae*.

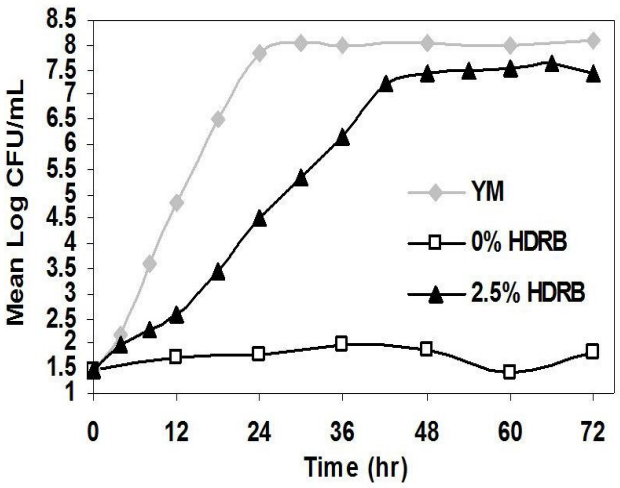

Figure 2: Mean growth of *Saccharomyces cerevisiae* ATCC 26603 in heat-stabilized defatted rice bran at low initial inoculum

[a]Results are the means and standard deviations of two replications. Mean values followed by same lowercase letters within row are not significantly different; mean values followed by same capitalized letter within column are not significantly different, ($p > 0.05$

initial inoculum level	sample media					
	YM		2.5% HDRB		0% HDRB	
	maximum cell population	growth increase	maximum cell population	growth increase	maximum cell population	growth increase
high (6.5 Log CFU/mL)	8.1 ± 0.3A	1.6 ± 0.2a	7.8 ± 0.2A	1.3 ± 0.1a	7.2 ± 0.2A	0.7 ± 0.3b
low (1.5 Log CFU/mL)	7.8 ± 0.1B	6.4 ± 0.1a	7.2 ± 0.0A	6.0 ± 0.0a	1.7 ± 0.2B	0.3 ± 0.2c

Table 3: Comparison of maximum cell population and growth increase of *S. cerevisiae* ATCC 26603 (mean ± stdev) Log CFU/mL with high and low initial inoculum level[a]

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