

# Study of Physico-Chemical Properties of Hom Mali Brown Rice Flour Extract Powder and Optimum Concentration in *Aspergillus niger* Culture Media

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## ABSTRACT

The objectives of this research were to determine the physico-chemical properties of Hom Mali brown rice flour (HMBRF) extract powder and the optimal concentration to include in ready-to-use media for culturing *Aspergillus niger* TISTR 3089. The HMBRF extract powder contained reducing sugar (3.45%) and total sugar (31.99%) with water activity, water absorption index and water solubility index being 0.18, 0.11 g/g and 81.75%, respectively. The concentrations of HMBRF extract powder used in liquid media (broth) and solid media (agar) for culturing *A. niger* were optimized by comparing cell dry weight and colony diameter of *A. niger* in liquid media and on solid media, respectively, with control media of potato dextrose broth (PDB) and potato dextrose agar (PDA). Results showed that cell dry weight of *A. niger* after growing for 4 d at 30±2°C in HMBRF extract powder solution at 7.5, 7.6 and 7.7% (w/v) was not significantly different ( $p>0.05$ ) from the control medium (PDB). Therefore, the optimum concentration of HMBRF extract powder as liquid medium for *A. niger* was used at 7.5% (w/v) to minimize cost. The colony diameter of *A. niger* on HMBRF extract solution at 5.0, 5.5 and 6.0% (w/v), each with 1.0% agar, was measured daily for 10 d and was significantly ( $p\leq0.05$ ) larger than that grown on the control medium (PDA) from the first day of measurement. The mycelium of *A. niger* on agar at these same concentrations of HMBRF extract powder reached the edge of the Petri dish (9.0 cm diameter) after cultivation for 7 d compared with 10 d on the control medium (PDA). Therefore, the optimum concentration for solid medium for *A. niger* was 5% (w/v) HMBRF extract powder with 1.0% agar.

**Key words:** physico-chemical properties, HMBRF extract powder, optimum concentration, culture media, *Aspergillus niger*

## INTRODUCTION

Traditionally microbiological evaluation depends on the ability to grow and maintain microorganisms on culture media under favorable

nutrient conditions in the laboratory that simulate environmental conditions (Prescott *et al.*, 2002). Favorable nutrients for culturing microorganisms include: an appropriate carbon source, a nitrogen source, availability of enzymes, vitamins, mineral

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elements, inorganic salt and water. Efforts to provide suitable nutrients for culturing microorganisms started with van Leeuwenhoek in 1675 using a fluid obtained by soaking peppercorn in water. A solid medium was developed thereafter by Koch in 1881, when he reported the use of boiled potatoes to culture *Mycoplasma* spp. (Khaled *et al.*, 1996; Olutiola *et al.*, 2000; Prescott *et al.*, 2002). However, information on recent studies of media formulation using locally available materials is scanty (Poopathi *et al.*, 2002). There was a preliminary study by Petchaboon *et al.* (2007), which found that *Phytophthora infestans* could be cultivated on culture media prepared from either glutinous rice (100 g/L) or japonica rice (100 g/L). Fungi, such as *Aspergillus niger*, *Fusarium moniliforme*, *Penicillium* spp. and *Curvularia paleescens*, which were isolated from onion, carrot, orange and maize, respectively, were cultured on agar media from corn meal dextrose agar (Adesemoye and Adedire, 2005), and also Thai and Japanese strains of *Pleurotus sajorcaju* were cultured on media from rice straw (Alfredo *et al.*, 2006). However, potato dextrose agar (PDA) and potato dextrose broth (PDB) are recommended for isolation and enumeration of yeasts and molds from dairy and other food products (David and Mary, 2003). There are two major types of PDA and PDB according to preparation method, either ready-to-use media or culture media prepared from fresh potatoes. Ready-to-use PDA is composed of 20.0% potato infusion, 2.0% dextrose and 1.5% agar, while PDB contains the same composition, but without agar (Alexopoulos and Beneke, 1961). PDA and PDB were prepared by boiling 200g of fresh potatoes in 500 mL of distilled water for 30 min on a hot plate, filtering with a filter cloth, adjusting the volume to 1 L with distilled water, adding 2.0% dextrose and adding 1.5% agar for the solid medium (Alexopoulos and Beneke, 1961; Booth, 1971; Harrigan and McCance, 1976). In Thailand, both types of media, (ready-to-use and culture

prepared from fresh potatoes), are usually used for yeast and mold enumeration. Ready-to-use PDA and PDB are completely imported at high cost and fresh media that use potato as a main raw material have problems associated with cost and the shortages of fresh potatoes, as well as the inconvenient preparation. Thus, it is necessary to look for new nutritional sources, which are locally available and have lower costs than potatoes for use as the raw materials in producing ready-to-use culture media for yeasts and molds. Broken Hom Mali brown rice (HMBR), which contain enough nutrients to culture yeast and mold and is a by-product from the rice milling process, can be used as a raw material in producing ready-to-use media. From previous studies, Hom Mali brown rice flour (HMBRF) extract powder was prepared by sieving broken HMBR at particle size 100 mesh, boiling with water in the ratio of 1:3.7 and digesting with  $\alpha$ -amylase 0.05% before filtration to get a clear liquid, which was then spray-dried (Srisoth and Piyachomkwan, 2003; Nukit, 2006). Therefore, the objectives of this study were to determine the physico-chemical properties of HMBRF extract powder and the optimal concentration to include in the ready-to-use media for culturing *Aspergillus niger*.

## MATERIALS AND METHODS

### Microorganisms and inoculums

*Aspergillus niger* TISTR 3089 obtained from Thailand Institute of Scientific and Technological Research (TISTR) was used throughout this study. Lyophilized *A. niger* was activated in Difco potato dextrose broth (PDB), incubated in a rotary shaker at 150 rpm and 30 $\pm$ 2°C for 48 h, then transferred using a sterile loop onto Difco potato dextrose agar (PDA) plates with three locations on each plate and incubated at 30 $\pm$ 2°C for 7 d. Mycelia of *A. niger* were transferred using a sterile needle to PDA slant, incubated for 7 d and stored at 4°C until used.

Before using in the experiment, *A. niger* was propagated at the center of a PDA plate for 7 d. A sterile cork borer (0.4 cm) was used to cut the tips of *A. niger* mycelia, which were transferred onto the formulated media (Sriswadskulmee, 2002).

### **Hom Mali brown rice flour extract powder preparation**

#### **Hom Mali brown rice flour extract process**

Broken HMBR was obtained from the Royal Chitralada Project and prepared by grading, drying, milling and sieving through a 100 mesh (Nukit, 2006). The extraction process for HMBRF was prepared according to the procedure of Pradistpong (1996), with some modifications (Figure 1).

#### **Spray dry process of HMBRF extract powder**

HMBRF extract was spray-dried, according to the procedure of Jitpisoot (2007), with some modifications (Figure 2). The amount of total soluble solids (TSS) of HMBRF extract before spray-drying was adjusted with distilled water to 15.0°Brix using a hand refractometer (ATAGO, Japan).

The physico-chemical properties of HMBRF extract powder were measured. Color (L\* a\* b\*) was measured by a Lovibond Reflectance Tintometer (RT100, PFX, UK), water activity by a Navasina (Model MS1, Switzerland) and moisture content according to AOAC (2000). In addition, the percentage of reducing sugar (RS) was measured using a spectrophotometer

Mix HMBRF with distilled water in the ratio 1:3.7

↓ ← Add  $\text{CaCl}_2$  solution with  $\text{Ca}^{2+}$  content

200 ppm

Adjust to pH 6.5 with 0.5 N NaOH

↓ ← Add  $\alpha$ -amylase 0.05%

Cover with aluminum foil and digest in water bath at 75°C for 30 min

↓

Cool down rapidly in ice bath

↓

Boil for 10 min

↓

Centrifuge at 5,000 rpm for 30 min

↓

HMBRF extract

**Figure 1** HMBRF extraction process, modified from Pradistpong (1996).

(UV9200, China) (Somogyi, 1952), the water absorption index and water solubility index (Pradistpong, 1996) and total sugar results were obtained from the Food Quality Assurance Service Center (FQA), Institute of Food Research and Product Development, Kasetsart University under AOAC (2000).

### **Study of optimum concentration of HMBRF extract powder in *Aspergillus niger* culture media**

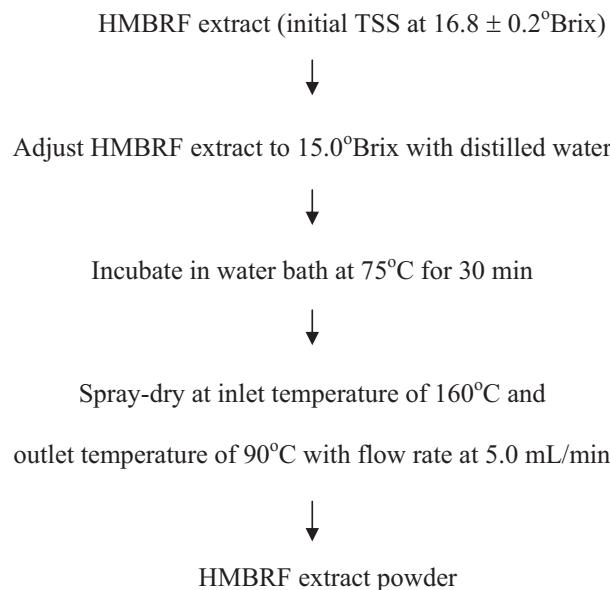
#### **Optimum concentration of HMBRF extract powder in liquid media**

HMBRF extract powder was dissolved in distilled water at 5.0, 6.0, 7.0 and 8.0% (w/v) and mixed thoroughly. Then, 50 mL of each concentration was placed in separate 250-mL Erlenmeyer flasks and sterilized in the autoclave at 121°C for 15 min. Sterile media were inoculated with a cork borer of *A. niger* culture and incubated in a rotary shaker at 150 rpm and 30±2°C. After 4 d incubation, the cells of *A. niger* were filtered through Whatman No.1 filter paper with a vacuum pump, washed twice with 50 mL distilled water, dried in a hot air oven at 80°C overnight, put into

desiccators and weighed at regular intervals until constant weight was achieved (Junlakun, 2002). Data were analyzed by analysis of variance (ANOVA) to determine the optimum concentration of HMBRF extract powder in the liquid media used for culturing *A. niger* and compared with the control medium (PDB).

#### **Optimum concentration of HMBRF extract powder in solid media**

HMBRF extract powder was dissolved in distilled water at 4.0, 4.5, 5.0, 5.5 and 6.0% (w/v), mixed thoroughly, 1% agar added and then stirred on a hot plate until the solution boiled and was homogenous. A sample (500 mL) of each concentration was placed in separate 1000-mL Duran bottles and sterilized in the autoclave at 121°C for 15 min. The media were cooled to 45-50°C, poured aseptically into sterilized Petri dishes and allowed to solidify before inoculation. Sterile media were inoculated with a cork borer of *A. niger* culture at the center of each Petri dish. Inoculated plates were incubated with mycelia at 30±2°C and colony diameter was measured daily in two directions at right angles to each other (Lopez-



**Figure 2** Spray dry process for HMBRF extract powder, modified from Jitpisoot (2007).

Malo *et al.*, 2005). Data were analyzed using ANOVA to determine the optimum concentration of HMBRF extract powder in the solid media used for culturing *A. niger* and compared with the control medium (PDA), which was prepared as indicated in the instructions and adjusted to pH 3.5 with the appropriate amount of sterile 10% tartaric acid.

## RESULTS AND DISCUSSION

### The physico-chemical properties of HMBRF extract powder

The yield of HMBRF extract powder was estimated as 57.23%. The physico-chemical properties of the HMBRF extract powder are shown in Table 1. The amount of reducing sugar in the HMBRF extract powder was 3.45%, which

was higher than in the potato extract (0.21%). The high amount of reducing sugar in the HMBRF extract powder provided an advantage by reducing the cost of the culture media, as there was no need to add 2% dextrose as indicated in the formulation of PDA or PDB and in the potato extract for culturing yeast and mold. Furthermore, its water solubility index was high (81.75%) indicating that its organic and inorganic compounds were easily dissolved, which increased the efficiency of microorganism cell metabolism.

### The optimum concentration of HMBRF extract powder in *Aspergillus niger* culture media

#### Optimum concentration of HMBRF extract powder in liquid media

The growth of *A. niger*, measured by cell dry weight, is shown in Table 2. The growth of *A.*

**Table 1** Physico-chemical properties of HMBRF extract powder.

| Physico-chemical property               | HMBRF extract powder |
|---|----------------------|
| Color                                   |                      |
| L*                                      | 84.52                |
| a*                                      | -0.52                |
| b*                                      | 7.68                 |
| Aw                                      | 0.18                 |
| Moisture content (%)                    | 2.49                 |
| Reducing sugar (%)                      | 3.45                 |
| Total sugar (%)                         | 31.99                |
| Water absorption index (g/g dry weight) | 0.11                 |
| Water solubility index (%)              | 81.75                |

**Table 2** Cell dry weight of *Aspergillus niger* in liquid medium of HMBRF extract powder compared with control.

| HMBRF extract powder (%w/v) | Cell dry weight (g/l)        |
|-----------------------------|------------------------------|
| 5.0                         | 3.78 $\pm$ 0.44 <sup>e</sup> |
| 6.0                         | 4.55 $\pm$ 0.61 <sup>d</sup> |
| 7.0                         | 6.02 $\pm$ 0.72 <sup>c</sup> |
| 8.0                         | 8.51 $\pm$ 0.81 <sup>a</sup> |
| Control                     | 7.11 $\pm$ 1.16 <sup>b</sup> |

Note:

Control = potato dextrose broth (Difco).

<sup>a-e</sup> = mean  $\pm$  standard deviation from three replications. Mean values within a column not followed by the same letter are significantly different ( $p \leq 0.05$ ).

*niger* in HMBRF extract powder at 5.0, 6.0, 7.0 and 8.0 % (w/v) was higher and significantly different ( $p \leq 0.05$ ) from the control medium (PDB). HMBRF extract powder solution at 8.0 % (w/v) had the highest cell dry weight. However, the cell dry weight of *A. niger* growing in the control was the same as that produced by HMBRF extract powder solution in amounts between 7.0 and 8.0 % (w/v). To narrow down the optimum concentration of HMBRF extract powder solution in the *A. niger* culture medium, the cell dry weight of *A. niger* was measured again in concentrations of HMBRF extract powder between 7.0 and 8.0% (w/v) and compared with the control (PDB). The results are shown in Table 3.

The growth of *A. niger* at 7.5, 7.6 and 7.7% (w/v) was not significantly different ( $p > 0.05$ ) from the control medium (Table 3). Therefore, the optimum concentration of HMBRF extract powder for liquid media was 7.5% (w/v) to minimize cost. Liquid medium for enumerating *A. niger* growth was prepared by suspending 75.0 g of the HMBRF extract powder in 1 L of distilled water, whereas control medium (PDB) was prepared by suspending 24.0 g of the powder in 1 L of purified

water. Normally, molds use simple sugars, especially monosaccharide (dextrose or glucose) or disaccharide, as the carbon source for growth and reproduction. Results from analysis of the physico-chemical properties showed that the amount of reducing sugar in the HMBRF extract powder was 3.45%, which was less than in the PDB control medium (6.19%) at the same concentration. This required a greater amount of HMBRF extract powder to provide the same amount of sugar available in the control medium.

#### Optimum concentration of HMBRF extract powder in solid media

Growth of *A. niger* in solid media as measured by colony diameter is shown in Table 4. The colony diameter of *A. niger* on HMBRF extract solution at 5.0, 5.5 and 6.0% (w/v), each containing 1.0% agar and measured daily for 10 d, showed significantly ( $p \leq 0.05$ ) larger colony sizes from those grown on the PDA control medium since the first day of measurement; the mycelia of *A. niger* on agar at those concentrations of HMBRF extract powder reached the edge of the Petri dish (9.0 cm diameter) in 7 d instead of

**Table 3** Cell dry weight of *Aspergillus niger* in liquid media of HMBRF extract powder at 7 to 8 % (w/v) compared with control.

| HMBRF extract powder (%w/v) | Cell dry weight (g/l)        |
|-----------------------------|------------------------------|
| 7.1                         | 5.78 $\pm$ 0.60 <sup>c</sup> |
| 7.2                         | 5.88 $\pm$ 0.80 <sup>c</sup> |
| 7.3                         | 5.92 $\pm$ 0.88 <sup>c</sup> |
| 7.4                         | 6.32 $\pm$ 0.70 <sup>c</sup> |
| 7.5                         | 7.50 $\pm$ 1.46 <sup>b</sup> |
| 7.6                         | 7.31 $\pm$ 1.05 <sup>b</sup> |
| 7.7                         | 7.70 $\pm$ 0.66 <sup>b</sup> |
| 7.8                         | 8.10 $\pm$ 0.56 <sup>a</sup> |
| 7.9                         | 8.50 $\pm$ 0.26 <sup>a</sup> |
| Control                     | 7.48 $\pm$ 0.80 <sup>b</sup> |

Note

Control was potato dextrose broth (Difco).

<sup>a-c</sup> = mean  $\pm$  standard deviation from three replications. Mean values within a column not followed by the same letter were significantly different ( $p \leq 0.05$ ).

10 d on the PDA control medium. Therefore, the optimum concentration for *A. niger* grown on solid medium was 5% (w/v) HMBRF extract powder with 1.0% agar because of the lower cost of preparation and shorter time required for mycelia to spread compared with the PDA control medium. In addition, results from a preliminary experiment showed that the colony diameters of *A. niger* on the solid medium containing HMBRF extract powder with the pH adjusted to 3.5 using sterile 10% tartaric acid compared with similar samples with unadjusted pH (5.85) were not significantly different ( $p>0.05$ ). Therefore, the preparation process using HMBRF extract powder in solid medium involved suspending 50.0 g of the powder in 1 L of distilled water, while for the PDA control medium the process involved suspending 39.0 g of the powder and adjusting the pH to 3.5 with sterile 10 % tartaric acid before use. However, in order to prepare the same amount of medium (1 L), the cost of media using HMBRF extract (liquid medium, 56.25 baht/L; solid medium 50.00 baht/L) was lower than commercial ready-to-use PDA media (PDA was more than 100 baht/L and PDB was more than 200 baht/L).

## CONCLUSION

The physico-chemical properties of HMBRF extract powder, namely water activity, reducing sugar, total sugar, water absorption index and water solubility index were 0.18, 3.45%, 31.99%, 0.11 and 81.75%, respectively. The optimum concentrations of HMBRF extract powder in liquid and solid media for the growth of *A. niger* were 75 g of HMBRF extract powder in 1 L of distilled water and 50 g of HMBRF extract powder in 1 L of distilled water with 1% agar, respectively. The preparation of the solid medium from HMBRF extract powder was more convenient than PDA because there was no need to adjust the pH. However, culture media using HMBRF extract powder should be tested with other fungi to assess efficiency.

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**Table 4** Colony diameter of *Aspergillus niger* in solid media using HMBRF extract powder compared with control.

| HMBRF extract powder (%w/v) | Diameter (cm)/day |           |           |           |           |           |           |
|-----------------------------|-------------------|-----------|-----------|-----------|-----------|-----------|-----------|
|                             | 1                 | 2         | 3         | 4         | 5         | 6         | 7         |
| 4.0                         | 0.50±0.1b         | 1.50±0.2b | 3.00±0.2b | 4.80±0.2b | 5.50±0.2b | 6.50±0.0c | 7.00±0.0b |
| 4.5                         | 0.50±0.2b         | 1.50±0.2b | 3.00±0.3b | 4.80±0.3b | 5.50±0.3b | 6.50±0.0c | 7.00±0.0b |
| 5.0                         | 1.00±0.2a         | 2.38±0.2a | 5.03±0.2a | 6.30±0.3a | 7.95±0.3a | 8.47±0.1b | 9.00±0.0a |
| 5.5                         | 1.00±0.1a         | 2.53±0.1a | 5.03±0.2a | 6.55±0.1a | 8.13±0.2a | 8.53±0.1b | 9.00±0.0a |
| 6.0                         | 1.00±0.2a         | 2.42±0.3a | 5.02±0.1a | 6.52±0.4a | 8.02±0.2a | 9.00±0.0a | 9.00±0.0a |
| control                     | 0.50±0.2b         | 1.48±0.2b | 3.03±0.3b | 4.97±0.6b | 5.45±0.5c | 5.97±0.4d | 6.50±0.5c |

Note:

Control was Potato dextrose agar (Difco).

<sup>a-d</sup> = mean ± standard deviation from three replications. Mean values within column not followed by the same letter were significantly different ( $p\leq 0.05$ ).

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