Nutrient compositions of distillers dried grain from rice husks with co-culture fermentation of \textit{Saccharomyces cerevisiae} with \textit{Candida tropicalis}

Tatang Sopandi*, Tini Surtiningshii and A. Wardahii

1Department Biology, Faculty of Mathematical and Biological Science. University of PGRI Adi Buana, Surabaya, Indonesia.
2Biology Department, Faculty of Science and Technology, Airlangga University, Indonesia.
3Department of Development Economic, Faculty of Economic, 17 Agustus 1945 University, Surabaya, Indonesia.

Email: tatang_sopandi@yahoo.co.id

Received 23 April 2018; Received in revised form 31 December 2018; Accepted 20 March 2019

ABSTRACT

**Aims:** Distillers dried grains are the nutrient rich co-product of dry-milled ethanol production. The present study aimed to prove that the nutritional composition of distillers dried grain from a crude hydrolysate of rice husk fermented by co-cultures of \textit{Saccharomyces cerevisiae} with \textit{Candida tropicalis} difference from unfermented crude rice husk hydrolysate and mono-cultured \textit{S. cerevisiae} or \textit{C. tropicalis}.

**Methodology and results:** The effects of mono- and co-cultures \textit{S. cerevisiae} with \textit{C. tropicalis} on the nutrient compositions of distillers dried grain were investigated. The crude rice husk hydrolysate in distilled water contained molasses, urea, sodium nitrate, ammonium nitrate, potassium phosphate and magnesium sulfate heptahydrate were fermented by mono- and co-cultures of \textit{S. cerevisiae} with \textit{C. tropicalis} for 7 days at 28-30 °C and stored with a relative humidity of 60-70% in the dark. A mono- and a co-culture fermentation of \textit{S. cerevisiae} and \textit{C. tropicalis} increased the crude protein, crude fat, crude fibres, ash, and calcium contents of the rice husk feedstock and decreased the metabolic energy reducing sugars.

**Conclusion, significance and impact of study:** Some nutrient components of the DDG crude rice husk hydrolysate performed higher than the non-fermentation of rice husks. The finding of this study will serve as a basic reference for future studies to utilize by-product of ethanol production from rice husks for animal feed formulation.

**Keywords:** Rice husk, distiller dried grain, \textit{Saccharomyces cerevisiae}, \textit{Candida tropicalis}

INTRODUCTION

The rapid development of the bioethanol industry has brought much attention to research on the use of distiller dried grain (DDG), a major product of the bioethanol industry, to meet the needs for nutrient sources, especially for animal feed proteins. DDG is known to be a source of protein, energy, water-soluble vitamins and minerals as well as good amino acid success for poultry (Purdum et al., 2014; Ezzat et al., 2015). Utilization of by-products as raw materials for feed is a strategy to maximize the benefits and efficiency of bioethanol production. The bioethanol industry can generate revenue from valuable DDG marketing to cover some of the production costs (Han and Liu, 2010).

In general, commercial bioethanol production uses food materials, such as corn, wheat, rice, tapioca, and sweet potatoes (Chum et al., 2013; Wadhwa and Bakshi, 2016; Wangpbor et al., 2017). Cheap, abundant and non-food raw materials as feedstock for bioethanol production continue to be used, especially those coming from agricultural waste, such as sugarcane bagasse, rice straw and wheat straw (Irfan et al., 2014). Preliminary studies show that the \textit{Saccharomyces cerevisiae} co-culture with \textit{Candida tropicalis} can produce bioethanol from rice husks (Sopandi and Wardah, 2015). Co-cultures of the \textit{S. cerevisiae} with \textit{C. tropicalis} also produce bioethanol in media containing phenolics and furfural as inhibitors of fermentation (Sopandi and Wardah, 2017). However, the evaluation of nutritional characteristics of distillers dried grain from rice husks fermented by mono- and co-cultures of \textit{S. cerevisiae} with \textit{C. tropicalis} has not been published. Knowledge of the nutrient composition of a material plays an important role in feed formulation in accordance with livestock needs. The aim of this study is to prove the nutritional composition of distillers dried grain from a crude rice husk hydrolysate a co-culture fermentation of \textit{S. cerevisiae} with \textit{C. tropicalis} difference from unfermented crude rice husk hydrolysate and mono-cultured \textit{S. cerevisiae} or \textit{C. tropicalis}.

*Corresponding author
MATERIALS AND METHODS

Preliminary treatment of rice husks

Local farm-sourced rice husks from Sidoarjo, Indonesia were air dried for 2 days to obtain rice husk with water content 20% and then ground to approximately 2-mm-diameter particles using a grinder mill (PM-15, Higao Tech, China). Preliminary treatment method followed the method described by Sopandi and Wardah (2017). The milled rice husks (900 g) were steamed at 130 °C for 3 h, cooled to room temperature, mixed with 15 L of 2.5% H₂SO₄ and autoclaved (All American, Wisconsin Aluminium Foundry Co. Inc.) for 15 min at 121 °C. To prevent changes in chemical content due to light and temperature, the crude rice husk hydrolysate (CRHH) was cooled, put in a glass container and stored in refrigerator (Samsung RT43H5001SA, Indonesia) at 1 to 5 °C in the dark condition until it was used.

Culture microorganism

Saccharomyces cerevisiae strain Food and Nutrition Culture Collection (FNCC) 3012 and C. tropicalis strain FNCC 3033 were obtained from the Microbiology Laboratories, PPAU Gadjah Mada University, Yogyakarta, Indonesia. Sabouraud agar (Oxoid, Thermo Scientific, UK) was used to maintain the strains. Working stock cultures were prepared from stocks in 7 days at 28 °C on a Sabouraud agar plate and subcultured from the master stock. The colonies were aseptically sampled by scraping the top with an inoculating loop and transferred to 10 mL sterile water. The inoculum stock suspensions were prepared from the working stock, diluted to 1.7 x 10⁶ spores/mL, and enumerated with a haemocytometer (Merk Assistant, Germany).

Fermentation

Our fermentation method followed the method described by Sopandi and Wardah (2016). Precisely 120 g of the CRHH was placed into a 5 L glass Erlenmeyer flask containing 3 L of distilled water, 20 g/L molasses, 7.5 g/L urea, 3 g/L NaNO₃, 5 g/L NH₄NO₃, 1 g/L KH₂PO₄, and 0.7 g/L MgSO₄·7H₂O, with shaking, and the pH of the media was adjusted by adding 0.1% HCl or NaOH until the pH reached 5.5. The mixture was divided by 5, and each division (1000 mL) was incorporated into an Erlenmeyer flask (2.5 L), covered with rubber and sterilized in an autoclave at 121 °C for 15 min. After cooling, 1 Erlenmeyer flask was inoculated with 10 mL liquid medium containing 10⁶ spores of S. cerevisiae/mL, 1 Erlenmeyer flask was inoculated with 10 mL liquid medium containing 10⁶ spores of C. tropicalis/mL, 1 Erlenmeyer flask was inoculated with 5 mL liquid medium containing 10⁶ spores S. cerevisiae/mL and 5 mL liquid medium containing 10⁶ spores of C. tropicalis/mL, and 1 Erlenmeyer flask was not inoculated with yeast. Each inoculated medium was divided into 5 portions and incubated for 7 days at 28-30 °C at a relative humidity of 60-70% in the dark. After fermentation, each division was evaporated until it was thick. The viscous part was dried at 60 °C to a constant weight. After fermentation, each division was evaporated until it was thick. The viscous part was dried in drying cabinet (Memmert, Brutschrank, Germany) at 60 °C to a constant weight. The unfermented crude hydrolysate of rice husk was also evaporated and dried at 60 °C to a constant weight.

Determination of crude protein

Determination of the crude protein of the CRHH and DDG rice husks was conducted using the Kjeldahl method (AOAC, 1990). One gram of sample was inserted into the Kjeldahl flask, and 10 g K₂SO₄, 0.7 g HgO and 20 mL sulphuric acid 90% were added. The Kjeldahl flask was paired with a digester and boiled until the mixture was clear, and the heating continued for 30 min. The formation of too much foam was prevented by the addition of paraffin oil. After cooling, distilled water was gradually added until the volume reached 90 mL, and then 25 mL sulphuric acid, glass beads and 80 mL sodium hydroxide 40% solution were added and stirred until two layers formed. The Kjeldahl flask was quickly discharged into the distillation unit and heated, and 50 mL distillate was placed in an Erlenmeyer flask containing 50 mL indicator solution. The distillate mixture was subjected to a standard chlorhydric acid solution until colour changes occurred.

Determination of amino acids

Amino acids in the CRHH and DDG rice husks were determined using high-performance liquid chromatography (Shimadzu Corporation, Kyoto, Japan). Precisely, 25 mg amino acid standards were dissolved in 0.01 M HCl, and tyrosine was dissolved in phosphate buffer (pH = 7.0) until the volume reached 25 mL; then, the solution was diluted again to reach 50 nmol/mL for post-column (Phenomenex, St. Torrance, CA, USA) derivatization and to reach 0.5 nmol/mL for pre-column derivatization. All the solutions were filtered through a 0.45 µm nylon filter (Merck Millipore, Burlingtonong, Massachusetts, United States) and injected. A total of 15 mg of sample was hydrolysed with 25 mL HCl 6 N and fed into a hydrolysis tube, frozen with liquid nitrogen, exposed to air, and sealed and heated at 110 °C for 6 h. After cooling, the HCl was removed by evaporation under infrared light. The residue containing the amino acid was dissolved in 0.01 M HCl, and tyrosine was dissolved in a phosphate buffer solution (pH 7.0). All the solutions were filtered through a 0.45 µm nylon filter. The mobile phase for the amino acid analysis was a borate buffer solution (pH 10.4). The column derivatization was performed with tetrahydrofuran containing 5 M Na₂HPO₄ and 0.05 M Na-acetate at pH 7.5 using acetic acid. The pre-column derivatization was prepared using 50 mg o-phthalaldehyde (OPA) in 4.5 mL ethanol and 50 mL 2-mercaptoethanol solution. One mL of the sample solution

DOI: http://dx.doi.org/10.21161/mjm.180115

ISSN (print): 1823-8286, ISSN (online): 2231-7538

174
was mixed with 2 mL of the derivatization solution and passed through a SEP-PAK C18 cartridge.

**Determination of crude fat**

Determination of the crude fat of CRHH and DDG rice husks was conducted using the Soxhlet method (AOAC, 1990). Precisely 5 g of sample was inserted into the thimble, 1.5 g of sand was added, and the sand and the sample were mixed with a glass rod. The glass rod was wiped with a piece of cotton wool, and the cotton wool placed in the top of the thimble. The DDG sample was heated in an oven at 102 °C for 5 h, and the sample was allowed to cool in a desiccator. The piece of cotton wool was taken from the bottom of the beaker, placed in the top of the thimble, and the thimble was inserted in a Soxhlet liquid/solid extractor. Precisely, 150 mL of the solution in the round bottom flask was mixed with approximately 90 mL of petroleum spirit in the flask. The extraction unit was assembled over an electric heating mantle, and the solvent was heated to boiling in the flask. The heat source was adjusted so that the solvent from the condenser dripped into the sample chamber at a rate of approximately 6 drops per sec. The extraction was continued for 6 h. The extraction unit was removed from the heat source, and the extractor and condenser were detached. The flask was replaced on the heat source, and the solvent was evaporated off. The flask was placed in an oven at 102 °C, and the contents were dried to a constant weight (2 h). The flask was cooled in a desiccator, and the flask and its contents were weighed.

**Determination of the metabolic energy**

The Sibbald method (Sibbald, 1976) was used to determine the CRHH and DDG metabolic energy using 20 broiler chickens (Arbor acres strain), aged 36 days. All the chickens were individually reared in a metabolic cage, maintained and fed commercial feed for 24 h for the environmental adaptation process. The chickens were left for 24 h without feeding but were still given drinks to empty the digestive tract of the remains of the previous feed. After the rest period, 10 chickens were divided into 2 group of fed 120 g/head/day for 4 days CRHH and DDG, respectively. The collection of excreta was conducted every 24 h for 5 days during the treatment period. The five remaining chickens from each group were starved for 24 h to measure endogenous energy and nitrogen, but drinking water was given ad libitum. Endogenous excreta were collected once after swallowing for 48 h. During the collection, the excreta were sprayed with a low concentration of H2SO4 (0.01 N) to allow nitrogen to bind and not to evaporate. The excreta samples were stored in the freezer for 24 h to prevent decomposition by microorganisms. For analysis, the excreta were removed from the freezer and the process of lubrication was performed. The diluted excreta were then put into a 60 °C oven for 24 h. The dry samples were then analysed for gross energy, crude protein, nitrogen and dry matter. The gross energy is the amount of heat liberated when a sample is totally oxidized in a bomb calorimeter containing oxygen at 25-30 atmospheric pressure (AOAC, 1995).

**Determination of reducing sugars**

The reducing sugars content in CRHH and DDG rice husks was analysed using the Nelson Somogyi method (Somogyi, 1952). Precisely, 10 g of the sample was mashed and dissolved in 250 mL distilled water. The solution was filtered, and the filtrate was centrifuged at 6000 rpm for 3 min. Arsenomolybdate reagent (1 mL) was added to the centrifuge and shaken until all the cuprous oxide was dissolved. The absorbance was measured at 540 nm with a spectrophotometer and compared to a standard curve of 0, 0.2, 0.4, 0.6, 0.8, and 1.0% of the glucose solution.

**Determination of crude fibre**

The crude fibre was analysed using the AOAC method (2005). Precisely, 3 g of the non-fat samples were put into 600 mL cup glasses, 50 mL 0.3 N H2SO4 was added, and the samples were heated on an electric heater for 30 min; then, 25 mL 1.5 N NaOH was added, and the heating continued for 30 min. The liquid was evaporated in an oven dryer at 105-110 °C for 1 h and placed into a Buchner funnel. Filtration was carried out into a suction flask connected to a vacuum pump. During filtration, the precipitate was washed successively with sufficiently heated aliquots of 50 mL 0.3 N H2SO4 and finally with 25 mL acetone. The filter paper and its contents were inserted into a porcelain dish and dried for 1 h at 105 °C and then cooled in an incubator and weighed. The porcelain cup and its contents were then burned or ignited in an electric furnace at a temperature of 400-600 °C until the ash was completely white. The sample was removed, cooled in a desiccator and weighed.

**Determination of ash**

The ash was analysed using the AOAC method (2005). The sample was dried in a drying oven at 105 °C for 1 h and cooled in a desiccator for 1 h. Precisely, 2 g of the sample was placed on a porcelain plate and inserted into an electric furnace that had been heated to 550 °C for 12 h. The porcelain grate containing the ash was placed into a dryer and heated at 105 °C for 1 h and then cooled in a desiccator and weighed.

**Determination of calcium**

The calcium was analysed using the AOAC method (2005). Precisely, 10.0 g of an ash sample was placed into a 300 mL glass beaker; then, 30 mL 96% HCl was added and heated on a hot plate until 1/3 remained, and 20 mL distilled water was added and boiled for 10 min. Once cooled to room temperature, the mixture was filtered and placed into a 200 mL volumetric flask. The filter paper was washed with water until 200 mL of the
filtrate was obtained. A 20 mL aliquot was pipetted into a 400 mL beaker, and 50 mL water was added. On a magnetic stirrer in a fume hood, 300 mg hydroxy naphtol blue indicator was added, and the pH was adjusted to 12.5±0.2 with KOH-KCN solution; then, 10 mL 0.02 M EDTA was added and mixed until the colour turned green. The solution was titrated with 0.02 M CaCO₃ to a permanent purple end point.

**Determination of phosphorus**

The phosphorus was analysed using the AOAC method (2005). Precisely, 10.0 g of the ash sample was placed into a 300 mL glass beaker, 30 mL of 96% HCl was added, and the sample was heated on a hot plate until 1/3 remained; then, 20 mL distilled water was added and boiled for 10 min. Once cooled to room temperature, the mixture was filtered and placed in a 200 mL volumetric flask. The filter paper was washed with water until 200 mL of the filtrate was obtained. A 0.5 mL aliquot of the sample solution was added to 4.5 mL of the molybdovanadate reagent, mixed and allowed to stand for 10 min. Aliquots of the working standard were transferred to 100 mL flasks containing 0.5, 0.8, 1.0 and 1.5 mg phosphorus. The absorbance was measured with a spectrophotometer at 400 nm and compared to a standard curve containing 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg phosphorus.

**Statistical analysis**

Tukey's test for significant differences of multiple comparisons and a paired samples t-test were used to identify significantly different treatment effects using SPSS 20 software. An analysis of variance (ANOVA) was performed to determine the differences between the experiments at a 5% level of significance (p>0.05).

**RESULTS**

All parameters of the CRHH and DDG rice husk nutritional components in this study are presented in Table 1. Some nutritional components including crude protein, crude fat, crude fibre, calcium and amino acids asparagine, lysine, isoleucine and glutamine of DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (2.83±0.07%) was significantly (p<0.05) higher than CRHH (2.21±0.06%). Yeast fermentation of the CRHH had a significant (p<0.05) effect on the content of some amino acids in DDG, as shown in Table 1. Asparagine in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (2.83±0.07%) was significantly (p<0.05) higher than CRHH (1.34±0.02%), but no significant (p>0.05) differences were seen in a monoculture *S. cerevisiae* (2.65±0.08%) or *C. tropicalis* (2.53±0.09%). A co-culture fermentation of *S. cerevisiae* with *C. tropicalis* increased the asparagine content of the rice husk feedstock by approximately 1.32%. Lysine in DDG from a co-culture *S. cerevisiae* with *C. tropicalis* (2.52±0.04%) was significantly (p<0.05) higher than CRHH (1.95±0.03%) or *C. tropicalis* (2.02±0.06%). A co-culture fermentation of *S. cerevisiae* with *C. tropicalis* increased the isoleucine content of the rice husk feedstock by approximately 1.34%. Glutamine in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (4.54±0.08%) was significantly (p<0.05) higher than CRHH (4.26±0.07%). However, no significant (p>0.05) differences were seen in the other amino acids except the fourth amino acid from a mono- and co-culture of *S. cerevisiae* with *C. tropicalis*.

**Crude fat**

Before fermentation, the crude fat content in CRHH is 2.35±0.25%. Yeast fermentation of the CRHH had a significant (p<0.05) effect on the DDG crude fat. The crude fat in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (6.85±0.73%) was significantly (p<0.05) higher than both a mono-culture of *S. cerevisiae* (4.33±0.49%) and CRHH, but no significant (p>0.05) differences were found in a mono-culture of *C. tropicalis* (5.55±1.48%). However, the crude fat in DDG from a mono-culture of *S. cerevisiae* was significantly higher than CRHH. A co-culture fermentation of *S. cerevisiae* with *C. tropicalis* increased the crude fat content in CRHH by approximately 4.50%.

**Amino acids**

Yeast fermentation of the CRHH had a significant (p<0.05) effect on the content of some amino acids in DDG, as shown in Table 1. Asparagine in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (2.83±0.07%) was significantly (p<0.05) higher than CRHH, but there were no significant (p>0.05) differences with a monoculture of *S. cerevisiae* (14.55±1.68%) or *C. tropicalis* (13.22±1.44%). However, both mono-culture fermentations with *S. cerevisiae* and *C. tropicalis* were significantly higher than CRHH.
Table 1: All parameters of the crude rice husk hydrolysate unfermented and distillers dried grain crude rice husk hydrolysate nutritional components; values and standard deviation (n = 5) with a superscript in same row were followed by Tukey’s test within the respective groups and indicate a significant difference a, b and c (p<0.05); values and standard deviation (n = 5) without a superscript in same row were followed by Tukey’s test within the respective groups and indicate a non-significant difference (p>0.05).

| Nutrient component | Crude rice husk hydrolysate unfermented | Mono-culture S. cerevisiae | Mono-culture C. tropicalis | Co-culture S. cerevisiae with C. tropicalis |
|---------------------|----------------------------------------|---------------------------|---------------------------|------------------------------------------|
| Crude protein (%)   | 10.51±1.24<sup>a</sup>                 | 14.55±1.68<sup>b</sup>   | 13.22±1.44<sup>b</sup>   | 14.89±1.23<sup>b</sup>                  |
| Amino acids         |                                        |                           |                           |                                          |
| Asparagine (%)      | 1.51±0.03<sup>a</sup>                 | 2.65±0.08<sup>b</sup>    | 2.53±0.09<sup>b</sup>    | 2.83±0.07<sup>b</sup>                  |
| Lysine (%)          | 1.34±0.04<sup>a</sup>                 | 2.48±0.02<sup>b</sup>    | 2.42±0.03<sup>b</sup>    | 2.52±0.05<sup>b</sup>                  |
| Histidine (%)       | 0.58±0.01                             | 0.68±0.03                | 0.73±0.06                | 0.64±0.07                              |
| Glycine (%)         | 1.12±0.03                             | 1.98±0.05                | 1.88±0.04                | 1.65±0.06                              |
| Alanine (%)         | 2.10±0.05                             | 2.25±0.08                | 2.06±0.07                | 2.30±0.04                              |
| Serine (%)          | 3.35±0.06                             | 3.95±0.05                | 3.91±0.08                | 3.88±0.03                              |
| Proline (%)         | 2.28±0.73                             | 2.35±0.68                | 2.43±0.82                | 2.85±0.69                              |
| Valine (%)          | 1.41±0.04                             | 1.27±0.07                | 1.34±0.05                | 1.35±0.03                              |
| Threonine (%)       | 1.22±0.07                             | 1.07±0.05                | 1.31±0.03                | 1.25±0.05                              |
| Isoleucine (%)      | 0.81±0.01<sup>a</sup>                 | 1.95±0.03<sup>b</sup>    | 2.02±0.06<sup>b</sup>    | 2.15±0.08<sup>b</sup>                  |
| Leucine (%)         | 3.22±0.06                             | 3.24±0.02                | 3.29±0.04                | 3.39±0.05                              |
| Methionine (%)      | 0.45±0.01                             | 0.52±0.02                | 0.47±0.03                | 0.54±0.04                              |
| Glutamine (%)       | 2.21±0.06<sup>a</sup>                 | 4.20±0.04<sup>b</sup>    | 4.26±0.07<sup>b</sup>    | 4.54±0.08<sup>b</sup>                  |
| Phenylalanine (%)   | 1.60±0.03                             | 1.62±0.04                | 1.59±0.06                | 1.63±0.06                              |
| Arginine (%)        | 0.96±0.04                             | 1.03±0.03                | 1.05±0.03                | 1.51±0.02                              |
| Tryptophan (%)      | 0.16±0.01                             | 0.17±0.02                | 0.18±0.01                | 0.21±0.03                              |
| Tyrosine (%)        | 0.84±0.02                             | 0.86±0.04                | 0.85±0.03                | 0.88±0.02                              |
| Cysteine (%)        | 0.37±0.01                             | 0.39±0.02                | 0.65±0.03                | 0.56±0.03                              |
| Crude fat (%)       | 2.35±0.25<sup>a</sup>                 | 4.33±0.49<sup>b</sup>    | 5.55±1.48<sup>b</sup>    | 6.85±0.73<sup>b</sup>                  |
| Metabolic energy (kcal/kg dry basis) | 2996.03±109.02<sup>b</sup> | 2630.08±124.93<sup>a</sup> | 2534.26±167.52<sup>a</sup> | 2469.93±165.72<sup>a</sup> |
| Reducing sugars (ppm/g) | 923.86±89.17<sup>b</sup> | 90.76±17.91<sup>b</sup> | 101.51±15.59<sup>a</sup> | 71.79±4.95<sup>a</sup> |
| Crude fibre (%)     | 19.13±0.91<sup>a</sup>                | 21.91±2.12<sup>b</sup>   | 23.09±2.59<sup>b</sup>   | 22.60±3.01<sup>b</sup>                 |
| Ash (%)             | 12.37±0.42<sup>a</sup>                | 15.95±0.15<sup>b</sup>   | 15.24±0.82<sup>b</sup>   | 15.19±0.78<sup>b</sup>                 |
| Calcium (%)         | 0.83±0.13<sup>a</sup>                 | 0.86±0.08<sup>b</sup>    | 0.91±0.11<sup>b</sup>    | 1.09±0.05<sup>b</sup>                  |
| Phosphorus (%)      | 0.79±0.09                             | 0.75±0.04                | 0.79±0.06                | 0.92±0.02                              |

Metabolic energy

Before fermentation, the metabolic energy content in CRHH is (2996.03±109.02 kcal/kg dry basis). Yeast fermentation of the CRHH had a significant (p<0.05) effect on the DDG metabolic energy. The metabolic energy of DDG from a co-culture of S. cerevisiae with C. tropicalis (2469.93±165.27 kcal/kg dry basis), a mono-culture of S. cerevisiae (2630.08±124.93 kcal/kg dry basis), and a mono-culture of C. tropicalis (2534.26±167.52 kcal/kg dry basis) was significantly (p<0.05) lower than the CRHH. However, no significant (p>0.05) differences were seen with mono- and co-cultures of S. cerevisiae with C. tropicalis. A mono-culture of S. cerevisiae, a mono-culture of C. tropicalis and a co-culture fermentation of S. cerevisiae with C. tropicalis decreased the metabolic energy by approximately 12.21%, 15.4%, and 17.56%, respectively.

Reducing sugars

Before fermentation, the reducing sugars content in CRHH is 923.86±89.17 ppm/g. Yeast fermentation of the CRHH had a significant (p<0.05) effect on the DDG reducing sugars. The reducing sugars in DDG from the co-culture of S. cerevisiae with C. tropicalis (71.79±4.95 ppm/g), a mono-culture of S. cerevisiae (90.76±17.91 ppm/g) and a mono-culture of C. tropicalis (101.51±15.59 ppm/g) were significantly (p<0.05) lower than CRHH. However, no significant (p>0.05) differences in mono- and co-cultures of S. cerevisiae with C. tropicalis were found. A mono-culture of S. cerevisiae, a mono-culture of C.
tropicalis and a co-culture fermentation of S. cerevisiae with C. tropicalis decreased the reducing sugars by approximately 90.18%, 89.01% and 92.23%, respectively.

**Crude fibre**

Before fermentation, the crude fibre content in CRHH is 19.13±0.91%. Yeast fermentation of the CRHH had a significant (p<0.05) effect on the crude fibre in DDG. The crude fibre in DDG from a co-culture of S. cerevisiae with C. tropicalis (22.60±3.01%), a mono-culture of S. cerevisiae (21.91±2.12%) and a mono-culture of C. tropicalis (23.09±2.59%) were significantly (p<0.05) higher than CRHH (19.13±0.91%). However, no significant (p>0.05) differences in mono- and co-cultures of S. cerevisiae with C. tropicalis were seen. A mono-culture of S. cerevisiae, a mono-culture of C. tropicalis and a co-culture fermentation of S. cerevisiae with C. tropicalis increased the crude fibre by approximately 14.53%, 20.70% and 18.14%, respectively.

**Ash**

Before fermentation, the ash content in DDG from a co-culture of S. cerevisiae with C. tropicalis (15.95±0.15%), a mono-culture of S. cerevisiae (15.24±0.82%) and a mono-culture of C. tropicalis (15.24±0.82%) was significantly (p<0.05) higher than CRHH (14.53%, 20.70% and 18.14%, respectively). Yeast fermentation of S. cerevisiae increased the ash content of DDG rice husk by approximately 38.57%, 25.90% and 41.81%, respectively. The increased ash content was mainly due to the contribution of the yeast cell mass. During fermentation, S. cerevisiae and C. tropicalis grow and convert fermentable sugars (i.e., glucose, xylose and arabinose) in the rice husk hydrolysate to ethanol, carbon dioxide and other compounds (Sopandi and Wardah, 2016). Yeast growth produces a cell mass that contains a much higher amount of protein (Russell, 2003). The proteins and other components are produced at the time of yeast cell autolysis, followed by cell death, intracellular proteolysis and other enzymatic activities (Han and Liu, 2010). Several investigators have reported variations in the protein content of an S. cerevisiae and C. tropicalis biomass. Onofre et al. (2017) reported that the crude protein content of a biomass S. cerevisiae from the beer manufacturing process was 49.63%. Aruna et al. (2017) reported that the crude protein content from a yeast fermentation by S. cerevisiae (BY4743) increased from 6.60% to 15.54%. Darwish et al. (2012) reported that the crude protein content from corn stalk fermented by P. ostreatus and S. cerevisiae increased from 3.60% to 11.80%. Golaghaiee et al. (2017) reported that the protein content of C. tropicalis biomass from media containing ammonium sulphate, iron sulphate, glycine and glucose was 52.16%.

**DISCUSSION**

The present study indicates that the crude protein, crude fat, crude fibre, ash, calcium and some amino acids, such as asparagine, lysine, isoleucine and glutamine, from mono- and co-culture fermentations of S. cerevisiae and C. tropicalis are higher than the CRHH. Fermented monocultures of S. cerevisiae, mono-cultures of C. tropicalis and co-cultures of S. cerevisiae with C. tropicalis increased the crude protein content of DDG rice husk by approximately 38.57%, 25.90% and 41.81%, respectively. The increased protein content was mainly due to the contribution of the yeast cell mass. During fermentation, S. cerevisiae and C. tropicalis grow and convert fermentable sugars (i.e., glucose, xylose and arabinose) in the rice husk hydrolysate to ethanol, carbon dioxide and other compounds (Sopandi and Wardah, 2016). Yeast growth produces a cell mass that contains a much higher amount of protein (Russell, 2003). The proteins and other components are produced at the time of yeast cell autolysis, followed by cell death, intracellular proteolysis and other enzymatic activities (Han and Liu, 2010). Several investigators have reported variations in the protein content of an S. cerevisiae and C. tropicalis biomass. Onofre et al. (2017) reported that the crude protein content of a biomass S. cerevisiae from the beer manufacturing process was 49.63%. Aruna et al. (2017) reported that the crude protein content from a yeast fermentation by S. cerevisiae (BY4743) increased from 6.60% to 15.54%. Darwish et al. (2012) reported that the crude protein content from corn stalk fermented by P. ostreatus and S. cerevisiae increased from 3.60% to 11.80%. Golaghaiee et al. (2017) reported that the protein content of C. tropicalis biomass from media containing ammonium sulphate, iron sulphate, glycine and glucose was 52.16%.

**Calcium**

Before fermentation, the calcium content in CRHH is 0.83±0.13%. Yeast fermentation of the CRHH had a significant (p<0.05) effect on calcium in DDG. Calcium in DDG from a co-culture of S. cerevisiae with C. tropicalis (1.09±0.05%) was significantly (p<0.05) higher than in a mono-culture of S. cerevisiae (0.86±0.08%), a mono-culture of C. tropicalis (0.91±0.11%) and CRHH. However, no significant (p>0.05) differences in a mono-culture of S. cerevisiae, a mono-culture of C. tropicalis and a non-fermentation culture were seen. A co-culture fermentation of S. cerevisiae with C. tropicalis increased the calcium content of the CRHH by approximately 0.26%.

**Phosphorus**

Before fermentation, the phosphorus content in CRHH is 0.79±0.09%. Yeast fermentation of the CRHH had no significant (p>0.05) effect on the phosphorus in DDG. The phosphorus in DDG from a co-culture of S. cerevisiae with C. tropicalis (0.92±0.02%) was not significantly (p>0.05) different from a mono-culture of S. cerevisiae (0.75±0.04%), a mono-culture of C. tropicalis (0.79±0.06%) or the CRHH.
of C. tropicalis cultivated in hydrolysed wheat bran, oat bran and rice husk media was poor in methionine and cysteine. Onofre et al. (2017) also reported that cysteine and tryptophan were at the lowest concentrations of the amino acids in a biomass of S. cerevisiae cultivated in a beer manufacturing process.

The increase in the crude fat content in DDG rice husks was thought to be due to the growth of yeast in the CRHH medium. The increased crude fat content was also due to the bioconversion of fermentable sugars (i.e., glucose, xylose, and arabinose) to fat in the CRHH. Several investigators have reported variations in the protein content of S. cerevisiae and C. tropicalis. Onofre et al. (2017) reported that the total fat content of a biomass S. cerevisiae from the beer manufacturing process was 4.64%. Aruna et al. (2017) reported that the crude fat content in yam peels fermented by S. cerevisiae (BY4743) increased from 1.12% to 2.09%. Siddique et al. (2016) reported that the fat content of a biomass of C. tropicalis SS1 cultured in agricultural waste was 5.08%. The crude fat content in DDG in the present study was lower than that of the DDG crude fat (8.8-12.4%) content reported by the US Grain Council (2012). Martinez-Amezgua (2007) reported that DDGS (distillers dried grain with solubles) from corn contained 9.75% crude fat. Hassan and Al-Aqil (2015) reported that the crude fat in DDGS corn ranged from 2.0-14.1%.

The increase in the crude fibre content in the DDG rice husks was thought to be due to the crude fibre content in the yeast cell biomass. Siddique et al. (2016) reported that the crude fibre content of biomass C. tropicalis SS1 from an aqueous extract of watermelon peel medium was 2.43%. Onofre et al. (2017) reported that the soluble fibre and insoluble fibre content of a biomass of S. cerevisiae from the beer manufacturing process were 9.12% and 2.87%, respectively.

The present study showed that the ash contents of DDG rice husk from mono- and co-cultures of S. cerevisiae with C. tropicalis were significantly higher than CRHH. The increase in the ash content was thought to be due to the contribution of the ash content in the yeast cell biomass. Yeast is considered an excellent source of selenium and chromium (Levander, 1989; Onofre et al., 2017). Onofre et al. (2017) also reported that the ash content of a biomass of S. cerevisiae from the beer manufacturing process was 7.96%.

The present study showed that the calcium content of DDG rice husk from mono- and co-cultures of S. cerevisiae with C. tropicalis were significantly higher than CRHH. Calcium in DDG rice husk from mono- or co-cultures of S. cerevisiae with C. tropicalis at 0.86-1.09%. The increase in the calcium content was thought to be due to the contribution of the calcium content in the yeast cell mass. Onofre et al. (2017) reported that the calcium content of a biomass of S. cerevisiae from the beer manufacturing process was 0.87 mg/100 g. Aruna et al. (2017) reported that the cassava ash content of fermented S. cerevisiae (BY4743) increased from 4.45% to 8.02%.

The present study shows that fermentation by mono- and co-cultures of S. cerevisiae with C. tropicalis causes a decrease in metabolic energy and reducing sugars in DDG rice husk ranging from 12.21-17.56% and 89.01-92.23%, respectively. The decrease in metabolic energy and reducing sugars is due to the consumption of sugars by S. cerevisiae and C. tropicalis, as carbon and energy sources for growth and these compounds are converted to ethanol during fermentation. Liu (2011) reported that starch will be converted by fermentation into sugars, then to ethanol and, finally, to carbon dioxide. Sopandi and Wardah (2015) reported that S. cerevisiae and C. tropicalis can grow, consume glucose, xylose and arabinose and produce ethanol from a rice husk hydrolysate medium.

The present study showed no significant differences in the phosphorus content in DDG rice husk from mono- and co-cultures of S. cerevisiae with C. tropicalis and those with no fermentation. These results indicate that the phosphorus content in DDG rice husk is derived from the rice feedstock and that there is no contribution from the yeast cell biomass. However, the DDG phosphorus (0.75-0.93%) content in this study is similar to that of the DDG phosphorus content reported by some investigators. Martinez-Amezgua (2007) reported that DDGS from corn contained 0.72 phosphorus. Deniz et al. (2013) reported that the phosphorus content of DDGS in corn ranged from 0.39-1.17%.

The present study indicates that the nutritional composition of DDG is different than that reported by previous investigators. Salim et al. (2010) reported that DDGS composition of corn consisted of crude protein (27.15%), fat (10.67%), fibre (6.21%), ash (4.54%), calcium (0.04%), and phosphorus (0.76%). Rahman et al. (2013) reported that the DDG composition of rice fermented by Aspergillus oryzae consisted of crude protein (21.5%), crude fat (4.5%), ash (0.9%), arginine (5.9%), histidine (2.4%), isoleucine (4.0%), leucine (8.2%), lysine (3.2%), threonine (4.4%) and valine (4.9%). Bae et al., (2015) reported that the DDG composition of rice fermented by A. oryzae consisted of crude protein (30.4%), crude fat (3.3%), ash (0.8%), arginine (4.1%), histidine (1.3%), isoleucine (3.5%), Leucine (7.5%), lysine (2.1%), threonine (4.1%) and valine (5.2%). The differences in DDG composition are thought to be due to the yeast species, the fermentation methods and the feedstock. Salim et al. (2010) suggests that the DDG nutritional composition varies depending on the diversity and quality of bioethanol feedstock production. Liu (2011) reported that variations in the DDG nutritional composition are influenced by differences in the main processes of fermentation, including the type and composition of the feedstock, the parameters and method used for the fermentation process, the amount of soluble compounds added to the distilled wet grains, the yeast fermentation effect and the analytical methodology.
CONCLUSION
Some nutrient components of the DDG crude rice husk hydrolysate performed higher than the non-fermentation of rice husks. The increased in some it components were mainly due to the contribution of the S. cerevisiae and C. tropicalis cell mass. The decrease in metabolic energy and reducing sugars was due to the consumption of sugars by S. cerevisiae and C. tropicalis, as carbon and energy sources for growth during fermentation.

ACKNOWLEDGEMENTS
This study was supported in part by a Competency Research Grant from Direktorat Riset dan Pengabdian Masyarakat. Kementerian Riset, Teknologi dan Pendidikan Tinggi, Indonesia.

CONFLICT OF INTEREST
No conflict of interest declared. All authors read and approved the final version of the manuscript.

REFERENCES
AOAC. (1990). Official methods of analysis. Association of Official Analytical Chemist, Washington D.C.
AOAC. (1995). Official methods of analysis. 16th Edn. Association of Official Analytical Chemist, Washington D.C.
AOAC. (2005). Official Methods of Analysis Association of Official Analytical Chemistry, Association of Analytical Chemists. 18th Edn. AOAC International. Maryland. USA.
Aruna, T. E., Awoh, O. C., Raji, A. O. and Olagunju, A. I. (2017). Protein enrichment of yam peels by fermentation with Saccharomyces cerevisiae (BY4743). Annals of Agricultural Sciences 62(1), 33-37.
Bae, K. M., Kim, K. W and Lee, S. M. (2015). Evaluation of rice distillers dried grain as a partial replacement for fish meal in the practical diet of the juvenile olive flounder Paralichthys olivaceus. Fisheries and aquatic sciences 18(2), 151-158.
Chum, H. L., Warner, E., Seabra, J. E. A. and Macedo, I. C. (2013). A comparison of commercial ethanol production systems from Brazilian sugarcane and US corn. Biofuels Bioproducts and Biorefining 8(2), 145-293.
Darwish, G. A. M. A., Bakr, A. A. and Abdallah, M. M. F. (2012). Nutritional value upgrading of maize stalk by using Pleurotus ostreatus and Saccharomyces cerevisiae in solid state fermentation. Annals of Agricultural Sciences 57(1), 47-51.
Deniz, G., Gencoglu, H., Gezen, S. S., Turkmen, I. I., Orman, A. and Kara, C. (2013). Effects of feeding corn distiller's dried grains with solubles with and without enzyme cocktail supplementation to laying hens on performance, egg quality, selected manure parameters and feed cost. Livestock Science 152 (2-3), 174-181.
Dimova, N. D., Iovkova, Z. S., Brinkova, M. and Godjevargova, Ts. I. (2014). Production of Candida biomass from hydrolysed agricultural biowaste. Biotechnology Biotechnological Equipment 24(1), 1577-1581.
Ezzat, M. A. E., Alagawany, M., Farag, M. R and Dhama, K. (2015). Use of maize distiller’s dried grains with solubles (DDGS) in laying hen diets: Trends and advances. Asian Journal of Animal and Veterinary Advances 10 (11), 650-707.
Golaghaiee, S., Ardestani, F. and Ghorbani, H. R. (2017). Microbial protein production from Candida tropicalis ATCC13803 in a submerged batch fermentation process. Applied Food Biotechnology 4(1), 35-42.
Han, J. and Liu, K. (2010). Changes in composition and amino acid profile during dry grind ethanol processing from corn and estimation of yeast contribution toward ddgs proteins. Journal of Agricultural and Food Chemistry 58(6), 3430-3437.
Hassan, S. M. and Al Aqil, A. A. (2015). Effect of adding different dietary levels of distillers dried grains with solubles (DDGS) on productive performance of broiler chicks. International Journal of Poultry Science 14 (1), 13-18.
Irfan, M., Nadeem, M. and Syed, Q. (2014). Ethanol production from agricultural wastes using Saccharomyces cerevisiae. Brazilian Journal of Microbiology 45(2), 457-465.
Levander, O. A. (1989). Selenium, chromium and manganese. In: Modern Nutrition in Health and Disease. 7th Edn. Shlis, M. D. and Vernon, R. Y. Lea and Febger (eds.). Philadelphia. pp. 263-267.
Liu, K. (2011). Chemical composition of distiller’s grains a review. Journal of Agricultural and Food Chemistry 59(5),1508-1526.
Martinez-Anezca, C., Parsons, C. M., Singh, V., Srinivasan, R. and Murthy, G. S. (2007). Nutritional characteristics of corn distillers dried grains with solubles as affected by the amounts of grains versus solubles and different processing techniques. Poultry Science 86 (12), 2624-2630.
Onofre, S. B., Bertoldo, I. C., Abatti, D. and Refosco, D. (2017). Chemical composition of the biomass of Saccharomyces cerevisiae - (Meyen ex E. C. Hansen, 1883) yeast obtained from the beer manufacturing process. International Journal of Environment, Agriculture and Biotechnology 2(2), 558-562.
Purdum, S., Hanford, K. and Kreifels, B. (2014). Short-term effects of lower oil dried distillers grains with solubles in laying hen rations. Poultry Science 93(1), 2592-2595.
Rahman, Md. M., Choi, J. and Lee, S.M. (2013). Use of distillers dried grain as partial replacement of wheat flour and corn gluten meal in the diet of juvenile black seabream (Acanthopagrus schlegeli), Turkish Journal of Fisheries and Aquatic Sciences 13, 699-706.
Russell, I. (2003). Understanding yeast fundamentals. In: The Alcohol Textbook-A Reference for The Beverage, Fuel and Industrial Alcohol Industries. Jacques, K. A., Lyons, T. P. and Kelsall, D. R. (eds). Nottingham, UK. pp. 85-119.

Salim, H. M., Kruk, Z. A. and Lee, B. D. (2010). Nutritive value of corn distillers dried grains with solubles as an ingredient of poultry diets: A review. World's Poultry Science Journal 66(3), 411-431.

Sibbald, I. R. (1976). A bioassay for true metabolizable energy in feedstuffs. Poultry Science 55, 303-308.

Siddique, S, Shakir, H. A., Qazi, J. I., Tabinda, A. B. and Irfan, M. (2016). Screening of some agri-wastes for economical cultivation of Candida tropicalis SS1. Punjab University Journal of Zoology 31(1), 031-037.

Somogyi, M. (1952). Notes on sugar determination. Journal of Biological Chemistry 195(1), 19-23.

Sopandi, T. and Wardah, A. (2015). Sugar consumption in mono and co-culture Saccharomyces cerevisiae and others selected microorganism for bioethanol production from stream rice husk medium. Asian Journal of Microbiology, Biotechnology and Environmental Sciences 17(3), 577-586.

Sopandi, T. and Wardah, A. (2016). Improving ethanol production by co-culturing of Saccharomyces cerevisiae with Candida tropicalis from rice husk hydrolysate media. African Journal of Microbiology Research 11(3), 65-74.

Sopandi, T. and Wardah, A. (2017). Ethanol production and sugar consumption of co-culture Saccharomyces cerevisiae FNCC 3012 with Candida tropicalis FNCC 3033 in media containing inhibitor fermentation. Journal of Microbiology, Biotechnology and Food Sciences 7(2), 160-167.

US Grains Council. (2012). A guide to distiller’s dried grains with solubles (DDGS). 3rd Edn. http://www.grains.org/images/stories/DDGS_user_handbook/2012/Complete_2012_DDGS_Handbook.pdf. [Retrieved on March 28, 2018]

Wadhwa, M. and Bakshi, M. P. S. (2016). Application of waste-derived proteins in the animal feed industry. In: Protein Byproducts: Transformation from Environmental Burden into Value-Added Products. Academic Press. pp. 161-192.

Wangpor, J., Prayoonyong, P., Sakdaronnarong, C., Sungpet, A. and Jonglertjunya, W. (2017). Bioethanol production from cassava starch by enzymatic hydrolysis, fermentation and ex-situ nanofiltration. Energy Procedia 138, 883-888.