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Nutrient compositions of distillers dried grain from rice husks with co-culture fermentation of *Saccharomyces cerevisiae* with *Candida tropicalis*

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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 *Saccharomyces cerevisiae* with *Candida tropicalis* difference from unfermented crude rice husk hydrolysate and mono-cultured *S. cerevisiae* or *C. tropicalis*.
Methodology and results: The effects of mono- and co-cultures *S. cerevisiae* with *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 *S. cerevisiae* with *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 *S. cerevisiae* and *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, *Saccharomyces cerevisiae, 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; Wangpor *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 *Saccharomyces cerevisiae* co-culture with *Candida tropicalis* can produce bioethanol from rice husks (Sopandi and Wardah, 2015). Co-cultures of the *S. cerevisiae* with *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 *S. cerevisiae* with *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 *S. cerevisiae* with *C. tropicalis* difference from unfermented crude rice husk hydrolysate and mono-cultured *S. cerevisiae* or *C.tropicalis*.

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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% H_2SO_4 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 1.7×10^6 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 $NaNO_3$, 5 g/L NH_4NO_3, 1 g/L KH_3PO_4, and 0.7 g/L $MgSO_4 \cdot 7H_2O$, 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 10^6 spores of *S. cerevisiae/*mL, 1 Erlenmeyer flask was inoculated with 10 mL liquid medium containing 10^6 spores of *C. tropicalis/*mL, 1 Erlenmeyer flask was inoculated with 5 mL liquid medium containing 10^6 spores *S. cerevisiae/*mL and 5 mL liquid medium containing 10^6 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 hydrolyzate 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 K_2SO_4, 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 (Pnenomenex, 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, Burlingtong, 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 Na_2HPO_4 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

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was mixed with 2 mL of the derivatization solution and passed through a SEP-PAK C1s 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 determinate 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 H_2S0_4 (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 centrifugate 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 H_2SO_4 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 H_2SO_4 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% HCI 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

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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 naphthol 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 CaCOs 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% HCI 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 rice husk were significantly (p<0.05) higher than CRHH. However, no significant (p>0.05) differences in mono- and co-cultures of *S. cerevisiae* with *C. tropicalis* were found. In this study, the metabolic energy and reducing sugars of DDG rice husk were significantly (p<0.05) lower than CRHH. This study also shows that the content of 14 amino acids does not have a significant difference between DDG of rice husk and CRHH.

Crude protein

Before fermentation, the crude protein content in CRHH is 10.51±1.24%. Yeast fermentation of the CRHH had a significant (p<0.05) effect on the DDG crude protein. The DDG crude protein from a co-culture *S. cerevisiae* with *C. tropicalis* (14.89±1.23%) 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.

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 (1.51±0.03%), 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 (1.34±0.02%), but no significant (p>0.05) differences were found in a monoculture *S. cerevisiae* (2.48±0.02%) or *C. tropicalis* (2.42±0.03%). A co-culture fermentation of *S. cerevisiae* with *C. tropicalis* increased approximately 1.18% of the asparagine content of the rice husk feedstock. Isoleucine in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (2.15±0.08%) was significantly (p<0.05) higher than CRHH (0.81±0.01%), but no significant (p>0.05) differences were found in a monoculture of *S. cerevisiae* (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 (2.21±0.06%), but no significant (p>0.05) differences were seen in a monoculture of *S. cerevisiae* (4.20±0.04%) or *C. tropicalis* (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%.

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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	Distillers dried grain crude rice husk hydrolysate		
		Mono-culture *S. cerevisiae*	Mono-culture *C. tropicalis*	Co-culture *S. cerevisiae* with *C. tropicalis*
Crude protein (%)	10.51±1.24[a]	14.55±1.68[b]	13.22±1.44[b]	14.89±1.23[b]
Amino acids				
Asparagine (%)	1.51±0.03[a]	2.65±0.08[b]	2.53±0.09[b]	2.83±0.07[b]
Lysine (%)	1.34±0.04[a]	2.48±0.02[b]	2.42±0.03[b]	2.52±0.05[b]
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[a]	1.95±0.03[b]	2.02±0.06[b]	2.15±0.08[b]
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[a]	4.20±0.04[b]	4.26±0.07[b]	4.54±0.08[b]
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[a]	4.33±0.49[b]	5.55±1.48[bc]	6.85±0.73[c]
Metabolic energy (kcal/kg dry basis)	2996.03±109.02[b]	2630.08±124.93[a]	2534.26±167.52[a]	2469.93±165.27[a]
Reducing sugars (ppm/g)	923.86±89.17[b]	90.76±17.91[a]	101.51±15.59[a]	71.79±4.95[a]
Crude fibre (%)	19.13±0.91[a]	21.91±2.12[b]	23.09±2.59[b]	22.60±3.01[b]
Ash (%)	12.37±0.42[a]	15.95±0.15[b]	15.24±0.82[b]	15.19±0.78[b]
Calcium (%)	0.83±0.13[a]	0.86±0.08[b]	0.91±0.11[b]	1.09±0.05[b]
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.*

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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 fiber 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 CRHH is 12.37±0.42%. Yeast fermentation of the CRHH had a significant (p<0.05) effect on the ash content in DDG. The ash content in DDG from a co-culture of *S. cerevisiae* with *C. tropicalis* (15.19±0.78%), a mono-culture of *S. cerevisiae* (15.95±0.15%), and a mono-culture of *C. tropicalis* (15.24±0.82%) was significantly (p<0.05) higher than CRHH. However, no significant (p>0·05) differences in the mono- and co-cultures of *S. cerevisiae* with *C. tropicalis* were seen. A co-culture fermentation of *S. cerevisiae* with *C. tropicalis* increased the ash content of CRHH by approximately 2.82%.

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.

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 mono-cultures 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 yam peel 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%.

The present study indicates that fermented mono- and co-cultures of *C. tropicalis* with *C. tropicalis* increased the asparagine, lysine, isoleucine and glutamine contents of DDG rice husk in a range from 67.55% to 87.41%, 80.60% to 88.06%, 140.74% to 162.96%, and 90.05% to 105.43%, respectively, compared to CRHH. The content of certain amino acids increased, especially lysine, isoleucine, asparagine and glutamine, due to the amino acid contribution of the yeast cell mass. Dimova *et al*. (2014) reported that a biomass *C. tropicalis* cultivated in hydrolysed wheat bran, oat bran and rice husk media was rich in lysine, isoleucine, glutamic acid and aspartic acid. Onofre *et al*. (2017) reported that glutamine and asparagine were the highest among amino acids in a biomass of *S. cerevisiae* cultivated in a beer manufacturing process.

The present study shows that CRHH and DDG rice husk contain three amino acids, methionine (0.45-0.54%), cysteine (0.37-0.65%), and tryptophan (0.21-0.16%), at the lowest concentrations compared to the other amino acids. Dimova *et al*. (2014) also reported that a biomass

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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-Amezcua (2007) reported that DDGS (distillers dried grain with soluble) 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 7.98%.

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-Amezcua (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.

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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.

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CONFLICT OF INTEREST

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

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