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ORIGINAL ARTICLE

Effect of fermentation using *Chrysonillia crassa* and *Monascus purpureus* on nutritional quality, antioxidant, and antimicrobial activities of used rice as a poultry feed ingredient

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ABSTRACT

Objective: This study was aimed at evaluating the effect of fermentation using *Chrysonillia crassa* and *Monascus purpureus* on nutritional qualities, antioxidant, and antimicrobial activities of the used rice as a poultry feed ingredient.

Materials and Methods: The used rice was soaked, steamed, and spread on a tray to cool. Suspension of *M. purpureus* or *C. crassa* was inoculated on the steamed used rice, and then mixed thoroughly. Afterward, the mixture was spread out on the tray, which was then covered with an aluminum foil. It was aerobically incubated for 7 and 4 days for the *M. purpureus*- and *C. crassa*-inoculated used rice, respectively. Subsequent to sun drying, the fermented used rice was grounded and analyzed.

Results: Crude protein and ash contents were higher ($p < 0.05$) in the used rice fermented with *C. crassa* or *M. purpureus* than in the unfermented. Conversely, carbohydrate content was lower ($p < 0.05$) in the fermented compared with the unfermented. Gross energy and energy from fat were higher ($p < 0.05$) in the used rice fermented with *M. purpureus* than the unfermented. Amino acids L-methionine, L-serine, L-glutamic acid, L-valine glycine, L-leucine, L proline, L-threonine, L-histidine, and L-Sistine were higher ($p < 0.05$) in *M. purpureus*-fermented used rice than in *C. crassa*-fermented and the unfermented used rice. However, amino acids L-isoleucine, L- alanine, L-lysine, and L-tryptophan were higher ($p < 0.05$) in the used rice fermented with both *C. crassa* and *M. purpureus*, compared with the unfermented. L-tyrosine content was higher ($p < 0.05$) in *M. purpureus*-fermented used rice than in the unfermented. Furthermore, the antioxidant activities of the fermented products were higher ($p < 0.05$) than that of the unfermented. In addition, the antimicrobial activities of the fermented products against *Staphylococcus aureus* were higher ($p < 0.05$) than that of the unfermented used rice.

Conclusion: In conclusion, the used rice fermented using *C. crassa* and *M. purpureus* improved the nutritional quality, as well as the antioxidant and antimicrobial activities of the products.

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Introduction

Feed represents about 70% of poultry production cost, and as a result, any increase in feed cost may consequently reduce the profit margin of farmers. In order to cut down this cost, many farmers are now exploring alternative cheap feedstuffs to be included in the poultry rations. Among the alternatives, used rice, which is one of the restaurant and household wastes, seems to be a good candidate. In Indonesia, it is abundantly available and the price is quite cheap [4]. Traditionally, such stuff has been fed to Indonesian endogenous chickens after being sun-dried. Apart from its availability, its nutritional quality seems to be much lower than original rice. When compared with original rice, the reducing sugar in the used rice was higher (0.48% *vs.* 0.39%), while protein content was lower (6.98% *vs.* 4.25%) [4]. Moreover, carbohydrate content was lower in the used rice compared with the original (8.31% *vs.* 10.7%) [3]. Taken together, it is, therefore,

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necessary to improve the nutritional quality of the used rice in order to be eligible as a poultry feed ingredient.

Fermentation is one of the simple methods used in improving the nutritional properties of materials. Several types of microorganisms may be exploited for the fermentation of the organic materials, one of which is fungus. Such microbe was capable of, respectively, decreasing and increasing the fiber and protein contents of the substrates [8], which may accordingly be beneficial for the poultry. In a previous study, we have identified and isolated the fungus *Chrysonillia crassa* from the gastrointestinal tract of the Indonesian endogenous chickens, and it exhibited probiotic activities *in vitro* [22,23]. The fungus was also reported to be able to decrease the fiber, and increase the protein content of cassava pulp in the study of Sugiharto et al. [18]. In light with this, it is, therefore, interesting to use *C. Crassa* as a fermentation starter in order to improve the nutritional and functional properties of the used rice.

Another fungus that may be used to ferment the used rice is *Monascus purpureus*. It has traditionally been used in the production of "angkak," a traditional medicine, which is made from fermented rice. The fungus has the ability to produce the natural red pigment as a secondary metabolite which includes isoflavones, edible pigments, fatty acids, dimerumic acid (antioxidant), enzymes, organic acids, monacolin K (lovastatin, anti-hypercholesterolemic agent), γ-aminobutyric acid (GABA, hypotensive agent), and vitamins [9]. In addition, the red pigment has also been proven to be antimicrobial to bacteria *Escherichia coli* and *Bacillus subtilis* [16].

It was previously documented that some fungi may act as antioxidant and antimicrobial agents. Sugiharto et al. [17] gave an example that the fungus *Acremonium charticola* may be a good source of these agents. Owing to this fact, it is, therefore, tempting to expect fungal fermentation to not only produce fermented products with improved nutritional characteristic, but also with functional properties such as antioxidant and antimicrobials. To the best of our knowledge, data documenting the fungal fermentation on used rice has been scarce so far. Hence, this present study is aimed at evaluating the effect of *C. crassa* or *M. purpureus* fermentation on nutritional qualities, as well as antioxidant and antimicrobial activities of the used rice.

Materials and Methods

Preparation of starter inoculum

The *M. purpureus* fungus was isolated from "angkak" bought from the local "Chinese drug store" in Semarang. One gram of "angkak" was poured onto potato dextrose agar (PDA) supplemented with chloramphenicol. After incubating at room temperature for 7 days, the pure *M. purpureus* cultures (the reddish culture) were transferred to a new PDA with chloramphenicol, where it was again incubated at room temperature for another 7 days. To prepare the starter inoculums, a petri dish of the grown *M. purpureus* was harvested using 10 ml of sterilized distilled water. This suspension was subsequently used in fermenting the used rice. For *C. Crassa*, the fungus was initially retrieved from the stock culture (preserved on PDA supplemented with chloramphenicol and kept at 4°C). Following incubation at 38°C for 2 days, the grown fungal culture was transferred to chloramphenicol-supplemented PDA, where it was again incubated at 38°C for another 2 days. Similar to *M. purpureus*, a petri dish of the grown *C. crassa* was harvested using 10 ml of sterilized distilled water, and the suspension was then used as a fermentation starter.

Fermentation procedures

The used rice was obtained from the local market of Semarang. About 500 gm of it was soaked in water for an hour, after which it was drained, steamed for 60 min, and allowed to cool on a tray. To elicit the fermentation process, 50 ml of the *M. purpureus* (7.0×10^7 cfu/ml) or *C. crassa* (2.0×10^{13} cfu/ml) suspension as prepared above was inoculated on the steamed rice and mixed thoroughly. The mixture was spread out on the tray and covered with a perforated aluminum foil. It was aerobically incubated for 7 and 4 days for the *M. purpureus*- and *C. crassa*-inoculated used rice, respectively. After sun drying for 2 days, the fermented product was grounded and sieved (1-mm sieve).

Assessments of parameters and data analysis

The proximate characteristics of the fermented products were determined according to the standard method AOAC [2], its amino acid contents were determined according to a standard ultra-performance liquid chromatography (UPLC) procedure [20], and its antioxidant activity was determined based on the 2.2'-azinobis-3-ethylbenzothiozoline-6-sulfonic acid (ABTS) radical decolourization assay [1]. To make the working solution of ABTS radical, ABTS (9.5 ml, 7 mM) and potassium persulfate (245 µl, 100 mM) were reacted. It was then added with up to 10 ml of distilled water. Then, the solution was kept at the room temperature in the dark for 18 h. Afterward, it was diluted in a potassium phosphate buffer (0.1 M, pH 7.4), whereas the target sample was diluted with methanol (dilutions ranged from 50 to 1.250 µg/ml), and 10 µl of the diluted sample was mixed with 2.99 ml of ABTS radical working solution. The absorbance of the mixture solution was recorded at 734 nm. The following formula was employed to calculate the percent of antioxidant activity: % antioxidant activity = $[(A_c - A_s)/A_c] \times 100$ (where A_c and

A_s are the absorbance of the control and sample, respectively). Methanol was used as the control, while the antimicrobial activity of the fermented product was determined using the agar well diffusion method [5]. The nutrient agar surface was inoculated by spreading 20 ml of the inoculums of *E. coli* and *Staphylococcus aureus* over the entire agar surface. A hole of 6-mm diameter was made by aseptically punching the inoculated agar plate with a sterile tip. Suspension of *C. crassa* and *M. purpureus* (50 ml) was then poured into the well, and the culture was incubated at 37°C for 24 h. Subsequent diameter of the inhibition zone (clear zone) was measured in millimeters. The analyses were conducted in triplicate. Data collected were analyzed using analysis of variance, followed by Duncan's multiple range test to assess the difference between mean values.

Results

Table 1 describes the proximate composition of the fermented used rice. Crude protein and ash contents were higher ($p < 0.05$) in the rice fermented with *C. crassa* or *M. purpureus* than in the unfermented. Conversely, carbohydrate content was lower ($p < 0.05$) in the fermented than in unfermented. Gross energy and energy from fat were higher ($p < 0.05$) in that which was fermented with *M. purpureus* as compared with the unfermented, but was not significantly different from the product fermented with *C. crassa.* Table 2 shows the amino acid contents. The contents of amino acids L-serine, L-methionine, L-glutamic acid, glycine, L-valine, L-leucine, L-proline, L-threonine, L-histidine, and L-sistine were higher ($p < 0.05$) in *M. purpureus*-fermented used rice than in *C. crassa*-fermented and the unfermented. Amino acids L-isoleucine, L-alanine, L-lysine, and L-tryptophan were higher ($p < 0.05$) in both *C. crassa* and *M. purpureus*-fermented as compared with the unfermented. The content of L-tyrosine was higher ($p < 0.05$) in the *M. purpureus*-fermented than in the unfermented used rice, but there was no substantial difference when compared with the *C. crassa*-fermented.

Furthermore, the antioxidant activities of the fermented products were higher ($p < 0.05$) than that of the unfermented product. These activities (expressed as percentage activities) of the unfermented, *C. crassa* and *M. purpureus*-fermented used rice were 3.06% ± 0.37%, 9.5% ± 1.77%, and 9.79% ± 0.45%, respectively. The antimicrobial activities of the fermented used rice are shown in Figures 1–3. Overall, these activities (indicated by diameter of clear zone) in the fermented products against *S. aureus* were higher ($p < 0.05$) than that of the unfermented. The diameter clear zones against *S. aureus* were 2.93 ± 2.90 mm, 19.3 ± 1.73 mm, and 9.38 ± 1.74 mm for the unfermented, *C. crassa*- and *M. purpureus*-fermented, respectively. In contrast, there was no significant difference in the diameter clear zones of fermented used rice against *E. coli* as they were 2.23 ± 3.85 mm, 0 mm, and 4.83 ± 5.30 mm.

Discussion

Fermentation is known to be one of the simplest techniques in improving the nutritional and functional properties of the raw product [8,13]. In this study, amino acids and crude protein contents of the used rice increased with fermentation using *C. crassa* or *M. purpureus*. This finding was in accordance with Martono et al. [12] showing the increased protein content in cassava flour following fermentation with the use of yeast. Likewise, Yafetto [21] showed that the protein contents of sterile fermented cassava pulp could be enriched using *Aspergillus niger*, given that after 8 days, protein contents increased by 22.61%, while those of non-sterile fermented cassava pulp were enriched by 21.54% when the substrates were kept at a moisture content of 50% w/v. The increased protein in the fermented products may be due to the increase in the extracellular protein produced by the fungi growing on the substrates [11]. Bayitse et al. [6] further suggested that the increased protein in the fermented product may be due to the potency of the fungi in producing an enzyme capable of degrading starch and polysaccharides into monosaccharides. The latter compound may easily be processed to protein by the fungi. It is further reported in this study that both fungal fermentations increased the ash content of the substrates. Similar findings were also documented

Table 1. Proximate composition of the fermented products.

Items	Unfermented used rice	*C. crassa*-fermented used rice	*M. purpureus*-fermented used rice
Carbohydrate (%)	77.4 ± 0.39^a	72.7 ± 1.12^b	67.1 ± 3.04^c
Gross energy (kcal/100 gm)	354.5 ± 0.32^b	358.8 ± 4.35^{ab}	372.2 ± 10.85^a
Total fat (%)	1.29 ± 0.66^b	1.98 ± 1.02^{ab}	3.86 ± 1.34^a
Energy from fat (kcal/100 gm)	11.6 ± 0.55^b	17.8 ± 9.14^{ab}	34.7 ± 12.06^a
Ash (%)	0.41 ± 0.01^b	0.77 ± 0.16^a	0.96 ± 0.11^a
Crude Protein (%)	8.31 ± 0.45^c	12.5 ± 0.32^b	17.3 ± 2.59^a

Values with different superscripts within the same rows are significantly different ($p < 0.05$).

Table 2. Amino acids content of fermented products.

Amino Acids (mg/kg)	Unfermented used rice	*C. crassa*-fermented used rice	*M. purpureus*-fermented used rice
L-Methionine	855 ± 69.65[b]	1,278.7 ± 164.71[b]	2,613.3 ± 638.88[a]
L-Serine	5,149.6 ± 626.0[b]	5,389.8 ± 633.69[b]	7,962.6 ± 1,292.49[a]
L-Glutamic acid	12,586.1 ± 361.09[b]	16,500.9 ± 2,167.22[b]	2,0573.3 ± 2,614.72[a]
L-Phenylalanine	6,503.2 ± 1,228.9[a]	6,077.3 ± 1,911.95[a]	7,730.0 ± 1,243.59[a]
L-Isoleucine	3,715.9 ± 175.11[c]	4,831.1 ± 142.14[b]	6,700.3 ± 755.36[a]
L-Valine	5,211.8 ± 238.21[b]	6,370.2 ± 257.41[b]	8,875.1 ± 350.18[a]
L-Alanine	4,670.4 ± 182.18[c]	6,521.6 ± 499.48[b]	8,506.1 ± 1,159.11[a]
L-Arginine	6,772.3 ± 793.82[a]	6,455.2 ± 1,119.64[a]	8,321.6 ± 1,119.05[a]
Glycine	4,625.1 ± 549.3[b]	5,669.3 ± 800.18[b]	8,241.9 ± 1,169.05[a]
L-Lysine	3,212.3 ± 125.12[c]	5,020.8 ± 717.28[b]	7,934.1 ± 1,030.92[a]
L-Aspartic acid	6,085.2 ± 223.09[b]	8,989.8 ± 1,777.95[a]	11,514.6 ± 1,517.43[a]
L-Leucine	7,079.5 ± 312.53[b]	8,025.9 ± 302.75[b]	11,172.3 ± 1,350.05[a]
L-Tyrosine	3,440.3 ± 685.69[b]	3,569.4 ± 1,081.54[ab]	5,633.8 ± 1,317.01[a]
L-Proline	3,748.7 ± 119.77[b]	4,730.7 ± 256.29[b]	7,516.4 ± 942.46[a]
L-Threonine	3,585.7 ± 473.59[b]	4,649.9 ± 419.97[b]	6,440.4 ± 947.69[a]
L-Histidine	2,376.8 ± 384.39[b]	2,532.0 ± 632.69[b]	3,900.2 ± 656.19[a]
L-Sistine	161.3 ± 00.0[b]	324.4 ± 48.95[b]	576.5 ± 174.13[a]
L-Tryptophan	782.6 ± 12.92[c]	1,522.1 ± 258.44[b]	2,011.6 ± 102.80[a]

Values with different superscripts within the same rows are significantly different ($p < 0.05$).

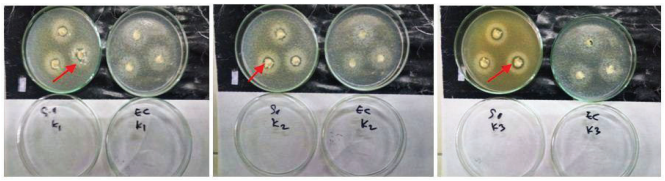

Figure 1. Antimicrobial activity of unfermented product against *S. aureus* (S) and *E. coli* (Ec).

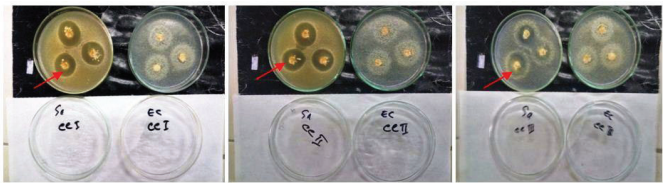

Figure 2. Antimicrobial activity of *C. crassa*-fermented used rice against *S. aureus* (S) and *E. col*i (Ec).

by Sukma et al. [19], in which it was illustrated that fermentation resulted in an increase in fat, protein, and ash contents by 58.5%, 124.5%, and 18.6%, respectively; on the other hand, carbohydrate content decreased by 25.6%. According to Gow et al. [7], the increased ash content in the fermented products may be attributable to the increase in

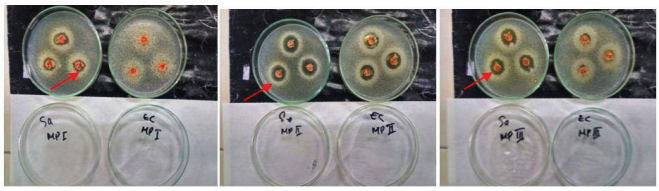

Figure 3. Antimicrobial activity of *M. purpureus*-fermented used rice against *S. aureus* (S) and *E. coli* (Ec).

fungal population in the substrates, as fungal cell wall is rich in minerals.

Result in this study also showed an increase in fat content in the used rice following fungal fermentation, irrespective of the fungal species. In line with the latter finding, Sukma et al. [19] revealed that the fermentation by fungus *Rhizopus oryzae* increased the fat content in rice bran. Given that cell wall and plasma membrane of the fungi are generally composed of fat (phospholipid and lipoprotein), any increase in the fungal biomass may, therefore, result in an increase in the fat content of the fermented products. It was apparent in this study that the fungal fermentation could increase amino acid content in the used rice. As regards *M. purpureus*, fermentation with this fungus was capable of increasing the essential amino acids (L-Arginine, L-Isoleucine, L-Histidine, L-Lysine, L-Leucine,

L-Threonine, L-Methionin, L-Tryptophan, and L-Valine) as well as non-essential amino acids (L-Alanine, L-Aspartate, L-Cysteine, L-Glutamate, L-Glutamine, Glycine, L-Proline, L-Serine, and L-Tyrosine). Likewise, *C. crassa*-fermentation increased the essential amino acids L-Isoleucine and L-Tryptophan as well as non-essential amino acid L-Tyrosine.

Our finding in this study was in accordance with Jannathulla et al. [8] reporting that the fungal fermentation resulted in higher content of amino acid in the fermented product when compared with the unfermented. Such elevated amino acid contents may be due to the activity of protease enzyme in the filamentous fungi to decompose protein to amino acids. This study also showed the carbohydrate content of the two fermented products to be lower than that of the unfermented product. The reason for such a decrease in carbohydrate content of the fermented products remains unclear. However, owing that carbohydrate is the main substrate for the fungal growth as Yafetto [21] suggested, fungi utilize a wide range of carbon sources (monosaccharides, disaccharides, and polysaccharides) for mycelial growth. Therefore, the decrease in the carbohydrate content seemed to be attributable to the use of carbohydrate by fungi in the course of the fermentation process.

It can be observed in this study that the antioxidant activity of the used rice was improved by the fungal fermentation. This finding was in accordance with Kwak et al. [10] which suggested that the fermentation can improve the antioxidant activity. The latter improvement may be due to the microbial hydrolysis or breakdown of plant cell walls into the various antioxidant compounds, i.e., phenolic and flavonoids. In this study, fungal fermentation was associated with the enhanced antimicrobial activity of the used rice. This was reasonable, as fermentation generally produces compounds (secondary metabolites) capable of acting as antimicrobial agents [15]. Indeed, *C. Crassa* and *M. purpureus* were the two fungi used in fermenting the used rice in this study and that they can produce secondary metabolites [16].

Overall, our obtained data indicated that *M. purpureus*-fermentation yielded results better than *C. crassa*-fermentation in terms of improving the protein, ash, and amino acid contents, as well as the antioxidant and antimicrobial activities. The reason for the latter condition was not exactly known, but the data contributed by Kim and Ku [9] indicate that *M. purpureus* has an ability to produce the natural red pigment as a secondary metabolite which contains some active ingredients such as isoflavones, fatty acids, enzymes, dimerumic acid (antioxidant), vitamins, monacolin K (lovastatin, anti-hypercholesterolemic agent), γ-aminobutyric acid (GABA, hypotensive agent), and organic acids. The pigment is also proven to be an antimicrobial to bacteria *E. coli* and *B. subtilis* [16].

Conclusion

Fermenting the used rice using fungi *C. crassa* and *M. purpureus* improved the nutritional quality, antioxidant, and antimicrobial activities of the products.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' contribution

Turrini Yudiarti designed, performed the work, and wrote the manuscript, Sugiharto Sugiharto, Endang Widiastuti, Hanny Indrat Wahyuni, and Tri Agus Sartono performed the work and revised the manuscript and Isroli Isroli performed the data analysis.

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