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PHYSICAL AND CHEMICAL PROPERTIES OF ORGANIC MINERAL ADDITIVE FOR RUMINANT THROUGH BIOLOGICALLY INCORPORATED BY *SACCHAROMYCES CEREVISIAE* IN DIFERENCE SUBSTRATES

SIFAT FISIK DAN KIMIA DARI ADITIF MINERAL ORGANIK UNTUK TERNAK HASIL FERMENTASI SACCHAROMYCES CEREVISIAE PADA SUBSTRAT YANG BERBEDA

Ahmad Sofyan[1], Hendra Herdian[1], Awistaros Angger Sakti[1], Gumilang Khairulli[2] and Anuraga Jayanegara[2]

[1] Division of Feed and Animal Nutrition,
Research Unit for Processes Development and Chemical Engineering (BPPTK),
Indonesian Institute of Sciences (LIPI),
Jl. Jogja-Wonosari Km. 31.5, Gading, Playen, Gunungkidul, D.I. Yogyakarta 55861
[2] Department of Animal Nutrition and Feed Technology, Faculty of Animal Sciences,
Bogor Agricultural University (IPB),
Jl. Agatis, Kampus IPB Darmaga Bogor 16680
*E-mail: sofyan_lipi@yahoo.co.id

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ABSTRACT

This research was conducted to evaluate physical and chemical properties of organic mineral additive incorporated by *Saccharomyces cerevisiae* which was cultivated on different growth media consisted of cassava flour and rice bran, respectively. Treatment was arranged on completely randomized design consisted of rice bran without inoculants (RBo), rice bran with inoculants (RBi), cassava flour without inoculants (CFo), cassava flour with inoculants (CFi). Substrates were fortified by microminerals contained of Cu (100 ppm), Mn (100 ppm), Zn (100 ppm), I (10 ppm), Fe (2.5 ppm) and Co (2 ppm). Inoculation of *S. cerevisiae* could reduce fungal contamination. Physical characteristic of cassava flour was better in flavour and texture than rice bran in which tends to rancidity. Nutrients composition was similar in cassava and rice bran however crude fibre content in rice bran tends to be higher after fermentation. Mineral content (Zn and Fe) relatively decreased and in substrate supplemented by inoculant and rice bran had higher than cassava. In summary, micromineral was incorporated in cassava flour with inoculated S. cerevisiae had better than rice bran.

Keywords: Organic mineral, cassava flour meal, S. cerevisiae, rice bran, ruminant.

ABSTRAK

Penelitian dilakukan untuk mengevaluasi sifat fisik dan kimia aditif mineral organik untuk ternak hasil fermentasi Saccharomyces cerevisiae pada media tumbuh masing-masing mengandung tepung singkong dan dedak padi. Perlakuan disusun dengan pola rancangan acak lengkap yang terdiri dari dedak padi tanpa inokulum (RBo), dedak padi dengan inokulum (RBi), tepung singkong tanpa inokulum (CFo), dan tepung singkong dengan inokulum (CFi). Media tumbuh difortifikasi dengan larutan mineral yang mengandung Cu (100 ppm), Mn (100 ppm), Zn (100 ppm), I (10 ppm), Fe (2.5 ppm) dan Co (2 ppm). Perlakuan inokulasi S. cerevisiae dapat mengurangi kontaminasi jamur. Sifat fisik tepung singkong memiliki aroma dan tekstur yang lebih baik daripada dedak padi yang cenderung beraroma tengik. Komposisi nutrien pada substrat tepung singkong dan dedak padi cenderung sama, namun kandungan protein kasar dalam dedak padi cenderung lebih tinggi setelah fermentasi. Kandungan mineral (Zn dan Fe) relatif menurun pada substrat dedak padi sebagai dengan inokulum dibandingkan substrat dari tepung singkong. Dapat disimpulkan bahwa inkorporasi mikromineral pada subsrat tepung singkong dengan inokulasi S. cerevisiae lebih baik dari dedak padi.

Keywords: Mineral organik, tepung singkong, S. cerevisiae, dedak padi, ternak ruminansia

INTRODUCTION

Livestock organically based development has been concerned in the recent decades. Due to it is not only support to ecological balance and animal welfare but also need to generate organic product for human better life. The use of organic feed additive increase nutrient utilization and minimize environmental contamination. Organic mineral supplementation had an important role in growth performance, nutrient utilization, trace mineral balance and serum mineral concentration. Lower dose of organic mineral showed the similar result as inorganic mineral with required amount therefore it reduced excretion as inorganic element and reduced soil or environmental toxicity[(1)].

Mineral bioavailability is defined as the ingested nutrient fraction that is absorbed and subsequently utilized for normal physiological functions of human or animal[(2)]. In order to increase its bioavailability, inorganic mineral could be incorporated with organic compound which formed organic mineral complexes or chelated mineral. Chelated mineral with the peptide compound is potential to prevent mineral deficiencies[(3)].

Substrates for yeast growth that was fortified by some minerals potentially absorbed into yeast cells. Addition of Zn^{2+} into medium supported the mycelia yeast growth. It would be associated with mineral incorporated by either cells or organic substances in the cells made chelated minerals[(4)]. Micromineral supplementation in organic form was improving broiler performance[(5)], feed digestibility[(6)], health and reproductive performance of ruminant[(7)].

Moreover, *Saccharomyces cerevisiae* is one of yeast species was wildly used for food and feed fermentation. *S. cerevisiae* was reported inhibited aflatoxin contamination in broiler feed[(8)] and reduced anti-nutrition factor (phytic acids) in soybeanmeal[(9)]. In ruminant addition of *S. cerevisiae* on feed could lead to increase the milk production of dairy cow[(10)] and stabilize ruminal pH that preventing sub acute ruminal acidocis[(11)]. However, organic mineral consisted of some microminerals involving yeast which was cultured on local feedstuff still limited reported. This research was conducted to evaluate physical and chemical properties of ruminant organic mineral additive for ruminant incorporated by *S. cerevisiae* cultivated on different growth media consisted of cassava flour and rice bran, respectively. The result of the experiment to contribute animal production sustainability based on local feed sources.

MATERIALS AND METHODS

Organic mineral was prepared involving fermentation processes by inoculation of *Saccharomyces cerevisiae* ATCC 9763. Substrates were used as yeast medium growth consisted of rice bran (*Oryza sativa*) or cassava flour meal (*Manihot* sp). Prior to inoculation, yeast was precultured on malt extract broth (Oxoid®) which was incubated during 48 hours then *S. cerevisiae* (5% v/w) inoculated into substrates. Substrates were fortified with microminerals contained of Cu (100 ppm), Mn (100 ppm), Zn (100 ppm), I (10 ppm), Fe (2.5 ppm) and Co (2 ppm). Formulation per kg substrate consisted of $CuSO_4.5H_2O$ (9.810 g), $MnCl_2.4H_2O$ (7.129 g), $ZnSO_4.7H_2O$ (12.646 g), KI (0.217 g), $FeCl_2.4H_2O$ (0.177 g) and $CoCl_2.6H_2O$ (0.192 g). Minerals were diluted with sterilized distilled water and added into substrate into moisture content about 40%. Mixture consisted of mineral and substrate was according to the treatment. Incubation was conducted in room temperature (25-30°C) and facultative condition.

Organic mineral was harvested on 7 days of facultative fermentation then dried in oven at 55°C (up to 24-48 h, DM 10%), followed by ground and sieved to pass a 1 mm of particle size. Chemical analysis of sample consisted of dry matter, organic matter, crude protein, crude fibre was conducted according to AOAC methods(12) and content of ether extract and nitrogen free extract was calculated by difference in dry matter basis. Sample was prepared in dry condition to prevent deterioration and fungal contamination. The concentration of trace elements was measured by atomic absorption spectrophotometer(5).

The experiment was arranged on completely randomized design with 4 treatments and 3 replications. Treatment consisted of rice bran without inoculants (RBo), rice bran with inoculants (RBi), cassava flour without inoculants (CFo), cassava flour with inoculants (CFi). Data from physical properties were analyzed descriptively and data from chemical properties were analyzed using analysis of variance (ANOVA). In order to compare between treatments mean, data were statistically analysed with Duncan Multiple Range Test using Co-STAT® Statistical Software (Cohort 2008)(13). Analysis of interrelationship parameters was performed by hierarchical clustering analysis/HCA(14). Visualization of HCA was analyzed using the dendogram-heatmap that constructed using `heatmap.2` function from `gplots library` in the R-statistical software(15). Pre-treatment of data were calculated based on the relative differential data from treatments and control according the formula (x_t/x_o-1) where x_o and x_t denote before and after fermentation data respectively.

RESULTS AND DISCUSSION

Evaluation of physical characteristics of organic mineral incorporated by *S. cerevisiae* (MEY) on different growth media was performed at the 7 days of incubation in facultative condition. Sample was treated by inoculation of yeast seemed to be better in sensory test in accordance with odour, texture and occurring fungal contamination (Table 1). Then, chemical composition of MEY showed that no difference in all parameters except for crude fibre and nitrogen free extract + ether extract (Table 2).

Table 1. Physical characteristics and fungal contamination of organic mineral incorporated by *S. cerevisiae* on different growth substrates

Observed Variables	RBo	RBi	CFo	CFi
Texture after harvesting	Agglomerated	Agglomerated	Agglomerated	Granulated
Odor	Rancid	Poor	Poor	Good
Fungal Contamination	Contaminated	Low-Contaminated	Contaminated	No-Contaminated
Visualization				

RBo: rice bran without inoculation, RBi: rice bran inoculated by S. cerevisiae, CFo: cassava flour without inoculation, CFi: cassava flour inoculated by S. cerevisiae.

Both substrates were fermented without yeast inoculation showed the contamination by fungi that was indicating not only visualized texture but also not good odour (off-flavour). Although substrates were sterilized before inoculation, fungal contamination might be occurred while oxygen presence in the facultative fermentation system. Moreover, rancidity was observed in rice bran that it could be associated with oxidative mechanism. Due to oxygen is possibly presence in the facultative fermentation and implied stimulation of lipolitic enzyme activity. Fatty acid in rice bran is readily oxidized by lipolitic enzymes such as lipase and lipoxgenase[(16)].

As a substrate for yeast growth, cassava reflected better physical traits with fungal contamination lower contamination. *S. cerevisiae* was grown on medium would compete and prevent other microbes to contaminate the substrates. *S. cerevisiae* could be used as microbial agent to minimize *Aspergillus flavus* contamination in animal feed[(8)]. Growth of *S. cerevisiae* seems favourably while substrate is readily available supplied from glucose[(17)]. Due to starch (complexes form of glucose) contained in cassava flour was higher than rice bran[(18)].

Some chemical parameters were changed after fermentation consisted of crude protein (CP), crude fibre (CF), ether extract (EE)+nitrogen free extract (NFE), ash whereas organic matter (OM) and dry matter showed not changeable (Figure 1). Characteristic of parameters change could be analysed using hierarchical cluster which was indicate similarities in each substrates. DM change was closely related to OM compared from NFE+EE. CP and CF had higher similarity compared from the interrelationship with other parameters (ash and EE).

Addition of yeast for incorporating microminerals was relatively increase value of CF, CP and ash when NFE+EE reduced. In this experiment, NFE+EE in cassava were observed higher than rice bran. Moreover, lipid content (EE) in cassava (0.7%) was lower than in rice bran (14.1%)[(18)]. Reducing starch and lipid caused by activity of *S. cerevisiae* secrete enzymes consisting α-amylase for degrading glucose/starch then lipase for degrading lipid[(19)] instead of protease enzyme. Organic matter of feedstuffs could be fractioned into structural (starch) and non-structural (crude fiber) carbohydrate. In term, NFE is non-structural carbohydrate that easily enzymatic degradation than crude fiber[(20)]. Consequently, some nutrients (CF and CP) which were not degraded by S. cerevisiae increased relatively while the other nutrients (NFE+EE) decreased.

Table 2. Nutrient composition of microminerals incorporated by *S. cerevisiae* on different growth substrates

Parameters	RBo	RBi	CFo	CFi
DM (%)	85.7[a] ± 5.0	85.9[a] ± 2.6	79.4[a] ± 3.2	84.8[a] ± 4.0
OM	81.6[b] ± 0.7	82.4[b] ± 1.0	98.3[a] ± 0.1	98.5[a] ± 0.1
Ash (%DM)	18.4[a] ± 0.7	17.6[a] ± 1.0	1.7[b] ± 0.1	1.5[a] ± 0.1
CP (%DM)	12.1[a] ± 1.2	13.2[a] ± 1.9	3.3[b] ± 1.3	3.4[b] ± 1.2
CF (%DM)	37.0[b] ± 5.0	43.5[a] ± 1.0	12.9[c] ± 3.8	16.7[c] ± 0.6
NFE+EE (%DM)	32.5[b] ± 6.9	25.7[b] ± 2.4	82.1[a] ± 4.6	78.5[a] ± 0.8

DM: Dry Matter, CP: Crude Protein, CF: Crude Fibre, NFE: Nitrogen Free Extract, RBo: rice bran without inoculation, RBi: rice bran inoculated by *S. cerevisiae*, CFo: cassava flour without inoculation, CFi: cassava flour inoculated by *S. cerevisiae.*

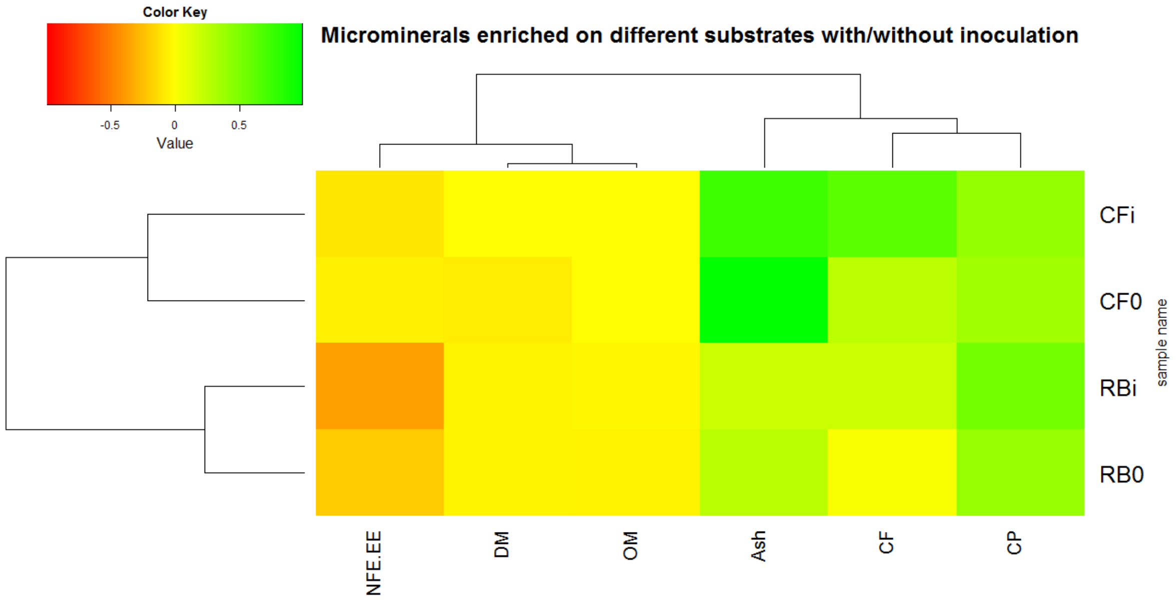

Figure 1. Hierarchical clustering analysis of nutrient composition change during fermentation. (Colour key consisted of green, yellow and red denote respectively for value of increase, no change, and decrease fold)

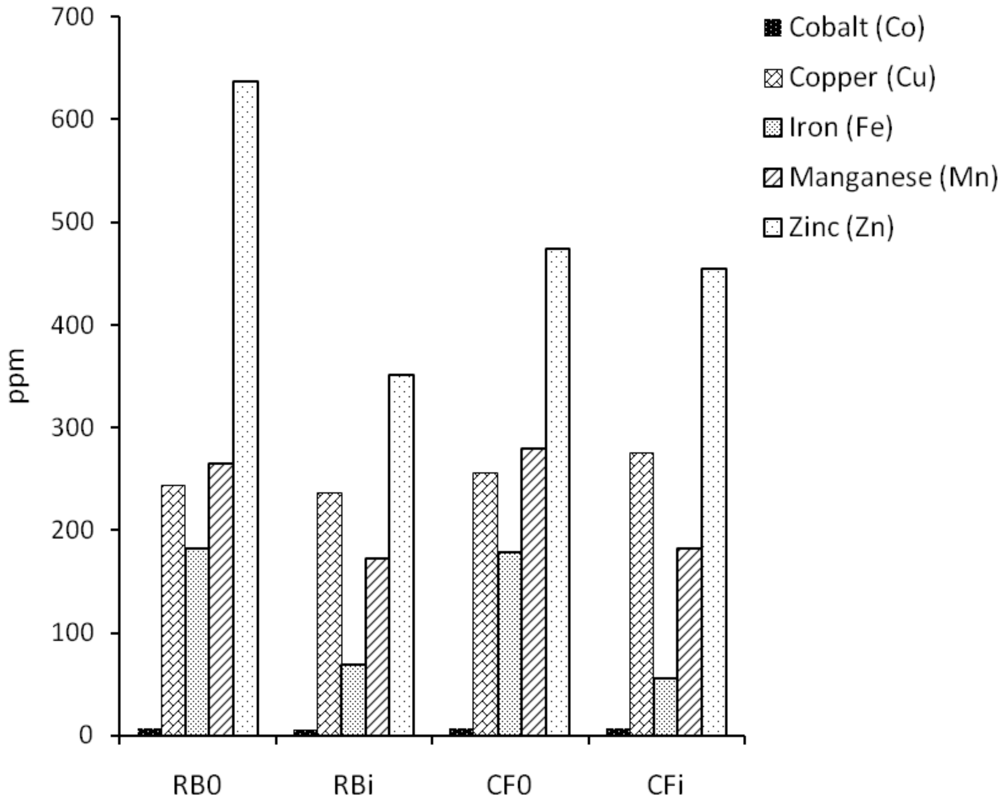

Figure 2. Micro-minerals content incorporated by *S. cerevisiae* on different substrates (RBo: rice bran without inoculation, RBi: rice bran inoculated by *S. cerevisiae*, CFo: cassava flour without inoculation, CFi: cassava flour inoculated by *S. cerevisiae*)

During the growth activity, S. cerevisiae metabolize NFE as sugar sources [(17)] and secreted lypolitic enzyme to metabolize lipid (EE)[(21)] to generate energy for maintenance and to produce secondary metabolites. Yeast fermented the substrate consisting of microminerals that could be incorporated into organic form (chelated minerals) and improved flavour of fortified substrate. S. cerevisiae had been widely used as bio-flavour producers to ferment sugar and generate volatile compound (e.g. β-pinene, β-terpineol, and D-limonene etc.) as flavour components[(22).]

After fermentation, microminerals (i.e. Zn and Fe) content in both substrates showed reduced in inoculation treatments (Figure 2). It related to the mineral utilization to support yeast activity. Other minerals (i.e. Co, Mn, Cu) seem similar with and without yeast inoculation. Incorporating inorganic microminerals into organic form might be involved by mechanism that mineral ions (i.e. Fe^{2+}, Zn^{2+}, Mn^{2+}, Cu^{2+} Co^{2+} and I^{-}) had a chance to bind organic compound as metabolic product or entry into yeast cells. This mechanism was supported that yeast *Aureobasidium pullulans* had capable to uptake Zn^{2+} and incorporated with mycelia cells[4]. Furthermore, *S. cerevisiae* supports binding system between Fe and organic compound such as amino acids, e.g. histidine, lysine and arginine[(23)].

Although addition of yeast in substrate fermentation tended to similar, inoculation of *S. cerevisiae* had beneficial for reducing anti-nutrient compound in substrate. Phytase enzyme degrades mineral complex-P (phosphorus) with phytic acid into myo-inositol phosphate, myo-inositol and inorganic phosphate[9] and increases availability in digestion system for animals. Therefore, supplementation of organic microminerals or chelated minerals could support animal productivity and health status[(5,24,25)].

CONCLUSION

Organic microminerals additive was incorporated by *S. cerevisiae* cultivated on cassava flour had better than rice bran in physical characteristic. However, composition of microminerals tended to similar in both substrates with or without inoculation. The further research need to be conducted to evaluate chelating mechanism involving yeast activity and influence of organic mineral on animal productivity.

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