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Improving the Quality of Tapioca by Product Through Fermentation by *Neurospora crassa* to Produce β Carotene Rich Feed

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Abstract: An experiment was conducted to improve the nutrient content of tapioca by product to produce β carotene rich feed as alternative poultry feed through fermentation by using carotenogenic fungi (*Neurospora crassa*) as inoculums. The experiment was determination of substrate composition (carbon source and nitrogen source) based on nutrient quality and quantity of these fermented products. The study was conducted in experimental methods, using the completely randomize design in factorial with 2 treatment were : 1. A factor, (tapioca by product as carbon source with nitrogen sources: A 1 = tapioca by product+tofu waste, A 2 = tapioca by product + palm kernel cake and A 3 = tapioca by product + rice bran. 2. B factor (Percentage of composition of carbon source with nitrogen source), B 1 = 90% : 10%, B 2 = 80% : 20%, B 3 = 70% : 30% and 60% : 40%). Results of study showed that optimum substrate composition of the fermentation by *Neurospora crassa* was the mixture 60% tapioca by product with 40% tofu waste. This conditions can increase β carotene and crude protein and also decrease crude fiber which made the nutritional value of the product based on dry-substance was 295.16 µg/g, β carotene 20.44% crude protein, 2.75% crude fat, 11.96% crude fiber, 0.24% calcium, 0.17% phosphor, metabolic energy 2677 Kcal/kg, 67.05% nitrogen retention and 35.44% fiber digestion.

Key words: Fermentation, Tapioca by product, nitrogen source, β carotene and *Neurospora crassa*

INTRODUCTION

The use of agro-industry waste (tapioca by product, sago waste, tofu waste and etc) is one of the alternatives for poultry feed. The waste of agro-industry must be processing beforehand to increase its quality. Tapioca by product is solid waste from meal tapioca processing which potential as animal feed and the amount is quite large in West Sumatera.

Tapioca by product can be use as a source of carbon in fermentation medium which contain 70.14% nitrogen free extractive but its crude protein still low which is 1.04% dry materials, that needed another feed as nitrogen source which is most needed for growth of fungi. The source of nitrogen can be obtained from high protein feed like tofu waste (25.15% crude protein), palm kernel cake (18.34% crude protein) and rice bran (13.21% crude protein) (Nuraini *et al*., 2007). The mixing tapioca by product and nitrogen source hoped to be able to cover the lack of nitrogen in tapioca by product, so that it could be used as fermentation medium for the growth of *Neurospora crassa*. This fungi need substrate as nutrient especially carbon and nitrogen source. The fermentation medium with imbalance nutrient contain will support the fungi to produce β carotene rich feed product.

β-carotene-rich feed can obtained from conventional feed like corn and its also can be obtained through fermentation by using carotenogenic fungi (*Neurospora crassa*) as inoculums. *Neurospora crassa* is the higher β carotene producer fungi then another carotenogenic fungi which had been isolated from corn cob (Nuraini and Marlida, 2005). Using of β carotene rich fermentation product can be use as alternative feed that can substituted corn.

MATERIALS AND METHODS

The materials used were: tapioca by product as carbon source in fermentation medium, tofu waste, palm kernel cake, rice bran as nitrogen source, *Neurospora crassa* as inoculum and chemicals for analysis. Equipments used were: oven, incubator, laminar air flow, kjehdal apparatus, fiber apparatus and spectrophotometer. The study was conducted in experimental methods, using the completely randomize design in factorial (Steel and Torrie, 1980) with 2 treatment were:

1. A factor, (tapioca by product as carbon source+nitrogen sources: A 1 = tapioca by product+tofu waste, A 2 = tapioca by product+palm kernel cake and A 3 = tapioca by product+rice bran).
2. B factor (Percentage of composition of carbon source: nitrogen source), B1 = 90% : 10%, B2 = 80% : 20%, B3 = 70% : 30% and B4 = 60% : 40%).

Substrates with certain composition according to the treatment, was mixed in plastic bag, aquadest was

added to maintain 70% moisture content and mixed thoroughly. It was then sterilized (121°C 15 min). After the temperature cooled down to 35°C, it was inoculated with 9% inoculum *Neurospora crassa*, mixed thoroughly, and then spread evenly with substrate thickness of 2 cm. After that it was incubated for 6 days. Fermented products were then analyzed for β-carotene (Gross, 1987), moisture, crude protein and crude fiber content (AOAC, 1990), amylase (Miller, 1959) and cellulase (Mandel *et al*., 1976). The best of fermented product (higher β carotene and crude protein content) then analyzed amino acid (Nur *et al*., 1992), nitrogen retention, metabolizable energy (Sibbald, 1976) and crude fiber digestion.

RESULTS

The effect of the composition substrate on β carotene, crude protein and crude fiber content fermented product: The average interaction between tapioca by product as carbon source+nitrogen sources and percentage of composition of carbon source with nitrogen source toward the average value of β carotene ,crude protein and crude fiber content of fermented product is shown in Table 1.

Amino acid content of fermented product: Amino acid content of fermented tapioca by product with nitrogen source mixture before and after the fermentation can be seen in Table 2.

Protein quality, metabolic energy and crude fiber digestion of fermented product: The quality of protein (Nitrogen retention), the amount of metabolic energy and crude fiber digestion of fermented product can be seen in Table 3 and 4. Cellulase activity (Unit/ml) *Neurospora crassa* is shown Table 5

DISCUSSION

DMRT results indicated that the treatment 60% tapioca by product and 40% tofu waste (Table 1) resulted in the highest β carotene content i.e. 295.16 µg/g. This happen because there were sufficient and balanced nutrients in the substrate, especially carbon and nitrogen sources (C/N 9:1), resulted in vigorous growth of *Neurospora crassa* which increased β-carotene and crude protein contents of the substrate. According to Litchfield (1992), suitable ratio of carbon and nitrogen for the growth of *Neurospora* was 7:1-15:1, while the ratio for the growth of fungi in general was 5:1-20:1. The yellow-orange colored spores of *Neurospora crassa* contain β-carotene (Wang *et al*., 2002; Perkins *et al*., 2002). Vigorous growth of *Neurospora crassa* could be seen from the abundance of spores resulted in increased protein content of the substrate. The increase of protein content after fermentation could be called as "protein enrichment process" or the enrichment of the substrate with protein from certain microorganisms because this process was identical with the production of single cell protein, in which the growing microbe cells could not be separated from the rest of the substrate. Ratledge (1994) stated that fungus body contained high protein content, i.e. 40-50%. Fungi with good growth and reproduction would be able to change more components of the media into cell mass that formed body protein of the fungi which in turn would increase protein content of the substrate. Yasril (2002) stated that *Neurospora* sp. that grew on substrate composed of 80% sago waste and 20% tofu waste for 8 days produced 246.43 µg/g of β-carotene.

The lowest crude fiber content i.e. 11.96% was obtained from the treatment with 60% tapioca by product and 40% tofu waste. This related with cellulase that reached its highest activity 14.40 Unit/ml at this treatment compared to other treatments (Table 5). High cellulase activity meant more cellulose was transformed into glucose, which decrease the cellulose content and lower the crude fiber content of fermented product. Romero *et al*. (1999) stated that *Neurospora* could produce cellulase that changed cellulose and hemicellulose into simple compound like glucose.

Amino acid content of the substrate (Table 2) with 60% tapioca by product+40% tofu waste after fermentation by *Neurospora crassa* was higher than before fermentation. According to Marathe *et al*. (1998); Yu and Weiss (1992), *Neurospora crassa* was able to produce the amino acids methionine, arginine, tyrosine, glutamic and tryptophan.

The average of nitrogen retention (Table 4) of fermented mixture of 60% tapioca by product and 40% of u waste was 67.05%. That number was bigger than the average of nitrogen retention another treatment and indicate that the quality of protein that fermented product better than other treatment. Nitrogen retention is one of the method to measure the quality of protein a materials or ration . Nitrogen retention of good ration for broiler is 65-67% (Wahju, 1997). At previous matter nitrogen retention just tapioca by product was 38.21%, according to Yunar (1990) having weakness as feed because high crude fiber and low palatabilitas; but after fermented with *Neurospora crassa*, hence nitrogen retention can be improved become 67.05%. According to Buckle *et al*. (1985) one of the advantage of fermentation was protein, fat and polisakarida in substrate hydrolysate constructively by enzyme microbe, so that fermented product have the higher quality compared to before fermented.

The average of metabolic energy (Table 3) of 60% tapioca by product and 40% tofu waste content mixture fermented by *Neurospora crassa* was 2717.63 kcal/kg. This number was bigger than metabolic energy before fermentation i.e. 2670.12 kcal/kg. This happened because increased glucose content which was the hydrolyses product from cellulose tapioca by product and tofu waste content mixture by the cellulase of *Neurospora crassa* during fermentation and later the

Table 1: Average nutrient content of fermented product by *Neurospora crassa*

		Composition of Substrate (% tapioca by product:% Nitrogen Source)				
Parameter	Tbp+N source	B1 (90%:10%)	B2 (80%:20%)	B3 (70%:30%)	B4 (60%:40%)	Mean
β Carotene (µg/g)	A1 (Tbp+Tw)	101.04^{Da}	162.35^{Ca}	220.23^{Ba}	295.16^{Aa}	194.70
	A2 (Tbp+Pkc)	96.20^{Db}	134.45^{Cb}	170.78^{Bb}	209.56^{Ab}	152.75
	A3 (Tbp+Rb)	95.45^{Db}	133.56^{Cb}	168.45^{Bb}	198.23^{Ab}	148.92
	Mean	97.56	143.45	186.49	234.32	
Crude Protein (%)	A1 (Tbp+Tw)	9.12^{Da}	12.62^{Ca}	16.39^{Ba}	20.44^{Aa}	14.64
	A2 (Tbp+Pkc)	8.08^{Db}	11.76^{Cb}	14.00^{Bb}	16.56^{Ab}	12.60
	A3 (Tbp+Rb)	5.11^{Dc}	6.03^{Cc}	8.70^{Bc}	9.89^{Ac}	7.43
	Mean	7.44	10.14	3.03	15.63	
Crude fiber (%)	A1 (Tbp+Tw)	15.18^{Ac}	14.12^{Bb}	12.99^{Cc}	11.96^{Dc}	13.56
	A2 (Tbp+Pkc)	16.70^{Aa}	16.40^{Aa}	15.21^{Ba}	14.52^{Ca}	15.70
	A3 (Tbp+Rb)	15.53^{Ab}	14.31^{Bb}	13.92^{Cb}	12.81^{Db}	14.14
	Mean	15.80	14.94	14.04	13.10	

Superscripted capital letter in the same row and lower case letter in the same column indicate highly significantly different ($P < 0.01$) results.

Table 2: Amino acid before and after fermented

			Tbp+Tw after fermented	
Amino acid (%)	Tbp (%)	Tbp+Tw Before fermented	(80% : 20%)	(60%:40%)
Alanine	0.23	0.68	0.71	0.95
Arginine	0.40	0.90	1.11	1.20
Asparagine	0.35	1.00	1.35	1.65
Glycine	0.27	0.78	0.82	0.95
Glutamine	0.90	2.45	2.75	3.15
Histidine	0.13	0.31	0.34	0.40
Isoleucine	0.25	0.56	0.63	0.72
Leucine	0.34	1.03	1.17	1.25
Lysine	0.13	0.23	0.94	1.58
Methionine	0.04	0.10	0.21	0.37
Phenylalanine	0.36	0.69	0.71	0.75
Valine	0.54	0.75	0.80	0.92
Proline	0.33	0.63	0.68	0.80
Threonine	0.43	0.50	0.57	0.70
Serine	0.38	0.65	0.69	0.75
Cysteine	0.04	0.12	0.15	0.20
Tyrosine	0.17	0.43	0.50	0.62
Tryptophan	0.02	0.05	0.09	0.13

Table 3: Average nitrogen retention, metabolic energy (TMEn) and crude fiber digestion broiler

Parameter	Nitrogen retention (%)	TMEn (kcal/kg)	Crude fiber digestion (%)
Tapioca by product (Tbp)	38.21	2865.73	20.18
Tbp+Tw before fermented	45.34	2670.12	23.29
Tbp+Tw after fermented	67.05	2717.63	35.84

glucose was counted as metabolic energy. Geharzt (1990) stated that cellulase was actually an enzyme complex that work gradually or simultaneously breaking down cellulose into glucose unit.

Evaluated on crude fibre digestion (Table 4) showed that fermented product of 60% tapioca by product and 40% tofu waste by *Neurospora crassa* was 35.84%, which higher than another treatment. This matter caused cellulase activity that treatment higher than others, so that crude fibre content and cellulose the treatment lower. According to Frazier and Westhoff (1984) that enzyme of cellulase hydrolysate cellulose become more simple like glucose, so that easier digested of crude fibre and used by livestock. Carlile and Watkinson (1995) expressing ferment can break complex components like carbohydrate, fat and protein become more simple, so that easy to digested.

Conclusion: Composition of substrate 60% tapioca by product and 40% tofu waste mixture was optimum condition for growth of *Neurospora crassa* based on nutrient quantity and quality. This fermentation process was able to increase β carotene and crude protein also decrease crude fibre, which made the nutritional value of the fermented product based on dry matter as follows: 295.16 µg/g β carotene, 20.44% crude protein, 11.96%

Table 4: Average nitrogen retention (%) and crude fiber digestion (%) broiler

Parameter	Tbp + N source	Composition substrate (% Tbp:% nitrogen source) B1 (90%:10%)	B2 (80%:20%)	B3 (70%:30%)	B4 (60%:40%)	Mean
Nitrogen retention (%)	A1 (Tbp+Tw)	61.85^{Da}	62.50^{Ca}	63.05^{Ba}	67.05^{Aa}	63.61
	A2 (Tbp+Pkc)	56.91^{Db}	57.14^{Cb}	59.82^{Bb}	60.71^{Ab}	58.65
	A3 (Tbp+Rb)	55.00^{Bb}	58.33^{Bb}	57.14^{Ab}	60.98^{Ac}	57.86
	Mean	57.92	59.32	60.00	62.91	
Crude fiber digestion (%)	A1 (Tbp+Tw)	30.11^{Da}	32.16^{Ca}	34.60^{Ba}	35.84^{Aa}	33.17
	A2 (Tbp+Pkc)	27.65^{Db}	29.05^{Cc}	29.88^{Bc}	32.13^{Ab}	29.68
	A3 (Tbp+Rb)	28.15^{Db}	30.02^{Cb}	32.18^{Bb}	33.90^{Ac}	31.06
	Mean	28.64	30.40	32.21	33.95	

Table 5: Cellulase activity (Unit/ml) *Neurospora crassa*

Parameter	Tbp +N source	Composition of Substrate (% tapioca by product : % Nitrogen Source) B1 (90%:10%)	B2 (80%:20%)	B3 (70%:30%)	B4 (60%:40%)	Mean
Cellulase activity (Unit/ml)	A1 (Tbp+Tw)	5.15^{Da}	9.13^{Ca}	12.85^{Ba}	14.40^{Aa}	10.38
	A2 (Tbp+Pkc)	3.58^{Db}	4.88^{Cb}	8.79^{Bb}	12.60^{Ab}	7.46^{A3}
	A3 (Tbp+Rb)	4.11^{Cb}	5.22^{Bb}	6.51^{Bc}	11.37^{Ac}	6.90
	Mean		4.28	6.54	9.38	12.79

Superscripted capital letter in the same row and lower case letter in the same column indicate highly significantly different (P<0.01) results

crude fibre, 67.05% nitrogen retention, 35.84% crude fiber digestion and 2717.63 kcal/kg metabolic energy.

Suggestion: Fermented product by *Neurospora crassa* require to evaluate by biologis to layer to know its influence to egg quality and production.

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