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Research Article

Physicochemical Properties of Fresh and Dry Powder *Bekasam* of *Catfish Clarias batrachus* (Linn, 1758)

Yanesti Nuravianda Lestari[1,5], Retno Murwani [1,2,3]*, and Tri Winarni Agustini [4]

[1]Master Program in Nutrition, Faculty of Medicine, Diponegoro University, Jl. Prof. Soedarto, Tembalang, Semarang, Indonesia
[2]Natural Product Laboratory, Centre of Research and Services, Diponegoro University, Jl. Prof. Soedarto, Tembalang, Semarang, Indonesia
[3]Faculty of Animal and Agricultural Science, Diponegoro University, Jl. Prof. Soedarto, Tembalang, Semarang, Indonesia
[4]Faculty of Fishery and Marine Science, Diponegoro University, Semarang, Indonesia
[5]Bachelor Program in Nutrition, Faculty of Health, Nahdlatul Ulama University, Surabaya, Indonesia

Abstract: *Bekasam*, a fermented fish product with high salt content and strong specific flavor, can be used as a flavor enhancer to stimulate appetite. It is traditionally made either of skipjack viscera or whole fish or from various types of fish. The aim of this study was to characterize the physicochemical properties of fresh bekasam and bekasam powder made of catfish [*Clarias batrachus* (Linn, 1758)]. Fresh catfish free of viscera and head were fermented in the presence of 10 % salt for 24 h at room temperature followed by addition of glutinous rice and fermented further for 6 d. The resulting bekasam was partly dried by cabinet drier to produce bekasam powder. The pH, organic acid, soluble protein, sodium, and glutamic acid content of fresh bekasam and bekasam powder were measured. Fresh bekasam has pH value of pH 6.95 ± 0.010; organic acid (8.40 ± 0.040) %; soluble protein (1.59 ± 0.006) mg · dL^{-1}; sodium content (4.67 ± 0.006) %; and glutamic acid (11.88 ± 0.025) mg · g^{-1}. Bekasam powder has lower pH, organic acid, and glutamic acid but higher soluble protein and sodium than fresh bekasam. The dry powder had lighter brown color and specific bekasam odor with less intensity which is physically better than fresh bekasam. Dry catfish bekasam powder therefore can serve as flavor enhancer.

Keywords: Flavor enhancer, monosodium glutamate, salt, fish fermentation

1. INTRODUCTION

Flavor enhancer has become a widely used food ingredient in various types of eatables, ranging from dry snacks to home cooked food. The most popular flavor enhancer in Indonesia is Monosodium Glutamate (MSG). Monosodium glutamate was also used to reduce salt use in cooking as it enhances the existing saltiness in food [1]. There was a long controversy regarding MSG use. A study of subcutaneous MSG injection in adult male mice showed that it disturbed lipid metabolism and the activity of antioxidant enzymes in blood tissue [2]. Another study showed that high MSG in a diet might increase blood pressure [3, 4]. In 1986, however, the Advisory Committee on Hypersensitivity to Food Constituent, FDA stated that MSG use was safe although there might be short term reaction in certain groups [5, 6]. Regardless of the controversy, there is a growing interest toward traditional flavor enhancer, one of which is a fermented fish product which is known to be appetizing when added to food. The interest is of natural consequences as glutamate is innately found in fermented foods. Bekasam is traditionally made of skipjack (*Katsuwonus pelamis* Linnaeus, 1758) viscera which is fermented with

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*Email: Retno Murwani: rmurwani@gmail.com

addition of salt [7–9], however other types of fish can be used. The product has a distinctive acidic and salty taste and aromas. Recently bekasam has gained interest as it showed an inhibitory activity against hipertensive enzyme, i.e., Angiotensin-I-Converting Enzyme (ACE). Bekasam made of milk fish and fermented for 6 d showed 51.77% ACE inhibitory activity [10]. The acidic, saltiness, and ACE inhibitory activity of bekasam made it a good candidate as flavor enhancer. In the following study fresh bekasam made of catfish [*Clarias batrachus* (Linn, 1758)] is investigated. Catfish is inexpensive, high in protein, essential fatty acids, lysine, leucine, and phosphor [11, 12]. The aim of this study was to characterize the physicochemical properties of fresh *bekasam* and *bekasam* powder made of catfish (*C. batrachus*).

2. MATERIALS AND METHODS

2.1 Fresh and Dry Powder Bekasam

Catfish (*C. batrachus*) from local market with an average weight of 200 g to 225 g was cleansed from viscera and head and split into two parts. They were then rinsed and added with 10 % salt (w/w), stored in close container and stood for 24 h at room temperature. It was then added with toasted glutinous rice (1:1) and then fermented for 6 d. The resulting *bekasam* was homogenized by waring blender, steamed for 40 min, and the water content was removed by pressing. The remaining bekasam was dried by cabinet dryer at 45 °C for 6 h. The dry *bekasam* was ground into a fine powder. Dry bekasam powder was kept in close container at room temperature with silica gel as moisture absorber prior to analysis.

2.2 pH Analysis

Approximately 20 g of each sample (fresh or powder) was added into 100 mL of distilled water, and homogenized by stomacher for 10 min. pH value was measured using a pH meter [10].

2.3 Sample Preparation to Determine Total Organic Acid, Soluble Protein, Sodium Content and Glutamic Acid

Fresh or dry powder samples were blended finely and diluted with deionized water (1:3 ratio). They were further homogenized for 10 min and centrifuged at 20 000 × g for 10 min [g = Relative Centrifugal Force (RCF)]. The supernatant was removed and filtered and ready for analysis.

2.4 Determination of Total Organic Acid

Previously prepared samples of 10 mL were added with three drops of phenolphthalein (PP) indicator and then titrated with 0.1 N of NaOH. Titrations were terminated when pink color appeared. Total organic acid was measured as percent of lactic acid according to the Equation below [10]:

$$\%\ Lactic\ acid = \frac{mL\ \ NaOH\ \times\ N\ \ NaOH\ \times\ \frac{1}{10}\ \times\ 90}{mL\ \ sample} \quad (1)$$

Titration was conducted in triplicates.

2.5 Soluble Protein Analysis

Soluble protein was determined by Lowry method. Sample of 10 mL was added with 5 mL of trichloroacetatic acid and centrifuged at 5000 × g for 20 min. The supernatant of 0.1 mL were added with 3.9 mL of distilled water and 5.5 mL of Lowry reagent. The mixture was left undisturbed for 10 min, then 0.5 mL of Folin reagent was added and incubated for 30 min at room temperature. The standard protein was Bovine Serum Albumin (BSA). Soluble protein content was measured at 600 nm. Determination was done in triplicates [10].

2.6 Sodium Content Analysis

Sodium content was determined by atomic absorption spectrophotometry (AAS) at 589 nm wavelength. 15 mL of HNO_3 was added to the prepared 15 mL aliquot and left for 24 h, after which it was heated for eight h at 80 °C until the solution became clear. After cooling at room temperature, the sample was diluted to 100 mL with double distilled water, then was filtered. The initial ~10 mL of filtrate was discarded and the remaining was kept in a glass bottle for chemical analysis. The analyses were done in triplicates [13].

2.7 Determination of Glutamic Acid

Glutamic acid content was measured by spectrophotometry at 565 nm wavelength. Sample

extract of 20 µL was mixed with 60 µL of buffer, 1 µL of enzyme, 5 µL of NAD, and 14 µL of MTT; and then homogenized. The mixture was incubated at room temperature for 30 min. Glutamic acid determination was done in three replicates and using 100 mM glutamate to construct a standard curve of 0-2.5 mM) [14].

2.8 Statistical Analysis

All data were analyzed to obtain the average value of each parameter and its standard deviation. A change in pH, organic acid, soluble protein, sodium, and glutamic acid content between fresh bekasam and bekasam powder were analyzed by pair t-test dan discussed descriptively.

3. RESULTS AND DISCUSSION

Fresh bekasam is of light brown color having a softer texture (thick paste) compared with fresh fish meat. The odor was dominated with sulphury meat like aroma, ammonia, acid vinegar, sour, and fishy. In this study, the fresh bekasam colour, odour, and texture were similar to the earlier studies of bekasam [7, 11]. However, bekasam powder was lighter in color (Fig. 1) and the odor was similar to typical fresh bekasam but with lighter intensity.

The result of this study showed that processing of fresh bekasam could alter the physicochemical properties (Table 1). pH value of fresh bekasam and

(a) (b)

Fig. 1. (a) Fresh bekasam, **(b)** Bekasam powder (before sieving).

bekasam powder were mildly acidic (pH 6.95 and pH 6.51 respectively), and similar to bakasang from home production in Manado and Bitung, North Sulawesi which showed a pH value range from pH 6.42 to pH 6.72 [11]. It is well known that the pH value is the result of microbial activity such as lactic acid bacteria Lactobacillus plantarum (Orla-Jensen, 1919) which breaks down carbohydrate via glycolysis in the absence of oxygen. In the absence of oxygen, piruvate is converted to lactic acid which decreases the pH. Mild pH value of fresh bekasam in this study was also associated with low use of salt (10 %). In bekasam made of mackerel (Rastrelliger sp.), as salt concentration increased from 30% to 50%, the decline in pH was larger [15]. Drying reduced pH slightly from pH 6.95 in fresh bekasam to pH 6.51 in dry bekasam powder which was possibly due to evaporation of some organic acid [16]. Such evaporation was also reflected by a higher decrease in total organic acid (Table 1).

Sodium content of fresh bekasam was 4.67 %

Table 1. Physicochemical characteristics of fresh and dry powder of catfish *bekasam.*

Physicochemical Characteristics	Fresh Catfish *Bekasam*		Catfish *Bekasam* Powder		Difference (Δ)	p value
	Wet Weight	100 % Dry Weight	Wet Weight	100 % Dry Weight		
Water content (%)	57.14	-	5.548	-	-	-
Dry matter (%)	42.86	-	94.452	-	-	-
pH	6.95 ± 0.01	-	6.51 ± 0.01	-	-	0.001*
Total organic acid (%)	3.60 ± 0.017	8.40 ± 0.040	2.60 ± 0.013	2.75 ± 0.014	5.65 ± 0.026	0.0001*
Sodium (%)	2.001 ± 0.003	4.67 ± 0.006	9.32 ± 0.005	9.87 ± 0.005	5.20 ± 0.011	0.0001*
Soluble protein (mg mL^{-1})	0.68 ± 0.003	1.59 ± 0.006	1.99 ± 0.002	2.11 ± 0.002	0.52 ± 0.007	0.0001*
Glutamic acid (mg g^{-1})	5.09 ± 0.008	11.88 ± 0.025	5.87 ± 0.008	6.22 ± 0.008	5.67 ± 0.036	0.0001*

All values are means ± SD ,
* Significant at $P < 0.05$

(dry weight) and increased to 9.87 % (dry weight) in bekasam powder. Drying would alter microscopic structure of food tissue which could lead to a change in macroscopic structure. During drying, heat and mass were transferred simultaneously. Heat transfer occurred in order to raise food temperature and evaporate moisture. Mass transfer occured due to moisture transfer from inner part to outer part of the food matrix which was finally evaporated. The loss of moisture during drying would damage cell wall and tissues of food matrix. Increase sodium content due to drying was common in various foods [17–20].

The soluble protein in fresh bekasam or bekasam powder was due to proteolitic bacteria during fermentation [21, 22]. The soluble protein content in this study (1.59 mg · mL^{-1} and 2.11 mg · mL^{-1}, respectively) was higher than the milkfish bekasam (0.74 ± 0.01 mg · mL^{-1}) [10]. Drying increased the soluble protein content due to reduced water content.

Glutamic acid content in bekasam was part of protein degradation product during fermentation by proteolytic bacteria to produce smaller peptide and free amino acids [22, 23]. Free glutamic acid can be produced effectively at optimal fermentation temperature of 32 °C to 37 °C. Increased production of glutamic acid was proportional to the increase of microbial growth. At optimum fermentation temperature, bacterial cell released the glutamic salt. The rate of glutamic acid production increased in line with the increase of fermentation temperature, but when fermentation temperature reached 42 °C, glutamic acid production declined. Increase temperature during fermentation could inhibit the formation of α-ketoglutarat dehydrogenase complex. This would change the production pathways of 2-oxoglutarate compound into glutamic acid so that the rate of glutamic acid production would increase [24].

Glutamic acid formation during fermentation was also affected by pH. Glutamic acid production by L. plantarum MNZ achieved the highest production at acidic condition (pH 4.5). However, further pH decline could reduce glutamic acid production. L. plantarum produced glutamic acid and the highest growth rate was achieved at pH 6.5 [25]. Drying fresh bekasam to produce dry powder reduced glutamic acid content due to lower water content.

4. CONCLUSIONS

Fresh catfish bekasam has light brown color and is softer in texture compared with fresh fish meat; its aroma is of sulphury-like meat, ammoniac acid vinegar like, sour, and slightly fishy. The dry powder produced from fresh bekasam has a lighter brown color with specific bekasam odor, but lesser in intensity, which is physically more attractive than fresh bekasam. Bekasam powder has lower pH, total organic acids, and glutamic acid but more soluble protein and sodium content compared with fresh bekasam. Further studies are warranted to compare bekasam dry powder as flavor enhancer in various foods with the flavor enhancer powders available in the market.

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6. REFERENCES

1. Jinap, S. & P. Hajeb. Glutamate: Its applications in food and contribution to health. *Appetite* 55: 1–10 (2010).
2. Singh, K. & P. Aeluwalia. Studies on the effect of monosodium glutamate (MSG) administration on some antioxidant enzymes in the arterial tissue of adult male mice. *Journal of Nutrition Science and Vitaminology* 49: 145–148 (2003).
3. Baad-Hansen, L., B. Cairns, M. Ernberg & P. Svensson. Effect of systemic monosodium glutamate (MSG) on headache and pericranial muscle sensitivity. *Journal of Cephalgia* 30(1): 68–76 (2010).
4. Shi, Z., B. Yuan, W. A. Taylor, Y. Dai, X. Pan & K. T. Gill. Monosodium glutamate is related to a higher increase in blood pressure over 5 years: Findings from the Jiangsu Nutrition Study of chinese adults. *Journal Hypertensy* 92(5): 846–853 (2011).
5. Food and Agricultural Products Center (FAPC). Studies find MSG is not harmful to health. [Online] From: http://fapc.okstate.edu (2003). [Accessed on

20 March, 2013].

6. FDA. Questions and answers on monosodium glutamate (MSG). [Online]. From: http://www.fda.gov/Food/Ingredients PackagingLabeling/FoodAdditivesIngredients/ucm328728.htm (2012). [Accesed on 30 March, 2016].
7. Ijong, G.F. & Y. Ohta. Physicochemical and microbiological changes associated with Bakasang processing: A traditional Indonesian fish sauce. *Journal of the Science of Food and Agriculture* 71: 69–74 (1996).
8. Purwaningsih, S., R. Garwan & J. Santoso. Karakteristik organoleptik Bakasang jeroan Cakalang (Katsuwonus pelamis, Lin) sebagai pangan tradisional Maluku Utara [Organoleptic characteristics of Innard Skipjack Tuna (*Katsuwonus pelamis*, Lin.) Bakasang as traditional food in North Maluku]. *Jurnal Gizi dan Pangan* 6(1): 13–17 (2011) [in Bahasa Indonesia].
9. Ingratubun, J. A., F. G. Ijong & H. Onibala. Isolasi dan identifikasi bakteri asam laktat pada Bakasang sebagai starter mikroba produk fermentasi [Isolation and identification of lactic acid bacteria in Bakasang as fermented microbe starter]. *Aquatic Science & Management (Jurnal Ilmu dan Manajemen Perairan)* 1: 48–56 (2013) [in Bahasa Indonesia].
10. Wikandari, P. R., Suparmo, Y. Marsono & E. S. Rahayu. Potensi bekasam Bandeng (*Chanos chanos*) sebagai sumber angiotensin I converting enzyme inhibitor [The potency of milkfish (*Chanos chanos*) *bekasam* as source of angiotensin I converting enzyme inhibitor]. *Biota* 16(1): 145–152 (2011) [in Bahasa Indonesia].
11. Harikedua, S. D., C. H. Wijaya & D. R. Adawiyah. Relationship between sensory attributes of Bakasang (a traditional Indonesian fermented fish product) and its physicochemical properties. *Fisheries Science* 78: 187–195 (2012).
12. Hidayati, L., L. A. Chisbiyah & T. M. Kiranawati. Evaluasi mutu organoleptik Bekasam ikan wader [Organoleptic quality evaluation of Bekasam from Silver rasbora]. *Jurnal Teknologi Industri Boga dan Busana* 3(1): 44–51 (2012) [in Bahasa Indonesia].
13. Pardede, vT. R. & M. D. S. Sri. Penetapan kadar kalium, natrium dan magnesium pada semangka (*Citrus vulgaris,* Schard) daging buah berwarna kuning dan merah secara spektrofotometri serapan atom [Determination of potassium, sodium and magnesium content in red and yellow watermelon (*Citrus vulgaris* Schard)]. *Jurnal Darma Agung* 2012: 1–7 (2012) [in Bahasa Indonesia].
14. Bio Assay System. EnzyChrom TM glutamate assay kit (EGLT-100): quantitative colorimetric determination of glutamate at 565 nm. [Online]http://www.gentaurpdf.com/pdf/EnzyChrom%20Glutamate%20Assay%20Kit.pdf (2009) [Accessed on 20 February 2013].
15. Desniar, D. P. & W. Wijatur. Pengaruh konsentrasi garam pada peda ikan kembung (*Rastrelliger sp.*) dengan fermentasi spontan [The influence of salt concentration on *peda* chub mackerel (*Rastrelliger sp.*) with spontaneous fermentation]. *Jurnal Pengolahan Hasil Perikanan Indonesia* 12(1): 73–87 (2009) [in Bahasa Indonesia].
16. Afoakwa, E. O., A. S. Budu, H. Mensah-Brown, J. F. Takrama & E. Akomanyi. Changes in biochemical and pysico-chemical qualities during drying of pulp preconditioned and fermented cocoa (*Theobroma cacao*) beans. *Journal of Nutritional Health & Food Science* 2(3): 1–8 (2014).
17. Özcan, M., D. rslan & A. Ünver. Effect of drying methods on the mineral content of basil (*Ocimumbasilicum* L.). *Journal of Food Engineering* 69: 375–379 (2005).
18. Ramos, I. N., T. R. S. Brandao & C. L. M. Silva. Structural changes during air drying of fruits and vegetables. *Food Science and Technology International* 9(3): 201–206 (2003).
19. Haghi, A. K. & H. Ghanadzadeh. A study of thermal drying process. *Indian Journal of Chemical Technology*, 12: 654–663 (2005).
20. Latapi, G. & D. M. Barret. Influence of pre-drying treatments on quality and safety of sun-dried tomatoes. Part I: Use of steam blanching, boiling brine blanching, and dips in salt or sodium metabisulfite. *Journal of Food Science* 71(1): 24–31 (2006).
21. Wikandari, P. R., Suparmo, Y. Marsono & E. S. Rahayu. Karakterisasi bakteri asam laktat proteolitik pada *bekasam*. [Characterization of Proteolitic lactic acid bacteria from Bekasam]. *Journal Natur Indonesia* 14(2): 120-125 (2012) [in Bahasa Indonesia].
22. Murwani, R., Supriyadi, Subagio, T. Agus & Ambariyanto. Isolation and identification of thermophilic and mesophylic proteolytic bacteria from terasi. In: Hadiyanto, H. Nur, A. Budiman, F. Iskandar & S. Ismadji (Eds.), *AIP Conference Proceedings, 16699*, 030005 (2015).
23. Ijong, G. F. & Y. Ohta. Amino acid compositions of

Bakasang, a traditional fermented fish sauce from Indonesia. *LWT-- Food Science and Technology* 28(2): 236–237 (1995).

24. Zareian, M., A. Ebrahimpour, A. Mohamed & N. Saari. Modelling of glutamic acid production by *Lactobacillus plantarum* MNZ. *Electronic Journal of Biotechnology* 16 (4): 2–16 (2013).
25. Zacharof, M. P. & R. W. Lovitt. Development of an optimized growth strategy for intensive propagation, lactic acid and bacteriocin production of selected strains of *Lactobacilli* genus. *International Journal Chemical Engineering and Application* 1(1): 55–83 (2010).