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THE PHYSICOCHEMICAL CHARACTERISTICS AND ANGIOTENSIN CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY OF SKIPJACK TUNA (*Katsuwonus pelamis*) "BAKASANG"

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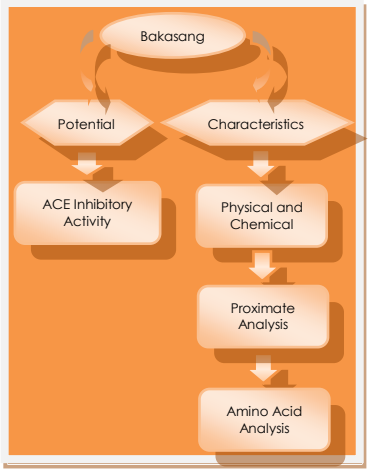
Graphical abstract



Abstract

Bakasang is a traditional fermented fish product which is often used as condiments. This study aimed to determine the physical and chemical characteristics and potential ACE (Angiotensin Converting Enzyme) inhibitor of bakasang. Color and viscosity were physical characteristics measured in which color was presented in value of L* (36.05), a* (18.76), b* (15.65), while viscosity value was 6 950 cP. The result of chemical characteristics including salinity, acidity, pH, TVB-N and LAB were 72 %, 2.56 %, 4.66, 36.88 mg N per 100 g and 3.32 log CFU g^{-1} respectively. Proximate and amino acid compositions analysis were also identified, resulting in 14.77 % protein, 1.11 % fat, 57.15 % moisture, 25.97 % ash and 1.00 % carbohydrate while the predominant amino acid found was histidine. The ACE inhibitory activity of the isolated bioactive peptides of bakasang was 68.80 %.

Keywords: Angiotensin Converting Enzyme (ACE) Inhibitor, bakasang, bioactive peptides, skipjack tuna [*Katsuwonus pelamis* (Linnaeus, 1758)]

1.0 INTRODUCTION

Bakasang is a traditional fermented fish product of Banda island, Maluku Province, Indonesia. It is made from Skipjack tuna meat with the addition of 20 % salt, followed by fermentation process under the sun for 7 d to 14 d and can then be stored for a few months.

Bakasang is a daily consumed food of Banda society as it is an inherited food which is still consumed up to now. It is usually used as condiment for cooking and flavoring agent for a number of food. Some studies have shown that fish sauce, similar to bakasang, contains nutrients that are beneficial for health. It contains about 20 g L^{-1} of nitrogen and 80 % of which are in the form of amino acids [1].

Hydrolysis of fish protein produces free amino acids, peptides and ammonia. High salt concentrations can control the growth of pathogens and produce preferred flavor and aroma. Some studies have shown that lactic acid bacteria are able to degrade protein into peptides that can inhibit Angiotensin Converting Enzyme (ACE) activity, the hypertension triggering enzyme. Inhibition of ACE activity would prevent hypertension. The research of ACE inhibitors in fermentation products has been widely carried out on milk and fishery products [2–8]. Some studies have shown that lactic fermentation on fish products provides ACE inhibitory activity [9–13].

This study aimed to determine the physical and chemical characteristics of bakasang and potential of isolated bioactive peptides as ACE Inhibitor.

2.0 EXPERIMENT

2.1 Raw Material and Sampling Method

Bakasang (Indonesia fermented fish) used in this study was obtained from Banda island, Maluku Province of Indonesia. Bakasang samples were made from Skipjack tuna [*Katsuwonus pelamis* (Linnaeus, 1758)] meat which was added with salt in the ratio 5:1 (w/w). Fermentation was conducted for 7 d to 14 d, after which it was stored for 2 mo to 3 mo. The sampling technique used purposive sampling method. Total samples prepared were 14 samples and each of it was packaged in a bottle containing 300 mL bakasang. However, only three samples were used for analysis by random selection, with assumption that whole samples are the same.

2.2 Preparation and Isolation of Bioactive Peptides from Bakasang

The isolation of bakasang bioactive peptides was conducted according to the method of Wang *et al*. [14] with some modifications. About 100 g of bakasang was dissolved in sterile distilled water with ratio 1:5 (w/v), followed by homogenization and inactivation at 90 °C for 10 min. The next step was centrifugation (Tomy MX–305) at 7 000 × g, 4 °C, for 20 min (× g = Relative Centrifugal Force [RCF]). After that, supernatant was added with ethanol in ratio 1:1 (v/v) while isolated bioactive peptides were added to the Tris-HCl buffer (pH 6.8) and stored at -20 °C before use.

2.3 Analysis Procedure

2.3.1 Determination of Physical Characteristics (Color and Viscosity)

The color of bakasang was measured with a color reader (Minolta CR–10) to determine the L*, a* and b* of the sample. Meanwhile, viscosity was measured with Rotational viscometer (Elcometer 2300).

2.3.2 Determination of Chemical Characteristics

2.3.2.1 pH and Acidity

The acidity of bakasang was analysed through pH measurement using a Senz pH digital tester (Trans instruments). The total acid was determined using titration method, by adding three drops of phenolphthalein indicator into 10 mL of suspension before titrated with 0.1 N NaOH solution [15].

2.3.2.2 Salinity

About 1 g of sample was added with 25 mL to 50 mL $AgNO_3$ and 20 mL of 0.1 N HNO_3. Then it was heated using a hot plate in the hood until all the solids dissolved except AgCl. The sample was cooled at room temperature and was added with 50 mL of halogen–free water and ferric indicator. Afterwards it was titrated with 0.1 N NH_4CNS until the solution turned light brown. Then a volume of 0.1 N NH_4CNS for titration was recorded [15].

2.3.2.3 Total Volatile Basic Nitrogen (TVB-N)

Total Volatile Basic Nitrogen (TVB-N) was analysed by mixing 5 g of bakasang with 15 mL of TCA 5 % and homogenized, then the solution was filtered with a filter paper. Boric acid (1 mL) was added to the conway cup in the inner chamber while 1 mL sample was put into the outer chamber. K_2CO_3 solution was included in the different sides of the outer chamber, and sealed. Blank solution was made with 3 % TCA. The cup was closed, shaken for 1 min and incubated for 2 h. After incubation, a solution of boric acid in the inner chamber was titrated with 0.01 N HCl until the color turned pink [16].

2.3.2.4 Total of Lactic Acid Bacteria (LAB)

Analysis of lactic acid bacteria was carried out by putting 10 g of bakasang into 90 mL of diluent (sterile 0.85 % NaCl) and homogenized. About 1 mL of the initial dilution was pipetted into 9 mL of diluent solution until the desired degree of dilution was reached. Through each degree of serial dilution, 1 mL sample was pipetted into a Petri dish and poured with 15 mL MRSA (Man Rogosa Sharp Agar) into a cup, shaken horizontally on a flat surface so that the sample could spread evenly. Then it was kept until frozen. The petri dish was incubated upside down at 37 °C for 48 h. The colonies grown on plates were calculated as the number of colonies per gram [17].

2.3.3 Analysis of Proximate and Amino Acid Composition

Proximate composition consisting of protein, fat, moisture and ash was analyzed based on Association of Official Analytical Chemists (AOAC) whereas carbohydrates was determined by different [14, 15]. Amino acid was analyzed by putting 0.5 g of sample into a beaker 25 mL then added with 10 mL of 6 N HCl. The beaker was heated at 100 °C for 24 h. After that, samples were filtered and the filtrate was taken.

The filtrate was added with 5 mL of dryer (methanol, picolotiocianat, triethylamine) and then dried. Derivative solution (methanol, Na-acetate, and triethylamine) was added and the sample was allowed to stand for 20 min. About 200 mL solution of 1 M acetate was added and the sample was injected into the HPLC. The HPLC instrument was conditioned as follows: at room temperature, use

pico tag column 3.9 × 150 mM, flow rate is 1.5 mL · min^{-1}, pressure limit is 3 000 psi (1 psi = 108.21976139216164 kPa), the gradient program, the mobile phase is 60 % acetonitrile and sodium acetate buffer 1 M, and UV detectors with a wavelength of 254 nM.

2.3.4 Determination of ACE Inhibitor

The ACE inhibitor activity assay was performed according to the method of Cushman and Cheung [18] with slight modification. About 50 mL of sample solution and 50 mL solution of ACE (≥ 2.0 units per mg protein, from Sigma-Aldrich Chemie) were pre–incubated at 32 °C for 10 min. The mixture was then incubated for 30 min at the same temperature with the addition of 50 mL of substrate (Hip-His-Leu 8 mM in buffer 50 mM HEPES from Sigma-Aldrich Chemie) containing NaCl 300 mM at pH 8.3. The reaction was terminated by the addition of 1 M HCl (200 mL). The solution was extracted with the addition of 1.5 mL ethyl acetate, and centrifuged (4 000 × g) for 15 min. After that, 1 mL of the supernatant was transferred to another test tube and was evaporated until dry (63 °C, for 45 min). Once dried, it was dissolved in 1 mL of distilled water and the absorbance was determined at a wavelength of 228 nM using a UV–Vis spectrophotometer (Thermo spectronic).

$$\%\text{Inhibition} = \frac{(B-A)}{(B-C)} \times 100\% \quad (1)$$

A = absorbance in the presence of the enzyme and angiotensin converting enzyme inhibitor compound
B = absorbance of the enzyme without inhibitor compounds
C = absorbance in the absence of enzymes and enzyme inhibitors.

3.0 RESULTS AND DISCUSSION

The characteristics of bakasang were determined through physical analysis (color and viscosity) and chemical analysis (pH, acidity, salinity TVB-N, LAB). In addition, proximate analysis to measure protein, fat, moisture, ash and carbohydrates content of *bakasang* was carried out, followed by analysis of amino acid composition. In order to determine the ACE inhibitory activity, bioactive peptides were isolated from bakasang.

3.1 Characteristics of Bakasang

3.1.1 Physical Characteristics (Color and Viscosity)

Color is one parameter determining the quality of food products. Colors are often used to observe changes in both physical and chemical properties of food product. One purpose of color measurement is to determine the value of L*, a*, and b*. L* (Lightness) indicates the level of brightness of a color in which 0 represents black and 100 represents white. Meanwhile, a* shows green and red colors, whereas b* shows blue and yellow colors. Bakasang color indicates the value of L* (36.05 ± 0.57), a* (18.76 ± 1.44), and b* (15.65 ± 0.53). Bakasang color shows the tendency of the same color, seen through insignificant differences in value of L*, a* and b*. The color of the fish sauce is influenced by the differences in the types, the proportions of fish meat and additives used, the manufacturing technique, the fermentation conditions and the preparation methods of fish meat [19].

Viscosity is one of the rheology properties that is important in food products. Viscosity is required for quality testing, standardization of quality and control process during the processing. The viscosity value of this research is considered high, which was 6 950 cP (1 cP = 1 mPa s). Bakasang is a semi–solid product with fairly high level of viscosity. Viscosity is also affected by the amount of salt added. The salt can draw water from the food that can reduce the moisture content and a_w of food products, then it will increase the level of viscosity. High salt concentrations can cause fish tissue hardens due to the increase of osmotic pressure.

3.1.2 Chemical Characteristics

Bakasang is a fish fermented product and the fermentation process is influenced by several factors such as pH, acidity and salinity. During the fermentation process, TVB-N and LAB values change and tend to increase. Chemical characteristics of bakasang can be seen in Table 1.

Table 1 The chemical characteristics of bakasang

Components	Contents*)
pH	4.66 ± 0.72
Acidity (%)	2.56 ± 0.58
Salinity (%)	13.72 ± 1.21
TVB-N (mg N per 100 g)	36.88 ± 2.03
LAB (log CFU · g^{-1})	3.32 ± 0.29

*) value ± se

The average pH value of bakasang is 4.66. In general, the fermentation product provides an acidic environment. Some research shows that the pH value of fermented fish product is low, ranging from 4.8 to 5.2; 4.90 to 6.23; 5.1 to 5.8 and 3.60 to 5.30 [1, 20–22]. The average salinity of the study was 13.72 %. Salinity mahyaveh (traditional fish sauce in Iran) ranged from 8.19 % to 17.1 % [23]. Salt in the fermentation process is used as a flavoring and preservative agent that inhibits the growth of spoilage bacteria and pathogens. The higher concentration of salt affected the shelf life of bakasang.

Total acid of bakasang in this research is 2.56 %. These results indicate a fairly high total acid, compared with fermented fish products in Thailand (Plasom) which ranged between 0.3 to 0.9 %, with fermentation time from 0 h to 8 h [20]. In this study, the fermentation time of bakasang was carried out in 7 d to 14 d and was kept for 2 mo to 3 mo. During the fermentation time, total acid will increase. The presence of organic acids in bakasang tends to lower the pH value and increase the total acid value, causing a sour taste in bakasang.

Fermentation process also changes the TVB-N value which increases during the process [24]. Several studies of fermented fish products result in different TVB-N value, depending on the raw materials used, the length of fermentation, and the fermentation conditions. TVB-N value indicates the hydrolysis presence of proteins by the activity of enzymes and bacteria which produce amino compounds that may lower the quality of product [25]. The average of total lactic acid bacteria produced was 3.32 log CFU g^{-1}. The total lactic acid bacteria of traditional fish sauce from Iran (mahyaveh) was ranged from 0 log CFU $\cdot g^{-1}$ to 5.54 log CFU g^{-1} [23].

LAB is the dominant microorganisms in a fermentation product of fish. In addition, LAB is a microorganism that is commonly used in food preservation techniques, such as fermentation. The use of lactic acid bacteria in preservation is mainly intended for providing the acidic condition which can suppress the growth of harmful bacteria and food poisoning [21].

3.1.3 Proximate Compositions

Bakasang is a semi solid product with high moisture content. It also contains nutritional composition including high amount of protein and ash, followed by fats and carbohydrates (Table 2).

Table 2 Proximate compositions of bakasang.

Components	Contents (%) *)
Protein	14.77 ± 3.05
Fat	1.11 ± 0.50
Moisture	57.15 ± 2.00
Ash	25.97 ± 5.00
Carbohydrate (by different)	1.00

*) value ± se

Table 2 shows that the ash content was higher than the other proximate parameter values after the moisture content. Bakasang moisture is derived from fish meat and total dissolved solids hydrolysis [25]. Additional salt can draw some water which can lower water content in bakasang. High ash content is probably derived from additional salt in the fish meat as a flavoring agent and to extend the product shelf life. In addition to salt, it is also sourced from minerals. The average protein content is 14.77 %. The protein content of bakasang is derived from fish meat and the hydrolysis during the fermentation process also produces a number of short chain protein.

3.1.4 Amino Acid Compositions

Bakasang contains essential amino acids and nonessential amino acids required by the human body. The amino acid compositions from bakasang are given in Table 3. During the fermentation process, protein is hydrolyzed into peptides and amino acids. The hydrolysis of proteins into peptides and amino acids is influenced by several factors such as the condition of fermentation, the fermentation time and the total LAB.

Table 3 Amino acid compositions of bakasang

Amino Acids	Contents (% · g^{-1} samples)
Valine*	0.91
Threonine*	1.25
Lysine*	2.72
Serine	1.33
Isoleucine*	0.81
Alanine	1.76
Histidine*	3.36
Phenylalanine*	1.18
Glutamate	3.30
Tyrosine	0.97
Proline	0.82
Arginine*	1.68
Glycine	1.62
Leucine*	2.06
Aspartate	2.30
Methionine*	0.85
Cysteine	0.04
Total	**26.97**

*Essential amino acids

According to amino acid composition, it can be seen that there are some dominant essential amino acids in bakasang, namely histidine (3.36 % g^{-1} of protein), lysine (2.72 % g^{-1} of protein) and leucine (2.06 % g^{-1} of protein). Meanwhile, the dominant nonessential amino acids were found as glutamate (3.30 % g^{-1} of protein), aspartate (2.30 % g^{-1} of protein) and alanine (1.76 % g^{-1} of protein).

Fermented fish products produce glutamic amino acid that serves as a flavor enhancer. Fish sauce (yu-lu) which is obtained through traditional fermentation process in China provides dominant amino acids, including glutamic acid, lysine, leucine, valine, proline, and a small amount of alanine [24]. Histidine of bakasang is higher than other amino acids due to high amount of histidine contained in the Skipjack tuna fish, species of scrombidae.

3.2 ACE Inhibitor Activity

The results of three samples of bakasang show the difference in ACE inhibitory activity, with average

value 68.80 % (Table 4). Compared with the other products, it has been found that ACE inhibitory activity of bekasam (Indonesian fermented fish) was 55.17 % [26] while douchi, a Chinese traditional fermented soybean contained 56.8 % to 76.3 % ACE inhibitory activity [27].

ACE inhibitory activity is produced by the growth of various lactic acid bacteria especially kinds of proteolytic lactic acid bacteria. *L. sakei*, *L. farciminis*, and *L. plantarum* have been involved in producing ACE inhibitory peptides [28]. In addition, *L. helveticus* has also been known to have high ability to produce bioactive peptide as ACE inhibitors [29]. The inhibitory effect of lactic acid bacteria depends on the species, the number of pathogenic bacteria and sanitation during the fermentation process.

The differences of percentage of ACE inhibitory activity might be influenced by the differences in the fermentation time. The ACE inhibitory activity of bakasang tested in this study has been kept for 3 mo. Related to the growth of LAB and protease activity, the ACE inhibitory activity should increase with fermentation time as protease will break down proteins into peptides [4, 30]. However, according to the ACE inhibitory activity of products aforementioned which are bekasam and douchi, it can be seen that the result was close to bakasang although the fermentation time was very short. Fermentation of bekasam was conducted for 6 d while douchi for 48 h to 72 h. It might be caused by the initial content of protein in raw material.

Table 4 ACE inhibitory activity of bakasang

Samples	ACE Inhibitor (%)
1	65.81
2	62.97
3	77.61
Average	**68. 80**

4.0 CONCLUSION

The physicochemical characteristics, proximate and amino acid compositions, and ACE inhibitory activity of fermented products were based on raw material, bioprocess, fermentation time and condition. This study showed that bakasang contained 68.80 % of ACE inhibitory activity, with physicochemical characteristics such as color value including L* (36.05); a* (18.76); b* (15.65), viscosity (6 950 cP), salinity (72 %), acidity (2.56 %), pH (4.66), TVB-N (36.88 mg N per 100 g) and LAB (3.32 log CFU g^{-1}). In addition, the proximate was composed of protein (14.77 %), fat (1.11 %), water (57.15 %), ash (25.97 %) and carbohydrate (1.00 %) while amino acid predominantly consisted of histidin (3.36 %) and glutamic acid (3.30 %). This result can be valuable information for further research and providing bakasang as a recommended condiment for wider society.

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References

[1] Tsai, Y. H.,C. Y. Lin, L. T. Chien, T. M. Lee, C. I. Wei and D. F. Hwang. 2006. Food Chemistry Histamine Contents of Fermented Fish Products in Taiwan and Isolation of Histamine-Forming Bacteria. *Food Chem.* 98(1): 64–70.

[2] Ichimura, T., J. Hu, D. Q. Aita and S. Maruyama. 2003. Angiotensin I-Converting Enzyme Inhibitory Activity and Insulin Secretion Stimulative Activity of Fermented Fish Sauce. *J. Biosci. Bioeng.* 96(5): 496–499.

[3] Je, J. Y., P. J. Park, W. K. Jung and S. K. Kim. 2005. Amino Acid Changes in Fermented Oyster (*Crassostrea gigas*) Sauce with Different Fermentation Periods. *Food Chem.* 91(1): 15–18.

[4] Zhang, J. H., E. Tatsumi, C. H. Ding and L. T. Li. 2006. Angiotensin I-converting Enzyme Inhibitory Peptides in Douchi, a Chinese Traditional Fermented Soybean Product. *Food Chem.* 98(3): 551–557.

[5] Rho, S. J., J. S. Lee, Y. I. Chung, Y. W. Kim and H. G. Lee. 2009. Purification and Identification of an Angiotensin I-converting Enzyme Inhibitory Peptide from Fermented Soybean Extract. *Process Biochem.* 44(4): 490–493.

[6] Pihlanto, A., T. Virtanen and H. Korhonen. 2010. Angiotensin I converting Enzyme (ACE) Inhibitory Activity and Antihypertensive Effect of Fermented Milk. *Int. Dairy J.* 20(1): 3–10.

[7] Ankolekar, C., M. Pinto, D. Greene and K. Shetty. 2012. In Vitro Bioassay Based Screening of Antihyperglycemia and Antihypertensive Activities of *Lactobacillus acidophilus* Fermented Pear Juice. *Innov. Food Sci. Emerg. Technol.* 13(January): 221–230.

[8] Moslehishad, M., M. R. Ehsani, M. Salami, S. Mirdamadi, H. Ezzatpanah, A. N. Naslaji, *et al*. 2013. The Comparative Assessment of ACE-inhibitory and Antioxidant Activities of Peptide Fractions Obtained from Fermented Camel and Bovine Milk by *Lactobacillus rhamnosus* PTCC 1637. *Int. Dairy J.* 29(2): 82–87.

[9] Lee, H. C. 2003. Creative Fermentation Technology for The Future [Online]. From: http://seafooduedavis.edu/iufost/lee.htm. [Accessed on 20 January 2015].

[10] Yin, S., L. J. Pan and C. L. Jiang. 2004. Effect of Lactic Acid Bacterial Fermentation on the Characteristics of Minced Mackerel. *J. Food Sci.* 67(2): 786–792.

[11] Itou, Y. and K. Akahane. 2004. Antihypertensive Effect of Heshiko, a Fermented Mackarel Product, on Spontaneously Hypertensive Rat. *Fish. Sci.* 1121(70): 1309−1323.

[12] Je, S. K. K., J. Young, J. Y. Park, W. K. Jung and P. J. Park. 2005a. Isolation of Angiotensin I Converting Enzyme (ACE) Inhibitor from Fermented Oyster Sauce, *Crassostrea gigas*. *J. Food Chem.* 90: 809–814.

[13] Je, S. K. K., J. Young, P. J. Park, H. G. Byun and W. K. Jung. 2005b. Angiotensin I Converting Enzyme (ACE) Inhibitory Peptide Derived from the Sauce of Fermented Blue Mussel, *Mytilus edulis*. *J. Bioresour. Technol.* 96: 1624–1629.

[14] Wang, N., G. Le, Y. Shi and Y. Zeng. 2014. Production of Bioactive Peptides from Soybean Meal by Solid State Fermentation with Lactic Acid Bacteria and Protease. *J. Food Sci. Technol.* 6(130): 1080–1085.

[15] Assosiation of Official Analytical Chemists (AOAC). 2005. *Official* Methods of Analysis. *17th ed.* Maryland: AOAC.

[16] Assosiation of Official Analytical Chemists (AOAC). 1995. *Official* Methods of Analysis. *Washington DC*: AOAC

[17] Jiang, S. T., L. J. Yin and C. L. Pan. 2002. New Technology for Producing Pasta-Like Fish Products Using Lactic Bacteria Fermentation. *J. Food Sci.* 65(8): 3114–3118.
[18] Cushman, D. W. and H. W. Cheung. 1971. Spectrophotometric Assay and Properties of the Angiotensin Converting Enzyme of the Rabbit Lung. *Biochem Pharmacol*. 20: 1637–1648.
[19] Riebroy, S., S. Benjakul, W. Visessanguan and M. Tanaka. 2005. Physical Properties and Microstructure of Commercial Som-fug, a Fermented Fish Sausage. *Eur. Food Res. Technol.* 220(5–6): 520–525.
[20] Park, J. N., Y. Fukumoto, E. Fujita, T. Tanaka, T. Washio, S. Otsuka, *et al*. 2001. Chemical Composition of Fish Sauces Produced in Southeast and East Asian Countries. *J. Food Compos. Anal.* 14(2): 113–125.
[21] Hwanhlem, N., S. Buradaleng, S. Wattanachant, S. Benjakul, A. Tani and S. Maneerat. 2011. Isolation and Screening of Lactic Acid Bacteria from Thai Traditional Fermented Fish (Plasom) and Production of Plasom from Selected Strains. *Food Control*. 22(3–4): 401–407.
[22] Desniar, I., A. Rusmana, Suwanto and D. N. R. Mubarik. 2013. Characterization of Lactic Acid Bacteria Isolated from an Indonesian Fermented Fish (Bekasam) and Their Antimicrobial Activity against Pathogenic Bacteria. *Emirates J. Food Agric.* 25(6): 489–494.
[23] Zarei, M., H. Najafzadeh, M. H. Eskandari, M. Pashmforoush, A. Enayati, D. Gharibi, *et al*. 2012. Chemical and Microbial Properties of Mahyaveh, a Traditional Iranian Fish Sauce. *Food Control.* 23(2): 511–514.
[24] Jiang, J. J., Q. X. Zeng, Z. W. Zhu and L. Y. Zhang. 2007. Chemical and Sensory Changes Associated Yu-lu Fermentation Process – A traditional Chinese Fish Sauce. *Food Chem.* 104(4): 1629–1634.
[25] Harikedua, S. D. 2009. Keterkaitan Profil Sensori Flavor Bakasang dengan Karakteristik Mutu Fisiko-Kimia dan Mikrobiologi [Correlation between Sensory Flavor Profile of 'Bakasang' with its Physicochemical and Microbiological Quality]. Thesis. Bogor: Bogor Agriculture University. [Bahasa Indonesia].
[26] Wikandari, P. R., Suparmo, Y. Marsono and E. S. Rahayu. 2011. Potensi Bekasam Bandeng (*Chanos chanos*) sabagai Sumber Angiotensin I Converting Enzyme Inhibitor [The Potency of Milkfish (*Chanos chanos*) Bekasam as Source of Angiotensin I Converting Enzyme Inhibitor]. *J. Biota.* 16(1): 145–152. [Bahasa Indonesia].
[27] Zhang, J. H., E. Tatsumi, C. H. Ding and L. T. Li. 2006. Angiotensin I-Converting Enzyme Inhibitory Peptides in Douchi, a Chinese Traditional Fermented Soybean Product. *Food Chem.* 98(3): 551–557.
[28] Basso, A. D., A. L. Picariello, G. Coppola, R. Tremonte, P. Musso and S. S. Luccia. 2004. Proteolytic Activity of *Lactobacillus sakei, Lactobacillus farciminis* and *Lactobacillus plantarum* on Sarcoplasmic Protein of Pork Lean. *J. Food Biochem*. 28: 195–212.
[29] Fuglsang, N. C., A. Rattray, F. P. Nilsson and D. Nyborg. 2003. Lactic Acid Bacteria: Inhibition of Angiotensin Converting Enzyme In Vitro and In Vivo. *Antonie Van Leeuwenhoek*. 83: 27–34.
[30] Rajwani, H. G. and L. Ananthanarayan. 2013. Angiotensin-I Converting Enzyme (ACE) Inhibitory Activity of Fermented idli Batter as Influenced by Various Parameters Prevailing During Fermentation. *J. of Food Ferment Technol*. 3(1): 71–77.