

INTRODUCTORY STUDY ON PROCESSING OF FERMENTED JACK BEAN (*Canavalia ensiformis*)

Widaningrum, Ermi Sukasih and Endang Yuli Purwani

Indonesian Center for Agricultural Post Harvest Research & Development
Jl. Tentara Pelajar No. 12 Bogor 16114
Email: widaningrum.adnan@gmail.com

(Diterima 09-01-2014; Disetujui 30-11-2015)

ABSTRACT

Tempe is a traditional fermented food in Indonesia and it is mainly made from soybean. There is imbalance between demand and supply of soybean for tempe production. Consequently, it has forced Indonesia to import large quantity of soybean to meet the local demand. In term of reducing soybean import, efforts must be done to substitute soybean with other local beans. Of one among many beans, jack bean (*Canavalia ensiformis*) had potency to be developed as material for making tempe. The objective of this research was to study physicochemical characteristics of jackbean tempe and did sensory evaluation. Soaking, drying, dehulling and splitting were applied prior to fermentation. Treatment done was fermentation time (12, 24, 36 and 48 hours). Value of pH, total count, and soluble protein were measured during fermentation process. The fungus grew well and jackbean cake tempe with dense mycelial growth was completely formed after 36 hrs of fermentation period. Soluble protein increased significantly from 0,24-0,26 mg/g during fermentation period, indicating that highly active proteolytic enzyme might exist. Sensory evaluation results showed that in the form of fried tempe, sensory properties of jackbean tempe was equivalent to soybean tempe, especially/particularly in terms of color, flavor, acidity, texture and overall acceptability. The results were significant to reduce dependency on soybean import by substituting it in the tempe production in Indonesia

Keywords : Soybean, jackbean (*Canavalia ensiformis*), tempe

ABSTRAK

Widaningrum, Ermi Sukasih dan Endang Yuli Purwani, 2015. Kajian awal pengolahan kacang Koro Pedang (*Canavalia ensiformis*) terfermentasi.

Tempe merupakan makanan fermentasi tradisional di Indonesia yang utamanya terbuat dari kedelai. Ada ketidakseimbangan antara permintaan dan pasokan kedelai untuk produksi tempe. Akibatnya, Indonesia terpaksa mengimpor kedelai dalam jumlah besar untuk memenuhi permintaan lokal. Dalam rangka mengurangi impor kedelai, perlu dilakukan upaya untuk mengganti kedelai dengan kacang-kacangan lokal. Kacang yang memiliki potensi untuk dijadikan bahan baku tempe yaitu kacang koro pedang (*Canavalia ensiformis*). Tujuan penelitian ini yaitu mempelajari sifat fisik, kimia dan sensori tempe yang dibuat dari kacang koro pedang. Perlakuan yang diterapkan yaitu waktu fermentasi (12, 24, 36 dan 48 jam). Parameter pengamatan yang diukur yaitu pH, total mikroba dan protein terlarut. Hasil penelitian menunjukkan bahwa kacang koro pedang cocok untuk produksi tempe. Ragi tumbuh dengan baik dengan pertumbuhan miselium padat terbentuk setelah 36 jam periode fermentasi pada tempe koro pedang. Protein terlarut meningkat secara signifikan dari 0,24 sampai 2,60 mg/g selama periode fermentasi, menunjukkan bahwa terdapat aktivitas aktif enzim proteolitik. Hasil analisis sensori memperlihatkan bahwa tempe koro pedang setara dengan tempe kedelai, terutama dalam hal warna, aroma, keasaman, tekstur, dan daya terima khususnya pada tempe yang disajikan setelah digoreng. Penggunaan koro pedang sebagai bahan baku tempe cukup signifikan untuk mengurangi ketergantungan pada impor kedelai dengan menggantikannya dalam produksi tempe di Indonesia.

Kata kunci : kedelai, koro pedang (*Canavalia ensiformis*), tempe

INTRODUCTION

Legume play a vital role as protein sources in developing countries such as Indonesia. Soybean is one type of legume that is used in Indonesia mostly for tempe. Tempe is a traditional fermented food in Indonesia and it is mainly derived from soybean. In Indonesia, more than 80% of soybean is processed into tempe and tofu. However, there was the imbalance between demand of soybean for tempe production and low production of soybean in the country. In 2011, national soybean demand was about 2.4 million ton, while soybean production was reduced from 974.51 thousand tons of dry beans in 2009 to 907.03 thousand in 2010¹. This condition has forced Indonesia to import large quantity of soybean to meet local demand. Use of indigenous sources to completely substitute soybean in high quality tempe production would help Indonesia to reduce dependence on expensive soybean import.

Researchers have tried to incorporate non oil-seed legume such as pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*) and tribal bean (*Canavalia virosa*) in the production of tempe^{2,3,4,5,6,7}. In the current study, jackbean (*Canavalia ensiformis* L.) was selected as substrate for tempe production due to its great potential to be developed. Jackbean is considered to be dispersed legume crop. Legumes are good sources of cheap and widely available proteins for human consumption. They are staple foods for many people in different parts of the world. Legume seeds have an average of twice as much protein as cereals and the nutritive value of the proteins are usually high. They range between the highly utilized legumes such as soybean, cowpeas to the lesser known ones. Studies have shown that the lesser known legumes together with other conventional legumes can be used for combating protein malnutrition prevalent in the third world⁸.

In Indonesia, jackbean grows under many different ecological conditions, and shows no marked seasonality compared to soybean. Jackbean was cultivated on marginal land especially in Central Java, East Java and Aceh. Potential production of jackbean cv. Tegak reached 2.9 to 6 tons/ha, much higher than the national soybean only 1.6 tonnes / ha. Other advantages, including the availability could be sustainable as an annual plant that can be harvested several times. Habitats of plants upright also does not require large tracts of land and loft as well as other jackbean, enough with trellis alone⁹.

The proximate composition of the jackbean seeds are 24-32% of crude protein, 1.8-9.6% of crude lipid,

4.65-10% of crude fibre, 2-4.6% of ash and 43-60% of carbohydrates¹⁰. Judging complete nutritional content, it is unfortunate that the jackbean has not been widely used by the public. Problems encountered in the use of the jackbean is the cyanogenic glucoside substances that pose less preferred flavor and reduce the bioavailability of nutrients in the body⁸. Cyanogenic glucosides act as precursors of free cyanide in jackbean, thus hydrolyzed perfect glucoside can produce free cyanide toxicity that can cause harmful effects. Accumulation of cyanide in the body lead to impair absorption of iodine and inhibits the absorption of protein. Therefore we need some treatment to reduce the cyanide content in jackbean used in food product that is safe for consumption. HCN maximum limit allowed by Food Agricultural Organization (FAO) to be consumed is <10 ppm cyanide at a safe level¹¹.

Jackbean is known to have anti-nutritional compounds such as concanavalin A, L-canavanine, canatoxin, polyamines, protease inhibitors, flatulence factors, cyanides, saponins, urease and L-Dopa⁸. Seed proteins of *Canavalia ensiformis* have considerably decreased cholesterol level in hypercholesterolemia rats¹². Marchetti et al.¹³ had also found inhibition of herpes simplex, rabies and rubella viruses by *C. ensiformis* lectins. In addition, Swaffar and Ang¹⁴ had also demonstrated the inhibitory effect of L-canavanine isolated from *C. ensiformis* against MIA PaCa-2 pancreatic cancer cells. The objective of the study was to investigate the physicochemical changes of jackbean in the production of tempe.

MATERIAL AND METHODS

Materials

Jackbean was supplied by farmers from Temanggung District, West Java. Tempe inoculum (*R. oligosporus*) in powder form was purchased from tempe producer at Rumah Tempe Indonesia, Bogor. Chemical used to analyze pH, protein, and proximate were purchased from local chemical stuff shop.

Physical Analysis

Physical characteristics of jackbean was determined by measuring the seed length, width and thickness and the weight of 10 seeds. The seed length, width and thickness were measured with a vernier calliper. Weight of seed was measured by analytical balance.

Dehulling of Jackbean

Jackbean was soaked for 24 hours, dried in convection drier at 60°C for 12 hours and dehulled mechanically using simple motor driven concrete disc impactors. The

bean was cut into smaller size (approximately 4 mm length in average).

Tempe Preparation

Jackbean tempe was prepared as following: dehulled jackbean was boiled for 20 minutes, and the boiling water was discarded. Boiled jackbean was soaked with tap water for 50 hours or until the pH value less than 5. Afterward, it was drained and steamed for 10 minutes, cooled to room temperature. Steamed jackbean was inoculated with tempe inoculum at the level of 0,1% of cooked bean, packed in perforated polyethylene bags (50 g cooked bean per pack) and incubated on a rack at 30°C for 12, 24, 36 and 48 hours. Time of fermentation became treatment.

Chemical Analysis

Both jackbean and tempe were subjected to chemical analysis. Major chemical component (moisture, fat, ash and protein) was analyzed according to AOAC method¹⁵. Soluble protein was analyzed according to Bradford Protein Assay (BioRad). The pH value was determined using a pH meter. Fungal growth was observed under light microscope. Total plate was analysed using PCA¹⁶.

Sensory Evaluation

Sensory evaluation of jackbean tempe was done using 30 panelists. Parameters tested were color, flavor, acidity, textures, taste, and general acceptability of panelists over jackbean tempe. For rating hedonic evaluation, panelists gave score of each jackbean tempe presented as follow: 5 = like, 4 = fairly like, 3 = neutral, 2 = fairly dislike, and 1 = dislike towards the jackbean tempe. For ranking evaluation, panelists gave 1 for the product they prefer most to be eaten, and gave 2 for the product they marked the second chance to be chosen.

Data Processing

Data achieved were analyzed using statistical analysis software SPSS 15.0.

RESULTS AND DISCUSSION

Physico-chemical Characteristic of Jackbean

Table 1. Physical characteristics of jackbean compared to soybean

Tabel 1. Karakteristik fisik koro pedang dan kedelai

Sampel	Mean length (cm)	Mean width (cm)	Mean thick (cm)
Jackbean	1.98	1.31	0.89
Soybean cv. Grobogan	0.86	0.70	0.58
Soybean cv. Kaba	0.67	0.55	0.41

Jackbean has different properties with soybean. Jackbean has a size of 2 to 3 times higher than soybean. Jackbean has an average length of 1.98 cm, 1.31 cm wide and 0.89 cm thick. Soybean cv. Grobogan have an average length 0.86 cm, 0.70 cm wide and 0.58 cm thick. As for soybean cv. Kaba, the average length is 0.67 cm, 0.55 cm wide and 0.58 cm thick. Soybean cv. Grobogan, suitable for making tempe because it has relatively large seed size, while soybean cv. Kaba suitable for the manufacture of tofu. Physical characteristics of jackbean and soybean are presented in Table 1.

Jackbean peel was bound tightly with its cotyledons. Soaking processed ease peeling process due to gum that serves as an adhesive skin with cotyledons, will dissolve. Jackbean seeds which were soaked will be swollen. The next process is drying to remove water. Dry jackbean beans peeled by commercial soybean peeling machine. Stripping is a method that considered very effective and efficient, because almost 100% of the peel can be removed. Jackbean seeds split into two pieces and still oversized for processing into tempe. Therefore, the split should be chopped to get smaller size.

Jackbean has been utilized as raw material in the tempe making¹⁷. However, it has only been tested under laboratory condition and has not implemented at industrial level. Physically, jackbean has big size and white color. For tempe production, jackbean need to be dehulled and cutted into smaller size. This step was designed to increase surface area of the jackbean which is necessary to obtain good growth of the mold. Breaking the seeds into smaller pieces enhanced tighter packing, but fungal growth did not improve, indicating that fungal growth may have been inhibited by other factors inherent to the seeds¹⁸.

Figure 1 shows that jackbean seed before and after modification. Dehulled jackbean can be prepared by farmers or farmers group and then tempe manufacturer can purchase the modified jackbean which is ready for use.

Cooking time is an important factor, because it is one step among the process of tempe preparation. Overview cooking time to cutted jackbean and soybean are presented in Table 2. Table 2 shows that soybean has cooking time for 40 minutes, which was characterized



Figure 1. Jackbean seed, dehulled jackbean and cutted jackbean

by the nut shell which was easily removable and has soft seeds, meanwhile the jackbean has shorter cooking time shorter, only 20 minutes. Shorter cooking time in jackbean probably caused by the smaller size of jackbean compared to soybean, after they had been cutted. Difference of cooking time indicates that jackbean can not be directly mixed in the preparation of tempe.

Table 3 presents physical characteristics and major chemical component of jackbean. Major component of jackbean consisting of approximately 34.58% of protein, 2.92% of ash, 1.13% of fat and 49.48% of carbohydrate. The major component of jackbean widely variative. The following gross composition of jackbean consisted of 21% protein, 4% of fat, 60% of carbohydrate was reported by Subagio et al¹⁹. He also reported that jackbean contained anti nutrition factor especially phytate (approximately 13.2 mg/g), small amount of HCN and trypsin inhibitor. Antinutrition factor consists of trypsin inhibitor, hemagglutinin, glukocida cyanogen, and oligosaccharida²⁰. The same thing also expressed by Oliveira et al.²¹, that jackbean are known to contain some toxic substances that may hinder their use as food for humans and animal feed such as concanavalin A lectin compound and the enzyme urease. Nevertheless, anti

nutrition factor can be reduced by soaking and cooking process that had been done in this research.

Phytate is the major storage form of phosphorus and it is negatively affects the bioavailability of essential minerals by forming insoluble mineral-phytate complexes and thereby inhibits absorption^{22,23,24}. Doss et al.⁸ reported that jackbean had anti-nutritional factors such as phenols, tannins, trypsin inhibitors and L-Dopa. Treatments like soaking, cooking, and autoclaving could reduce the amount of those anti-nutritional factors. Soaking and cooking (boiling) processing methods employed in his study were found to reduced significant levels of various antinutritional compounds such as total free phenolics (53% and 67% respectively), tannin (45% and 64% respectively), L-Dopa (35 and 52% respectively), trypsin inhibitor activity (30 and 62% respectively). Among the various processing methods employed, autoclaving was found to be more effective in reducing the maximum levels of total free phenolics (78%), tannin (83%), and L-Dopa (71%). In this research, soaking as long as 50 hours had been done to jackbean to decrease pH. This process was subsequent to boiling (cooking) process to produce softer jackbean that were ready to be fermented

Table 2. Determination of cooking time from soybean and jackbean

Tabel 2. Determinasi waktu masak kedelai dan koro pedang

Sample	Time (minutes)	Visual observations
Soybean	10	Skin easily peeled, hard
	15	Skin easily peeled, hard
	20	Skin easily peeled, fairly hard
	25	Skin easily peeled, fairly hard
	30	Skin easily peeled, fairly soft
	35	Skin easily peeled, fairly soft
	40	Skin easily peeled, soft
Jackbean (split)	5	Hard
	10	Fairly hard
	15	Fairly soft
	20	Soft
	25	Very soft

Table 3. Physico characteristics and major chemical component of jackbean

Tabel 3. Karakteristik fisiko dan komponen kimia koro pedang

Characteristics	Value
Physical:	
- Length (cm)	1.98
- Width (cm)	1.31
- Thickness (cm)	0.89
- Weight of 10 seed (g)	15.91
Major component*:	
- Moisture (%)	18.17
- Ash (%)	2.92
- Fat (%)	1.13
- Protein (% db)	34.58
- Carbohydrate by different (%)	49.48

*) based on dehulled seed

into tempe product.

Jackbean Tempe Making

Basically, fermentation process for tempe production is similar for all substrates. It includes soaking, dehulling, boiling and fermenting. The main differences between different substrates used in tempe fermentation are the selection of optimal pre-treatments such as modifying surface substrate, optimal soaking time and boiling time, optimal strain and inoculation level of *R. oligosporus*, and fermentation period.

Boiling was applied in order to soften the jackbean. After boiling, the hot water was discarded immediately, especially to remove anti microbial substances and bitter compound. Jackbean was then soaked in tap water. This step was important in providing acidic condition to inhibit

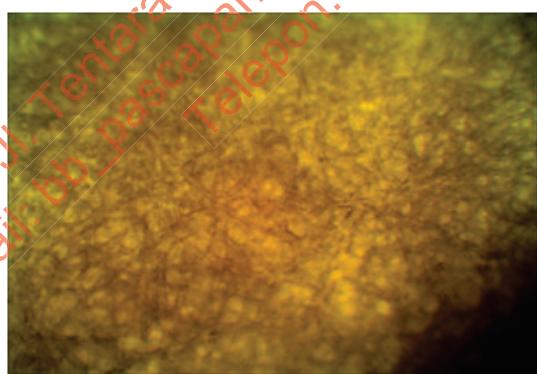
the growth of undesirable microorganism in subsequent step. The jackbean has to reach pH < 5. We found that the time for boiling, soaking and fermentation of jackbean tempe was comparable with those for soybean tempe as reported by Nuraida et al²⁵.

Microbiological and Biochemical Changes during Fermentation Process

The microbial and biochemical changes upon fermentation were intensely evaluated. Visual observation revealed that jackbean was suitable for tempe production. Jackbean was completely covered with dense mycelial growth after 24 hrs of fermentation period. Figure 2 presents the mycelial growth observed under light microscope (1000 times magnification) and visual appearance of jackbean tempe.

During fermentation process, the pH value remained relatively stable, the total plate count and soluble protein increased significantly (Table 4). These data indicate that bacteria especially lactic acid bacteria involved in the fermentation process (total count). In contrast, the pH of soybean tempe significantly increases from pH 5 to pH 7 due to the release of ammonia in the final product²⁶. An increased concentration of free ammonia might kill the tempe fungus, leading to a reducing quality of the final product²⁷. Increasing of soluble protein indicating that highly active proteolytic enzyme might exist. It also brings nutritional consequences such as increase in digestibility of jackbean tempe. Fermentation process of tempe increases the nutritional values of some nutrients, development of vitamins, phytochemicals and antioxidative constituents. Fermentation process of tempe decreases the phytic acid and enhances the bioavailability of minerals such as calcium, zinc and iron²⁸.

Major chemical component of jackbean tempe is



(a)



(b)

Figure 2. Mycelial growth of mold under light microscope with 1000 times magnification (a) and visual appearance of jackbean tempe (b)

Table 4. pH value, total plate count and soluble protein of jackbean tempe during fermentation

Tabel 4. Nilai pH, total mikroba dan protein terlarut pada tempe koro pedang saat fermentasi

Fermentation (hour)	pH	Total count (Log cfu/g)	Soluble protein (mg/g)
12	4.57a	6.46b	0.24a
24	4.86b	7.12a	0.97a
36	4.39b	7.56a	1.39a
48	4.93a	7.26a	2.60a

Table 5. Major chemical component of jackbean tempe compared with soybean tempe

Tabel 5. Perbandingan komponen kimia tempe koro pedang dengan tempe kedelai

Chemical component	Value	
	Jackbean tempe	Soybean tempe
Moisture (%)	67,02 ± 0,39	63,69±0,21
Ash (%)	0,18 ± 0,10	0,73±0,02
Fat (%)	0,68 ± 0,50	1,90±0,90
Protein (% db)	28,29 ± 0,05	19,38±0,43
Carbohydrate (%), by different	22,83 ± 0,70	12,59±0,89

Table 6. Median of hedonic test on jackbean tempe sensory evaluation

Tabel 6. Evaluasi sensori median uji organoleptik tempe koro pedang

Sample		Color	Flavor	Acidity	Texture	Taste	Acceptability
Raw form	Jackbean	5a	4a	3a	4a	-	4a
	Soybean	4b	4a	4b	4a	-	4a
Fried form	Jackbean	4a	4a	4a	4a	4a	4a
	Soybean	4a	4a	4a	4a	4a	4a

Remarks: 5= Like; 4= Fairly like; 3 = Neutral; 2= Fairly dislike; 1= Dislike

Table 7. Evaluation based on ranking test on jack bean tempe compared to soybean tempe

Tabel 7. Perbandingan evaluasi berdasarkan uji rangking pada tempe koro pedang dan kedelai

Sample		Color	Flavor	Acidity	Texture	Taste	Acceptability
Raw form	Jackbean	1	1	2	1	-	1
	Soybean	2	2	1	2	-	2
Fried form	Jackbean	1	2	2	1	2	2
	Soybean	2	1	1	2	1	1

Remarks: 1= the most preferred by panelists; 2 = the second preferred by panelists

presented in Table 5. Protein content of jackbean tempe was lower (28% dry basis) than jackbean seed (34% dry basis). The mycelium, basically mycoprotein may contribute to the decrease of the protein. The results are significant as the development strategies to broaden the food base in developing countries, by improving traditional food products based on indigenous raw material such as jackbean. The health benefit of jackbean tempe is currently under investigation.

Jackbean tempe has a compact structure as well as soybean tempe. However the nature of the "hard" varies depending on the substrate. The nature of change

occurred over the period of the fermentation process. At the beginning of the formation of a compact structure (24 hours of fermentation), jackbean tempe has the hard texture and soft characteristics, which were changed into the longer fermentation period. Nevertheless, the opposite happened in soybean tempe, the texture even harder when fermentation process was longer.

Sensory Characteristic

The test results showed that the hedonic sensory properties of jackbean tempe equivalent to soybean tempe (Table 6 and Table 7). However, there was one

piece of parameters that still have not yet preferred by panelists. This was acidity. There was significant difference in panelists evaluation. They most prefer soybean tempe rather than jackbean tempe. Beside of lectin and concanavalin contained in jackbean that contribute to the acidity in jackbean tempe, panelis disliking to the jackbean tempe was understandable because the panelists were more familiar to soybean tempe than jackbean tempe.

CONCLUSION

Jackbean was potentially used as a substrate for tempe production. The fungus grew well and jackbean cake tempe with dense mycelial growth was completely formed after 36 hr of fermentation period. Soluble protein increased significantly indicating that highly active proteolytic enzyme might exist. The results of this research were significant as the development strategies to reduce dependency on soybean import by substituting it in the tempe production in Indonesia.

ACKNOWLEDGMENT

Thanks to Indonesian Agency for Agricultural Research & Development (IAARD) – Ministry of Agriculture that has supported and provided funding for this research.

REFERENCES

1. [BPS]. Badan Pusat Statistik. 2011.
2. Damardjati D, Widowati S. Prospek pengembangan kacang gude di Indonesia. Jurnal Litbang Pertanian. 1995; IV(3):53-59.
3. Indrasari SD, DK Sadra, DS Damardjati. Evaluation of producer acceptance on soypigeonpea tempe prodction in Puwakarta District, Indonesia. Proceedings of the 4th ASEAN Food Conference. 1992. Jakarta. Indonesia. pp. 604-615.
4. Richana N, Damardjati DS. Karakteristik fisiko-kimia biji kacang tunggak (*Vigna unguiculata* (L) Walp) dan pemanfaatannya untuk tempe. Penelitian Pertanian Tanaman Pangan. 1999;18(1): 72-77.
5. Purwani et al. 2006. Teknologi Pemanfaatan Kacang-Kacangan untuk Produk Tempe. Laporan Penelitian. BB Pascapanen Bogor.
6. Haliza W, EY Purwani, R Thahir. Pemanfaatan Kacang-kacangan Lokal sebagai Substitusi Bahan Baku Tempe dan Tahu. Buletin Teknologi pascapanen Pertanian. 2007;3.
7. Djaafar TF, Cahyaningrum N, Purwaningsih H. Physico-Chemical Characteristics Of Tribal Bean (*Canavalia Virosa*) And Its Alternative Tofu And Tempe Food Products. Indonesian Journal of Agricultural Science. 2010;11(2): 74.
8. Doss A, Pugalenth M, Vadivel V, Subhashini G, Anita Subash R. 2011. Effects of processing technique on the nutritional composition and antinutrients content of underutilized food legume *Canavalia ensiformis* L.DC.
9. Anonymous. 2014. Ekspedisi 9: Koalisi dengan koro pedang substansi kebutuhan kedelai.<http://jatim.litbang.pertanian.go.id/ind/index.php/berita/janganlupa/746-ekspedisi-9>. BPTP.Jatim.
10. Janardhanan K, Vadivel V, Pugalenth M. 2003. Biodiversity in Indian underexploited/tribal pulses. In P.K. Jaiwal, & R. P. Singh (Eds.), Improvement strategies for Leguminosae biotechnology (pp. 353e405). The Netherlands: Kluwer Academic Publishers.
11. Pambayun R. "Hydro Cianic Acid and Organoleptic Test on Gadung InstantRice from Various Methods of Detoxification". Prosiding Seminar Nasional Industri Pangan Surabaya. PAU Pangan dan Gizi Universitas Gajah Mada. 2000. Yogyakarta.
12. Marto EK, Wallace P, Timpo G, Simpson BK. Cholesterol lowering MIA PaCa-2 pancreatic cancer cells is not due to conversion to its toxic metabolite canaline. Anticancer Drugs. 1990;10:113-118.
13. Marchetti M, Mastromarino P, Rieti S, Seganti L, Orsi N. Inhibition of herpes simplex, rabies and rubella viruses by lectins with different specificities. Research in Virology. 1995;146:211-215.
14. Swaffar DS, Ang CY. Growth inhibitory effect of L-canavanine against MIA PaCa-2 pancreatic cancer cells is not due to conversion to its toxic metabolite canaline. Anticancer Drugs. 1999;10:113-118.
15. [AOAC] Association of Official Analytical Chemistry. 1999. Official Methods of Analysis. Washington DC: AOAC Int.<http://www.aoac.org/vmeth/page1.htm> [26 Desember 2011]
16. [BAM]. Bacteriological Analytical Manual. Bacteriological Analytical Manual Chapter 3: Aerobic Plate Count. U.S. Food and Drug Administration. Diakses tanggal 8 Februari 2011.
17. Nout MJR, JL Kiers. Tempe fermentation, innovation and functionality: update into the third millenium. Journal of Applied Microbiology. 2005; 98:789.
18. Azeke M, Barbara F, Hans B. Comparative effect of boiling and solid substrate fermentation using the tempe fungus (*Rhizopus oligosporus*) on the flatulence potential of African yambean (*Sphenostylis stenocarpa* L.) seeds. Food Chemistry. 2007;103:1420–1425.
19. Subagio A, WS Windrati, Y Witono, A Nafi. Development of non-oilseed legumes as a source of protein to strengthen in Food Security in Marginal Area. In Nuraida et al. (Eds). Investing In Food Quality, Safety and Nutrition. International Conference Proceeding Investing in Food

- Quality, Safety & Nutrition: Lessons Learned from Current Food Crisis, Jakarta. 2008; June:27-28.
20. Ekanayake S, ER Jansz, Baboo M. Nair. Literature review of an underutilized legume: *Canavalia gladiata* L. Plant Foods for Human Nutrition. 2004;55(4): 305–321.
21. Oliveira AEA, MP Sales, OLT Machado, BKVS Fernandes, J Xavier-Filho. The Toxicity of Jackbean Cotyledon and Seed Coat Proteins to The Cowpea Weevil. *J. Entomologia Experimentalis et Applicata*. 1999;92(3): 249-255.
22. Hallberg L, Brune M, Rossander L. Iron-absorption in man - ascorbic-acid and dose-dependent inhibition by phytate. *American Journal of Clinical Nutrition*. 1989;49:140-144.
23. Sandström B, Sandberg AS. Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *Journal of Trace Elements and Electrolytes in Health and Disease*. 1992;6:99-103.
24. Bohn T, Davidsson L, Walczyk T, Hurrell RF. Phytic acid added to white-wheat bread inhibits fractional apparent magnesium absorption in humans. *American Journal of Clinical Nutrition*. 2004;79:418-23.
25. Nuraida L, Suliantari, Andarwulan N, Adawiyah DR, Noviar R, Agustin D. Evaluation Of Soybean Varieties On Production And Quality of Tempe. <http://seafast.ipb.ac.id/publication/prosiding/isbn-978-979-19919-0-2-p1-15.pdf>. 2008. Diakses tanggal 28 Maret 2013.
26. Sparringa RA, Owens JD. Causes of alkalinization in tempe solid substrate fermentation. *Enzyme and Microbial Technology*. 1999;25:677-681.
27. Steinkraus KH, Cullen RE, Pederson CS, Nellis LF, Gavitt BK. Indonesian tempe and related fermentations. In *Handbook of indigenous fermented foods*. Edited by K.H. Steinkraus, R.E. Cullen, C.S. Pederson, L.F. Nellis & B.K. Gavitt. New York, Marcel Dekker. 1983:1-94.
28. Astuti M, Dalais FS. Tempe, a nutritious and healthy food from Indonesia. *Asia Pacific Journal of Clinical Nutrition*. 2000;9:322–325.

Hak cipta © 2015 BB-Pascapanen
Kampus Penelitian Pertanian
Jl. Tentara Pelajar no 12A, Cimanggu,
Bogor, Jawa Barat, Indonesia
Email: bb_pascapanen@yahoo.com ,
Telepon: (0251) 8321762 , Faksimili: (0251) 8350920
Jl. Tentara Pelajar no 12A, Cimanggu, Bogor, Jawa Barat, Indonesia
Email: bb_pascapanen@yahoo.com ,
Telepon: (0251) 8321762 , Faksimili: (0251) 8350920