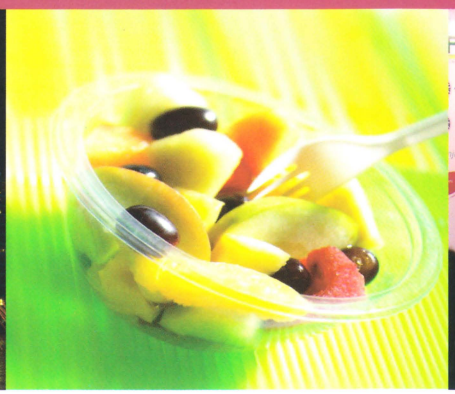
Proceeding

INDONESIAN ASSOCIATION OF FOOD TECHNOLOGISTS (IAFT)
International Conference

Future of Food Factors



Editor:
Harsi D. Kusumaningrum
Lilis Nuraida
Ratih Dewanti-Hariyadi
Desty Gitapratiwi
Purwiyatno Hariyadi

Southeast Asian Food & Agricultural Science & Technology (SEAFAST) Center
Bogor Agricultural University

Contamination of Fecal Coliform and *Staphylococcus* sp. on Green Grass Jelly (*Premna oblongifolia* Merr) in Bogor and Effect of Steaming on Microbiological and Physical Properties of the Product

Antung Sima Firlieyanti, Nidya Pramitasari

Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University
E-mail: ant.sima@yahoo.com

ABSTRACT

Green grass jelly is one of potential functional food due to its content of bioactive compounds such as flavonoids and chlorophyll. Despite its health benefit, the safety of the products is of concern since the production process is susceptible to microbial contamination. Microbiological analysis of 14 samples of green grass jelly in Bogor showed that the samples contained aerobic plate count between 1.6×10^4 to 2.4×10^6 CFU/g, fecal coliform 3.0×10^2 to 4.4×10^3 CFU/g, and *Staphylococcus* sp. 2.5×10^1 to 2.0×10^3 CFU/g, hence indicating a high level of microbial contamination during processing and marketing of the products. Steaming treatment effectively reduced the aerobic plate count up to 1.10 log CFU/g, fecal coliform 1.18 log CFU/g, and *Staphylococcus* sp. 0.71 log CFU/g. However, the physical characteristics of green grass jelly were adversely affected by steaming treatment. The color of the jelly was changed from its original dark green to brownish green. Steaming treatment also slightly increased the green grass jelly syneresis (9.03%) and decreased the gel strength (2.99 g/cm^2). The results indicated the prospective use of thermal in improving microbiological quality and safety of green grass jelly. Yet, further study on thermal design and application on green grass jelly is essential to achieve optimal microbiological properties while preserving favorable physical and sensory characteristics of the product.

Keywords: *green grass jelly, contamination, Coliform, Staphylococcus sp.*

INTRODUCTION

Green grass jelly (*Cyclea barbata* L. Miers and *Premna oblongifolia* Merr) has been intensively explored for its functional properties. Previous researches have revealed the high content of fibre (6.2 g per 100 g), phenol, saponin, flavonoids, and chlorophyll as well as antioxidant capacity of green grass jelly (Heyne, 1987; Zakaria et al, 2001). Traditionally, the plant has been used as antipyretic and treatment for gastritis and hypertension (Sunanto, 1995). Other known functional properties of green grass jelly were anti-cancer and induced proliferation of lymphocite cells (Zakaria et al, 2001), free radicals scavenger (Handayani, 2000), immunostimulant and immunosuppressan (Handayani, 2000; Zakaria et al, 2001), anti allergy (Rachmini, 2000), and increase the antioxidant capacity of lymphocite cells (Koessitoresmi, 2002). Additionally, studies on toxicity of the plants showed that green grass jelly is non-toxic, thus its save for consumption (Arisudana, 2003; Nugrahenny, 2003).

Despite its prospective values, the quality and safety of green grass jelly are still concerning. Until to date, commercial green grass jelly have been only available mostly at street vendors in the form of iced drinks. Most of the products are traditionally prepared by the sellers themselves. The production process of green grass jelly has been involving the intensive physical contacts of the producers and no heating can be applied since the non-heat stable characteristic of green grass jelly. The quality and safety in term of microbiology has been relied mainly on the application of sanitation and hygiene during processing, storage, and distribution. Poor sanitation and hygiene practices lead to unacceptable microbial load and consequently shorten the product's shelf life and limiting its marketing area. Moreover, the safety of the product is also at a significant risk of the possible presence of pathogenic microorganisms. Therefore, it is important to achieve the microbiological properties that can assure appropriate level of safety as well as to prolong product's shelf life and expand the market scope and value.

In this research, the level of microbial contamination on commercial green grass jelly and the level of sanitation and hygiene applied during product selling were being evaluated. The evaluation was emphasized on bacteria closely correlated to sanitation and hygiene practices, i.e. fecal coliform and *Staphylococcus* sp. The occurrence of fecal coliform, with *Escherichia coli* as the major group member, always be a reliable hint of fecal contamination

resulted from improper sanitation and hygiene practices, such as used of non-boiled water and involvement of non-hygienic handlers. While *Staphylococcus* sp. indicates the risks of *Staphylococcus aureus toxigenic strains* due to unhygienic food handlers. The research was also aimed to study the effect of heating treatment (steaming) on physical and microbiological properties of the product. The resulting data of microbiological content of existing commercial products as well as the possibility of applying thermal on the product are of value as the basis for further improvement of the green grass jelly's quality and safety.

METHODOLOGY

Samples

The samples used in the research were green grass jelly (*Premna oblongifolia* Merr) sold by 14 iced drinks vendors throughout Bogor area.

Media and Reagents

The media and chemical used were Plate Count Agar (PCA), Eosin Methylene Blue Agar (EMBA), Baird Parker Agar (BPA), Egg Yolk Tellurite (EYT), and KH_2PO_4 (phosphate buffer).

Equipment

Equipment used were autoclave, stomacher, incubator 37°C, vortex, micropipette, and texture analyzer.

Research Stages

The research was conducted in two consecutive stages as follows:

1. Microbiological analysis of commercial green grass jelly and observation of sanitation and hygiene practices during product selling

   The green grass jelly samples from 14 vendors in Bogor were analyzed for their content of Aerobic Plate Count, fecal coliform, and *Staphylococcus* sp. The vendors were also been observed to gain their general profile and data on sanitation and hygiene practices as reffered to the Minister of Health's Verdict (Keputusan Menteri Kesehatan RI) No.942/Menkes/SK/VII/2003 about Guideline of Hygiene and Sanitation Requirements for Food Vendors which includes food handler; equipment; water, food

ingredients, additives, and serving; facilites and location as parameters of observation.

2. Study on Effect of steaming on physical and microbiological properties
   Samples from 2 vendors were further treated by steaming application for 5 minutes. The physical properties i.e. color, syneresis, and gel strength and microbiological properties (Aerobic Plate Count, fecal coliform, and *Staphylococcus* sp.) were measured and compared between non-steamed and steamed samples.

Sampling

Each sample was collected in a single serving size (approximately 100 g) using plastic bag provided by the vendor. The sample was then preserved at low temperature using an ice box until analysis was being performed (maximum 4 hours after sample collection).

Sample Preparation

Sample for microbiological analysis was weighed aseptically 25 g into a sterile plastic bag. A 225 ml of phosphate buffer was added into the sample and the sample was then mashed using stomacher. The next serial dilution was made by transferring 1 ml sample suspension into 9 ml phosphate buffer.

Methods of Analysis

Microbiological Analysis

Analysis of Aerobic Plate Count (Bacteriological Analysis Method, FDA, 2001) and fecal coliform was performed by pour plate technique using PCA and EMBA medium respectively. Analysis of *Staphylococcus* sp. (BAM, FDA, 2001) was done by spread technique on solidified BPA containing EYT. All plates were incubated at 37°C for 48 hours in inverted position. Plates containing 20-250 typical colonies were counted and used for calculation of Colony Form Unit (CFU) per gram.

Gel Syneresis (AOAC, 1995)

Syneresis analysis was conducted by storing the samples at 10°C in 24 hours, and then measuring weight loss during storage. Syneresis was quantified using formula:

$$\text{Gel syneresis} = \frac{A-B}{A} \times 100\%$$

where: A = Sample weight before storage (g)
B = Sample weight after storage (g)

Gel Strength

Gel texture was measured using Stevens LFRA Texture Analyser. Measurement setting used in this research was referring to setting for pectin texture measurement, i.e. test speed 0.5 mm/s, distance 8.00 mm, triger force 10 g, probe diameter 0.5 inch cylinder Delrin. The measurement parameter were gel rupture strength/gel strength which was calculated using the following formula:

$$\text{Rupture strength (g/cm}^2\text{)} = \frac{\text{Load of rupture}}{\text{Surface area of probe base}}$$

RESULT AND DISCUSION

Sanitation and Hygiene Practices of Green Grass Jelly Vendors

The quality of water and food ingredients, quality of air, food handler practices, and cleanness of equipments were considered as factors affecting the microbiological properties of the green grass jelly samples since they could be a considerably source of contamination. Water used in green grass jelly production shall meet the potable water requirement. Failing to meet the requirement indicates a potential contamination of some enteric microorganisms, such as *E. coli, Vibrio cholerae, Salmonella sp,* protozoa, and Norovirus. There are no available data regarding microbial flora on green grass leaf, however, the used of fresh green grass leaf could also be a prospective contaminant source when it is not cleaned and sanitized properly. Contaminated air in processing and marketing area would contribute some extent of microbial contamination, particularly in term of mould, yeast, and *Bacillus* sp., on the jelly product. Whereas unhygiene practices of food handler could specifically increase the risk of *Staphylococcus aureus* and enteric bacteria or virus contamination.

Through direct observation and interviews with the seller, it was identified that the majority of the vendors applied poor sanitation hygiene practices in term of food handler; equipment; facilites; and location. For examples, almost none of food handler did not wear appropriate outfit, such as apron and head cover, and did not practicing hand washing during food handling. Equipments were cleaned, sanitised, dried and placed unproperly. The vendors were also not equipped with clean water, sealed and clean container for equipment, wash basin, and garbage bin. 13 out of 14 vendors were street vendors which were located on place susceptible to physical, chemical, and microbiological contamination. Only 1 vendor was counted to be better than others by the used of proper outfit by food handler and located in a proper place (indoor outlet) thus received less contamination from the environment and food handler. There were no observations conducted during production of green grass jelly. However, as all seller claims, for water, food ingredients, additives, and serving aspects, good practices were applied, including the used of boiling water for production, separated storage of raw and cooked materials, the used of clean and food grade packaging materials, and protection of food using closed container to avoid cross contamination during storage.

Microbiological Analysis of Commercial Green Grass Jelly

Microbiological analysis confirmed some extent of contamination of aerobic microorganisms, fecal coliform, and *Staphylococcus* sp. as shown in Figure 1, 2, and 3.

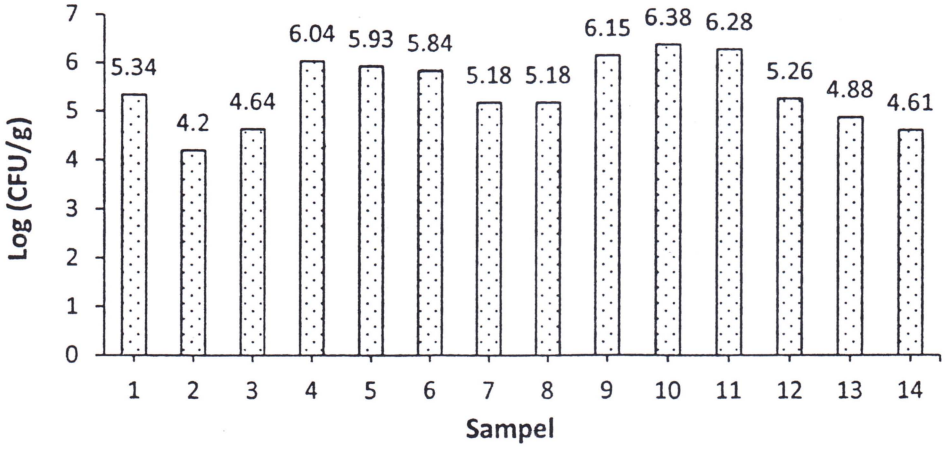


Figure 1. Aerobic Plate Counts of green grass jelly samples

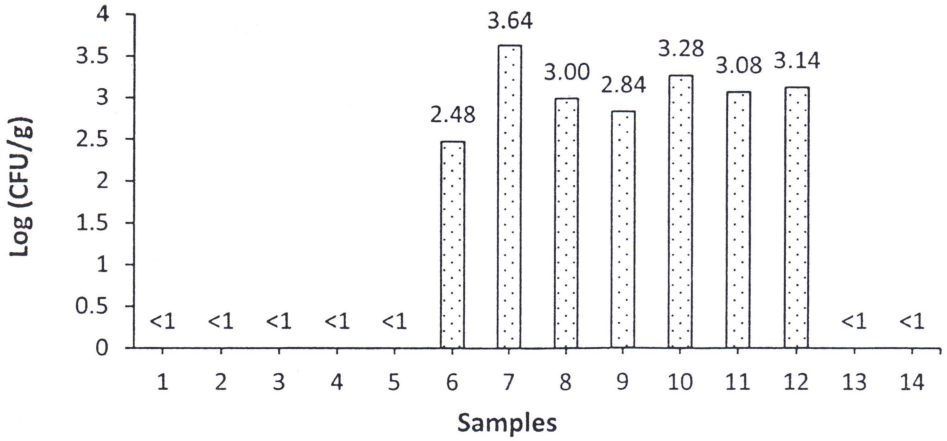


Figure 2. Fecal coliform content of green grass jelly samples

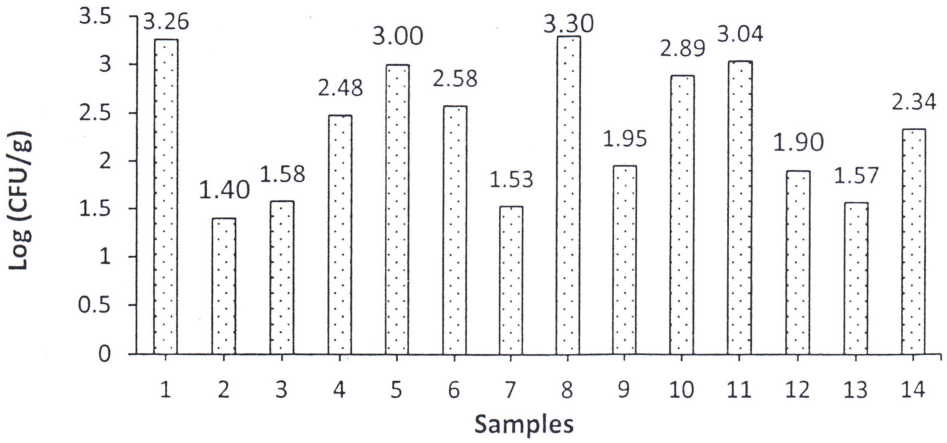


Figure 3. *Staphylococcus* sp. content of green grass jelly samples

Referring to Food and Environmental Hygiene Department, Hongkong (2001), green grass jelly can be categorized as ready to eat food level 2. In regards to classification in Table of Microbiological Limits for Assessment of Microbiological Quality of Ready to Eat Foods, in term of Aerobic Plate Counts, *E. coli*, and *Staphylococcus* sp., 71% samples were unsatisfactory (APC $\geq 10^5$) whereas 29% samples were classified as acceptable (APC=10^4–10^5); 50% samples were satisfactory (*E. coli* < 20) and the other 50% were unsatisfactory (*E. coli* ≥ 100); and 43% were acceptable *(Staphylococcus* sp. 20 - < 100) while 57% were unsatisfactory *(Staphylococcus* sp. 100 - < 10^4).

It has been common awareness that *E. coli* and other coliforms fecal bacteria act as indicators of fecal contamination. Such role leads to a range of significance consequences as the incidents of fecal in food could also be a warning of the occurence of enteric pathogenic bacteria and viruses such as Salmonella sp., Vibrio cholerae, and Hepatitis A virus. Although E. coli was previously known to be harmless, more recent studies have been identifying some pathogenic strains of E. coli hence extending the unfavored feature of the bacteria.

Staphylococcus sp. are commensals of the body surfaces of human and warm-blooded animals. The bacteria is frequently associated with skin infection through open wounded skin. Most species are not food borne pathogens, however S. aureus is an opportunistic pathogen with an ability to produce enterotoxin causing symptom of nausea, vomiting, abdominal cramps, and diarrhea when digested via contaminated food. *Staphylococcus* sp. is prevalence in ready to eat food and usually caused by recontamination from food handler pasca processing.

The relatively high number of fecal coliform and *Staphylococcus* sp. indicated poor sanitation and hygiene practices during production and marketing. This was partly confirmed by observed practices of the vendors which in general showed improper implementation of sanitation and hygiene principles as explained in the previous part of this paper. Interestingly, practices of sanitation and hygiene apllied during marketing did not always comply with the results obtained from microbiological analysis. The best instance was the fact that 1 vendor was found to apply better sanitation and hygiene compared to others. Yet, the results of analysis showed that the products sold by such vendor contain a high microbial load which is not significantly differents with other vendors (sample 11). This is because marketing is not the only factor affecting contamination level and seemed as not the major source of contamination. The main contamination would probably occured during processing, which involves intensive contacts of handler in hand-pressing green grass leaves into pulp, the potential used of non-boiled water, improper cleanness of equipments, etc. Still, it would need further study to confirm the assumption.

The ability of the bacteria to grow in the products was also encouraged by the characteristics of products which of favor both bacteria. Green grass extract contains carbohydrate, vitamins, and minerals useful as nutrient

sources for the bacteria (Heyne, 1987). Green grass jelly have an a_w of 0.98 and pH 5.5 which suits the growth condition of *E. coli* and *Staphylococus* sp. which require minimum a_w 0.96 and 0.86 respectively and pH 4 - 9 (Jay, 2000). The condition was worst by the fact that all products were kept at ambient temperature (25-30°C) for a prolonged period (9-10 hours) until the were consumed.

There are no similar research regarding microbial contamination on street vended jelly product in Indonesia. However, the results of this research are similar to that obtained by Sule et al. (2007) who found the contamination level of *S. aureus* in bean pudding were ranged 1.2×10^3 - 2.0×10^6 CFU/g and number of Total Plate Count were 3.1×10^4 - 3.2×10^6 CFU/g.

Effect of Steaming on Microbiological and Physical Properties of Green Grass Jelly

Considering the health function of green grass jelly and the prominent effectivity of heating in controlling microbiological contamination on food product, it is worthwhile to study the potential application of thermal on green grass jelly. As to obtain preliminary data on how heating could affect the characteristict of products, steaming treatment was employed on two samples from previous recearch stage. Microbiological analysis on steamed products resulted in reduced number of aerobic plate count, fecal coliform, and *Staphylococcus* sp. as shown by Figure 4.

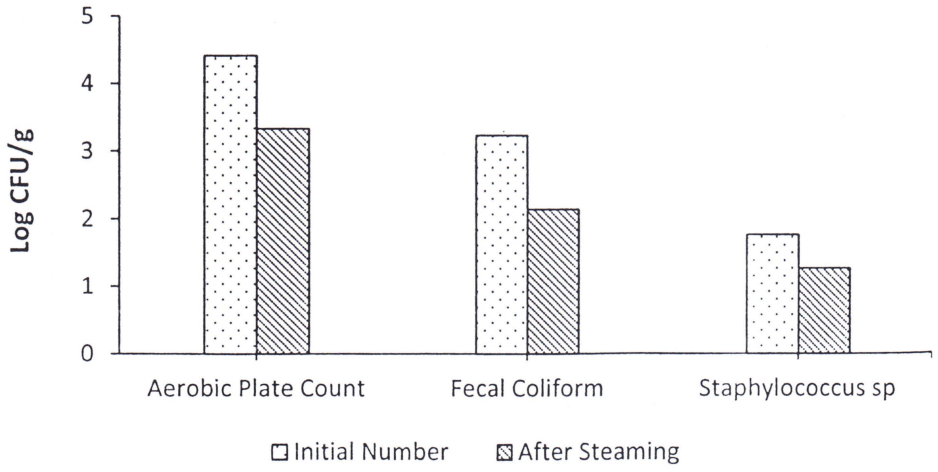


Figure 4. Reduction of Aerobic Plate Count, fecal coliform, and *Staphylococcus* sp. on steamed green grass jelly

E. coli and *Staphylococus* sp. are sensitive to heat with maximal growth temperature is 45-46°C which is below the steaming temperature. However, the relatively slight reduction of the bacteria, approximately 1 log, would be not sufficient to assure the products quality and safety. Therefore, design and application of pasteurisation aimed to achieve 5-6 log reduction of *E. coli* or *S. aureus* will give more level of quality and safety assurance. Sanitation and hygiene practices must also properly employed during processing and marketing to restrain the initial load of target bacteria.

Inspite of potential effectivity of heating to reduce microbial content, subjective visual observation and physical analysis found the unfaroble change of jelly in term of color, syneresis, and gel strength (Table 1). The jelly's color was visually changed from dark green to brownish green. This could be happened by the degradation of chlorophyll into pheophytin or phaeophytin, termed pheophytinase, which is induced by heat. Under high temperature, Mg^{2+} ion contained in a chlorophyll will detached and form magnesium free-chlorophyl or best known as pheophytin (Gross 1991). This event would be intensified in prolonged heating process. Another factor promoting pheophytinase is acidic condition which assumably was also occured in steaming process of green grass jelly. High temperature results in release of volatile organic acids which could react with chlorophyll to form pheophytin when the process occurs in a closed condition preventing volatile compound to escape in to the air.

Table 1. Changes in Physical Characteristics of Jelly as Affected by Steaming

Parameters	Treatments		
	No Steaming	Steamed jelly	% Changes
Color	Dark green	Brownish green	-
Syneresis (%)	37.40	45.83	22.54
Gel strength (g/cm^2)	140.43	137.95	1.77

Some researches have proved the bioactivity of chlorophyll as antioxidant. Chlorophyll and its derivatives could act as anticancer, antiimfalamtion, and antigenotoxic (Egner et al, 2001). The structure of

chlorophyll is very much alike with haemoglobin structure accept for the content of magnesium or Fe (iron) atom. This similarity leads to the similar function of both cells as free radicals scavenger. Chlorophyll degaradion in steamed green grass jelly, is then, not favorable as will lessen the functionality of the product.

Steaming was also negatively affected other physical properties of green grass jelly i.e. syneresis and gel strentgh. Steamed jelly loss 10% more water compared to unsteamed product. So, in steamed samples, water loss was as high as 47.10% which was categorized as unacceptable. There was also a decrease in gel strength of both samples of 129.68 g/cm^2 to 126.69 g/cm^2 and 151.18 g/cm^2 to 149.21 g/cm^2. The main component of green grass jelly is low methoxy pectin (Artha, 2001). Pectin is polimers of D-galacturonate acid with β-1,4 glycocidic bonds. Some carboxyl groups in pectin polymer undergo methyl esterification forming methoxyl or pectinic acid. Low methoxy pectin is pectinic acid with majority of carboxyls group are not esterified. The high content of free carboxyl is a major factor of gel vulnerability on heat. High temperature will break the crossbonding of gel polimers, forming a non rigid structure which manifests the softened texture of gel. Heat could also result in an increase of fibriler density thus pressing trapped water out of the gel matrix which is shown as syneresis.

CONCLUSION

Observation on 14 green grass jelly vendors showed that most vendors did not meet sanitation and hygiene requirements for food vendors. Microbiological analysis of 14 samples confirmed the indication of massive contamination during processing and marketing with number of Aerobic Plate Counts, fecal coliform, and *Staphylococcus* sp. was ranged between 1.6 x 10^4 - 2.4 x 10^6 CFU/g, 3.0 x 10^2 - 4.4 x 10^3 CFU/g, and 2.5 x 10^1 - 2.0 x 10^3 CFU/g, respectively. Steaming effectively reduced the aerobic plate count up to 1.10 log CFU/g, fecal coliform 1.18 log CFU/g, and *Staphylococcus* sp. 0.71 log CFU/g. Nevertheless, the physical properties of green grass jelly were unfavorably affected by steaming treatment. The color of the jelly was changed from its original dark green to brownish green. Steaming treatment also slightly increased the green grass jelly syneresis (9.03%) and decreased the gel strength (2.99 g/cm^2). Based on the results, thermal process could be one

effective approach to improve the microbiological quality and safety of green grass jelly. However, the application of thermal process should be optimized to minimize the negative effect on the physical properties of the product.

REFERENCES

AOAC. 1995. Official Methods of Analysis of The Association of Official Analytical Chemist. Washington D. C.

Arisudana IG. 2003. Mempelajari toksisitas subkronis bubuk gel daun cincau hijau (*Cylcea barbata* L. Miers dan *Premna oblongifolia* Merr.) [*skripsi*]. Bogor: Fakultas Teknologi Pertanian IPB.

Artha IN. 2001. Isolasi dan karakteristik sifat fungsional komponen pembentuk gel cincau hijau (*Cyclea barbata* L. Miers) [*disertasi*]. Bogor: Program Pascasarjana IPB.

BAM. 2001. Aerobic Plate Count. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm063346 [2 April 2011].

BAM. 2001. *Staphylococcus aureus*. http://www.fda.gov/Food/ScienceResearch/LaboratoryMethods/BacteriologicalAnalyticalManualBAM/ucm071429 [2 April 2011].

Depkes RI. 2003. Keputusan Menteri Kesehatan RI No. 942/Menkes/SK/VII/2003 tentang Pedoman Persyaratan Higiene Sanitasi Makanan Jajanan, Jakarta.

Egner AP, Stephen JG, Thomas WK. 2001. Chlorophyllin Intervention Reduces Aflatoxin DNA Adducts in Individuals at High Risk for Liver Cancer. PNAS on Line Medical Science.

Gross J. 1991. *Pigments in Vegetable, Chlorophylls, and Carotenoids*. New York: Van Nostrand Reinhold.

Heyne K. 1987. Tumbuhan Berguna Indonesia Jilid III. Jakarta: Yayasan Sarana Wana Jaya.

Handayani DM. 2000. Mempelajari Pengaruh Ekstrak Cincau Hijau (*Cyclea barbata* L. Miers) terhadap Produksi Radikal Bebas Makrofag Peritoneal Mencit Secara *In Vitro* [Skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Jay JM. 2000. Modern Food Microbiology. Aspen Publ., Inc., Gaithersburg, Maryland.

Koessitoresmi A. 2002. Kapasitas Antioksidan Ekstrak Batang dan Daun Cincau Hijau (*Cyclea barbata* L. Miers) pada Sel Limfosit Manusia Secara *In Vitro* [Skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Nugrahenny D. 2003. Pengaruh Seduhan Tea Cincau Hijau *Cyclea barbata* L. Miers dan *Premna oblongifolia* Merr. terhadap Kadar Sitokrom P-420 dan Aktivitas Glutation S-Transferase dari Hati Tikus [Skripsi]. Bogor: Fakultas Teknologi Pertanian, Institut Pertanian Bogor.

Rachmini M. 2000. Mempelajari Efek Penghambatan Ekstrak Tanaman Cincau Hijau (*Cyclea barbata* L. Miers) terhadap Alergi [Skripsi]. Bogor: Fakultas Teknologi Pertanian, IPB.

Sule EI, Lawal AO, Damisa, D. 2007. Enterotoxicity of *Staphylococcus aureus* Isolated from Beans Pudding. Science World Journal 2(2):9-11.

Sunanto. 1995. Budidaya Cincau.Yogyakarta: Penerbit Kanisius.

Zakaria FR, Prangdimurti E, Ananta E, Pandoyo AS. 2001. Aktivitas Antikanker Gel Cincau HIjau (*Cyclea barbata* L. Miers). Di dalam Nuraida L dan RD Hariyadi (ed.). Seminar Nasional Pangan Tradisional : Basis Bagi Industri Pangan Fungsional dan Suplemen. Pusat Kajian Makanan Tradisional IPB. Bogor, 14 Agustus. P 179-193.