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Kerandang (*Canavalia virosa*) Seed as Raw Material for Non-Dairy Milk: The Effect of Soaking and Boiling on Physics-Chemical Characteristics

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Kerandang plants were found in the land of sand beach in Kulon Progo and Bantul Regency, Yogyakarta, Indonesia. Kerandang seeds can make into tempeh, tofu or non-dairy milk, as well as soybeans but contains HCN (anti-nutrition). Soaking and boiling is a necessary stage the seeds processing because its treatment can be decreased the anti-nutrition. The objectives of this research are to know the physics-chemical characteristic change during soaking and boiling treatment of seeds and to give the information about the isoflavones content in beans and non-dairy milk of Kerandang. Water absorption ability, protein, cyanide, saccharides, total phenolic and isoflavones has been analyzed. The results showed that the water absorption of seeds reaches the optimal point on 3 h soaking in water (27° C) and constant after 3 h. Kerandang seeds were content oligosaccharides, disaccharides and monosaccharide (raffinose, stachyosa, sucrose, glucose, fructose and galactose). Soaking treatment for 3 h doesn't cause a decrease of saccharides. The combination soaking and boiling treatment led to decrease of protein about 8.32% to 14.99%. Combination treatment (soaking and boiling) led to decrease of cyanide acid content significantly of 59.50 to 93.04%. Kerandang seed contains total phenolic of 7.42 mg GAE/100 g seed. Kerandang beans contain isoflavones as much as 195.02 mg/g of dry beans and Kerandang non-dairy milk is content isoflavones of 192.91 mg/100 ml. The soaking and boiling treatment were can reduce the cyanide content in Kerandang seeds, so that safely to used as raw material of non-dairy milk.

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Introduction

Kerandang (*Canavalia virosa*) plant belonging Leguminosae, were found in the sand land in Kulon Progo and Bantul Regency, Yogyakarta Province, Indonesia. It's grow and producing seed about 909 kg/ha/year. People in Kulon Progo regency were utilization this plant as alternative food. The flowers and young pods were utilization as vegetable, while the old seeds were use as raw material in tempeh processing. However, until now the kerandang seeds has not been optimally use as food. One reason is kerandang seeds containing anti-nutrition compounds such as tannins, phytic acid, L-DOPA, cyanide acid (HCN), oligosaccharides as flatulence factor, concanavalin A, and canavavin (Thangadurai et al., 2001; Sridar and Seena, 2006; Doss et al., 2011).

The leguminous beans are cheap protein source but contain anti-nutrition compounds. The anti-nutrition compounds in beans i.e. flatulence factor, phytic acid, polyphenol, tannin, lectin, cyanogens, protease inhibitor, lipase inhibitor, α-amylase inhibitor (khokhar and Apenten, 2016). Several studies have shown that antinutrition compounds are soluble and heat stable. Physical and chemical method are employed to reduce or remove the anti-nutrition factors, including soaking, cooking (boiling), autoclave, microwave, germination, fermentation and enzyme treatment (Mubarak, 2005; Khokar and Apenten, 2016). Yasmin et al., (2008) was studied about the effect of different processing methods (soaking in water or sodium bicarbonate solution and citric acid solution, soaking plus cooking and germination) on anti-nutrition factors (phytic acid, total polyphenols, tannins and hydrocyanic acid) of red kidney bean. The anti-nutritional factors were reduced significantly (P<0.001) with processing techniques. Cyanide content were most effectively (25%) reduced by cooking after soaking in sodium bicarbonate solution, followed by germination.

Soaking of beans before cooking is a common practice to soften texture and hasten the cooking process. Soaking has also been suggested for reducing anti-nutritional substances and improving cooking quality (Rehman et al., 2001). In traditional cooking, beans are first soaked overnight to increase availability of water in the seeds before cooking and thereby accelerate chemical reactions such as starch gelatinization and protein denaturation during cooking. Hydration of dry beans before cooking or canning usually considered necessary to decrease cooking time and increase drained weight (Bellido et al., 2006).

Khokhar and Apenten (2016) also said that, a single technique application is often insufficient for effective treatment and so combinations are commonly employed. Thus the most effective methods for reducing saponin contents have been reported to be soaking and cooking. Using an abrasion and soaking combination methods, the amount of quinoa saponin was reduced by up to 100%. Acording to Oboh et al., (2000), soaking for nine hours significant reduction the oligosaccharide content in white lima beans, African yam beans and pigeon peas. The combinations soaking and boiling treatment, led to increase oligosaccharides losses on lima beans, African yam beans, pigeon beans and jackbeans.

In fact, according to Djaafar et al., (2010), in the processing of kerandang seed into tempeh, HCN content can be eliminated as much as 97.62%. Djaafar et al., (2013) also said that Kerandang seeds containing oligosaccharide raffinose and stachyose, so need the pre-treatment such as soaking and boiling to eliminate raffinose and stachyose. As long as there is no information about the effect of soaking and boiling treatment on change of physic chemical characteristic in kerandang beans. Soaking and boiling are the pre-treatment to eliminate the anti-nutrition compounds in Kerandang seeds so that the Kerandang seed to be safe for consumption. Thus, in terms of food safety, kerandang beans potentially consumed as food alternatives, especially as raw material for non-dairy milk. Based on these, the objectives of this research are to know the effect of soaking and boiling treatment on physic-chemical characteristic of Kerandang beans and to give the information about isoflavones content in beans and non-dairy milk of kerandang.

Materials and Methods

Materials

Kerandangs were harvested from wildly grown plants on the sand beach land in the Bugel Village, Panjatan District, Kulon Progo Regency, Yogyakarta, Indonesia. The others materials were using for analysis such as natrium hydroxide (NaOH), galic acid, saccharide standart (glucose, fructose, galactose, sucrose, stachyose and raffinose) and isoflavone standart (daidzin, genistine, daidzien and genistein).

Methods

Soaking and boiling treatment were conducted based on method by Akpapunam (1985) and Nwaoguikpe et al., (2011), modified. Peeled Kerandang beans (water content ± 10%) was soaking in water (27 ºC) during 0 h, 3 hours, 6 hours, 12 hours and 24 hours in ratio seeds and water is 1:6 (w/v). After that, was done boiling during 0 min and 10 min at 80-90 ºC. The ratio seeds and water for boiling is 1:5 (w/v). Furthermore, the seeds were dried until the water content of ±10% and stored in a covered jar until needed for analysis.

Analysis were conducted to ability of water absorption of peeled Kerandang seed (Correa et al., 2010), protein (AOAC, 1990), monosaccharide, disaccharide and oligosaccharide content by HPLC (Vega, 2009), Cyanide (HCN) (AOAC, 1990), total phenolic with Folin-Ciocalteau method (Radix et al., 2009) and isoflavones glycosides, isoflavones aglycone using *Ultrahigh Performance Liquit Chromatograph* (UPLC) based on Tsangalis et al., methdos (2002). The completely randomized design was use in this study with five replications. The data was expressed as a mean values.

Results and Discussion

Water absorption

Water absorption of beans during soaking mainly depends on soaking time and water temperature. Throughout the immersion, water spreads slowly into the beans and eventually reaches a constant level of moisture content (Ranjabri et al., 2011). The ability to water absorb of kerandang beans shown in Fig. 1. The main purpose of soaking is to soften the seed so to facilitate the destruction of seed. In addition, soaking beans also aims to eliminate anti-nutrition such as HCN compounds.

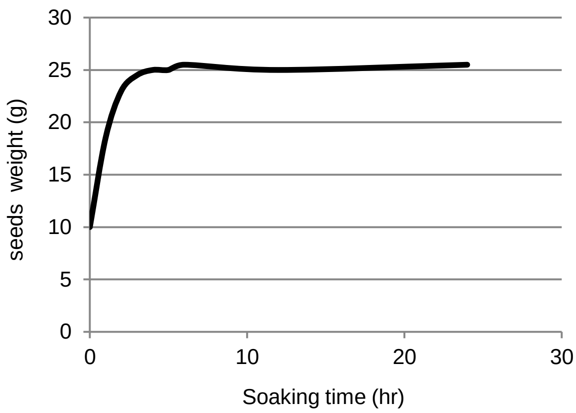

Fig. **1**. Water absorption ability of Kerandang beans during soaking

Water absorption at 1-2 h during was rapid; water absorption percentage was 85% to 130%. Water absorption of kerandang beans was reaches the optimal point on a 3 h soaking in water at 27 °C (150%) and water absorption ability is constant after 3 h soaking. Nyombaire et al., (2016) reported that water absorption of red kidney beans is 80% during the first hour of soaking at 77 °C. The water absorption ability is influenced by grain hardness, the attractiveness of the cotyledons, elasticity, porosity and colloidal properties and enzyme activity (Esteves et al., 2002 in Correa et al., 2010). Acording to Zamindar et al., (2013), soaking and boiling treatment significant effect on water absorption of red beans lines, reached 101.2% during 24 h soaking. Shafaei et al., (2016) explained also that water absorption increase with increasing the water temperature. The time of soaking needed to reach saturation moisture content was shorter in higher water temperature, in the case of chickpea.

In this study, the soaking treatment was conducted against peeled kerandang beans so that the water will more easily diffuse into the beans tissue. As a result of water absorption will be rapid and achieve constant at 3 h soaking. Based on these data, the kerandang non-dairy milk processing will begin with soaking treatment peeled kerandang beans for 3 h.

Protein

Protein is one of the determinants of milk quality so in the non-dairy milk processing attempted prevents reduction of protein. Soaking and cooking/boiling in the processing of Kerandang non-dairy milk plays an important role on protein changes. Kerandang beans contain a protein of 39.17% (db). Change of protein content during soaking and boiling shown in Fig. 2. During soaking for 3 to 12 h, do not decrease protein content. Soaking treatment for 24 h can decrease of 3.83% protein content. Combination soaking 24 h and boiling at 80-90 ºC for 10 min causes decrease protein content in the Kerandang beans significantly. This is caused by proteins that are soluble such as albumin will diffuse out of the cell tissue. This decrease trend of protein content is similar to obsevation by Udensi et al., (2010) who reported that protein content decrease in ukpa (*Munuca flagellipes*) during soaking. Obasi and Wogu (2008) also reported that protein content decrease yellow maize during soaking.

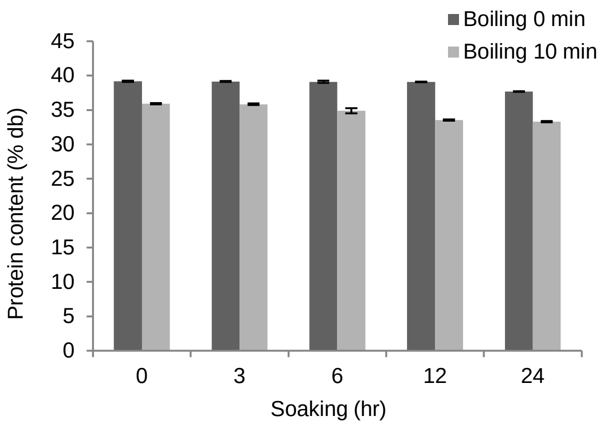

Fig. **2.** Protein content change of Kerandang seeds during soaking and boiling

The boiling and combination treatment (soaking and boiling) caused decrease of protein content significantly. A decrease of protein content in this treatment ranges 8.32 to 14.99%. Besides dissolved in water, the protein will also damage during boiling by high temperatures. Udensi et al., (2010) also explained that reduction of protein content of ukpa (*Munuca flagellipes*) will be increase with soaking and boiling time increasing.

Monosaccharide, disaccharide and oligosaccharide

Oligosaccharides (stachyose, raffinose and verbascose) are present in legume (Reddy et al., 1980; Onigbinde and Okinyele et al., 1980; Vega et al., 2009; Khokhar and Apenten, 2016). They produce flatulence factor in man and animals due to the absence of the enzyme α-galactosidase which is needed for hydrolysis of the α-1.6 galactosidic linkage of these oligosaccharides in the lower intestine. These sugars then undergo anaerobic fermentation by microorganism producing carbon dioxide, hydrogen and small amount of methane gas that cause flatulence which is characterized by abdominal rumblings, cramps, diarrhea and nausea (Price et al., 1988).

Monosaccharide, disaccharides and Oligosaccharides content of kerandang beans during soaking and boiling treatment are presented in Table 1. Kerandang beans containing oligosaccharides raffinose higher than stachyose. Total oligosaccharides in kerandang beans are 1.90 g/100 g of dry beans. Suarez et al., (1999), suggests that consumption of oligosaccharides at least as much as 1.32 g and consumption of soy milk conventional process minimum of 100 ml can potentially cause flatulence, whereas gas production in the intestine occurs in large numbers then removed through the rectum.

Table 1. Oligosaccharides, disaccharide and monosaccharide content of Kerandang seed on soaking and boiling treatment

Soaking (h)	Boiling (min)	Saccharides type (g/100 g dry beans)					
		Raffinose	Stachyose	Sucrose	Glucose	Fructose	Galactose
0	0	1,73 ± 0,11	0,17 ± 0,03	0,83 ± 0,05	nd	0,12 ± 0,01	nd
	10	1,69 ± 0,11	0,12 ± 0,01	0,80 ± 0,03	0,09 ± 0,00	0,15 ± 0,01	nd
3	0	1,71 ± 0,12	0,16 ± 0,01	0,84 ± 0,05	nd	0,14 ± 0,01	nd
	10	1,64 ± 0,10	0,12 ± 0,02	0,85 ± 0,07	0,08 ± 0,00	0,15 ± 0,01	0,01 ± 0,00
6	0	1,66 ± 0,11	0,15 ± 0,03	0,86 ± 0,02	nd	0,16 ± 0,01	0,02 ± 0,00
	10	1,18 ± 0,10	0,09 ± 0,01	1,08 ± 0,11	0,08 ± 0,00	0,31 ± 0,02	0,11 ± 0,00
12	0	0,75 ± 0,04	0,06 ± 0,00	0,30 ± 0,01	0,13 ± 0,01	0,21 ± 0,01	0,24 ± 0,01
	10	0,78 ± 0,04	0,04 ± 0,01	0,26 ± 0,03	0,04 ± 0,00	0,35 ± 0,01	0,28 ± 0,01
24	0	0,90 ± 0,06	0,05 ± 0,00	0,87 ± 0,02	nd	0,49 ± 0,01	nd
	10	0,69 ± 0,03	0,03 ± 0,00	1,01 ± 0,06	nd	0,51 ± 0,02	nd

nd = not detected; Detection limit of glucose = 0,002; detection limit of galactose = 0,003

Kerandang beans also contain sucrose and fructose, whereas glucose and galactose is a monosaccharide that is not detected in kerandang seeds. Hou et al., (2009) suggested that the glucose content in soybean seed is very small and it ranged between 0.003 g/100 g to 0.187 g/100 g and depending on soybean varieties.

In Table 1, can be seen that the pre-treatment of kerandang beans in kerandang non-dairy milk processing by soaking and boiling will reduce the content of oligosaccharides, but a significant reduction was obtained at 12 and 24 h soaking. Soaking during 12 h caused reduce the raffinose and stachyose content respectively of 56.65% and 64.71%. Likewise with 24 h soaking cause reduce raffinose and stachyose content respectively of 47.98% dan 70.59%, which is in agreement with the data reported by Oboh et al., (2000) on selected Nigerian legume seed (Africa yam beans, white lima beans, red lima beans, cream pigeon peas, jackbeans, and brown pigeon peas). Nyombaire et al., (2016) also reported that soaking for 12 h at 77 °C resulted in 80.83% raffinose reduction and cooking of red kidney beans (*Phaseolus vulgaris* L.) for 14 min at 99,3 °C further reduced raffinose content significantly ($p<0.05$) but had no such effect on stachyose reduction.

A decrease in the oligosaccharide content during 12 to 24 h soaking caused by the occurrence of spontaneous or natural fermentation, so microbes which grow can hydrolyze raffianose and stachyose into sucrose, glucose, fructose and galactose. Therefore, in Table 1 can be seen that glucose and galactose decrease during soaking for 24 h. Djaafar et al., (2013) reported that the lactic acid bacteria can hydrolysis raffinose into sucrose, glucose, fructose and galactose during fermentation of Kerandang milk and then use of them for growth. The content of some types of sugar in kerandang beans is potential for further processing into fermented products, especially fermented using lactic acid bacteria. This is due lactic acid bacteria can use various types of sugars as a carbon source for growth and produce useful metabolites such as enzymes and antibacterial compounds such as bacteriocins.

Cyanide (HCN)

Cyanide is anti-nutrition which toxic. Cyanide concentration during soaking and boiling were decrease. A decreased of cyanide during soaking for 3 to 24 h significantly namely 41.04% to 79.99%. Likewise during boiling without soaking, a decrease of cyanide significantly (33.87%). The combination of soaking and boiling treatments also can significant ($p<0.05$) reduced the HCN content, about 59.50% to 93.04%.

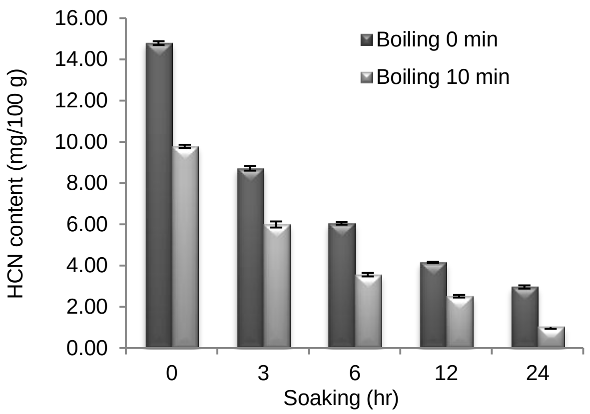

Fig. **3.** Change of HCN content in kerandang seed during soaking and boiling

The decline of HCN content relates to the HCN properties is readily soluble in water. The longer soaking time, more HCN dissolved into the water. In addition, soaking and boiling treatment combination at 80-90 ºC causes cell tissue of beans experienced shrinkage so that cyanide will be out of cotyledons and dissolved in water (Nwaoguikpe et al., 2011). The HCN content reduction during soaking and boiling processing also explained by Effiong and Umeron (2011) that HCN content in horse eye beans (*Mucuna urens*) was significant ($p<0.05$) reduced from 56.30 mg/100 g in the raw sample to 15.20 mg/100 g at 72 h soaking at 37 ºC and 60 min cooking (100 ºC). Yasmin et al., (2008) also reported that cyanide content in red kidney beans was most effectively (25%) reduced by cooking after soaking in sodium bicarbonate solution, followed by germination. Thus soaking and boiling of bens is the right way to reduce the HCN content in Kerandang beans so that the beans safe to consumed. In humans, the clinical signs of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pain, vomiting, diarrhea, mental confusion, twitching and convulsions. Death due to cyanide poisoning can occur when the cyanide level exceeds the limit an individual is able to detoxify. The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5 to 3.5 mg per kg of body weight. Children are particularly at risk because of their smaller body size (Kwok, 2008).

Total phenolic

The polyphenol compounds are secondary metabolite of plants. These compounds classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acid, flavonoids, stilbenes and lignans (Khokhar and Apenten, 2004). The present the polyphenol (total phenolic) are associated with the antioxidant capacity of beans, anticancer and anti-mutagenic (Heimler et al., 2005; Fernandez et al., 2006; Fernandez et al., 2008).

Methanol extract of Kerandang seed has a total phenolic content of 7.42 ± 0,02 mg galic acid equivalents/100 g peeled Kerandang seed (mg GAE/100 g). Total phenolic content of kerandang beans (*Canavalia virosa*) is smaller than the *Canavalia ensiformis* L.DC have a total phenolic content of 3.83% or 3.83 g GAE / 100 g dry beans (Doss et al., 2011).

Change of total phenolic compounds during soaking and boiling of kerandang beans shown in Fig. 4. In Fig. 4 can be seen that soaking for 3 min not significant ($p<0.05$) effected on total phenolic content, a decrease about 2.16%. The soaking for 6-24 h can decrease total phenolic about 22.91% to 54.18%. Likewise the boiling treatment at 10 min can decrease the total phenolic content about 27.49% to 38.41%. Sharma et al., (2013) reported that soaking treatment of soybeans in distilled water overnight at 25 °C had reducing effect on total phenolic compounds about 30.94%. The decrease of total phenolic during soaking may simply be due to leaching out into soaking solution (Ramakrishna et al., 2006).

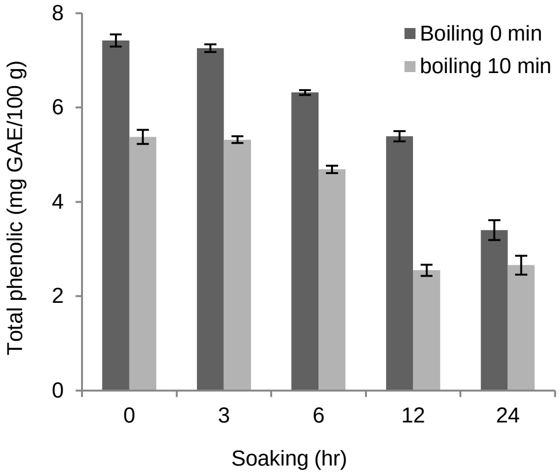

Fig. **4**. Change of total phenolic in kerandang seed during soaking and boiling

The combination of soaking and boiling treatment can decrease total phenolic content about 34.10% to 75.34%. Sowndhararaja et al., (2010) also shown that soaking and boiling treatment can decrease total phenolic content on *Bauhinia vahlii* Wight and Arn. A decrease the total phenolic content of this legume is quite large, about 48% of the soaked and heated beans than the total phenolic of fresh beans. Acording to Xu and Chang (2008), boiling process of pinto beans may cause a decline in the total phenolic content about 63-77%, while for black beans reduction in total phenolic approximately 61-74%. The heating (boiling) process can cause degradation of polyphenols and release of phenolic component, so that during processing, especially heating will decrease the phenolic content of foodstuffs.

Isoflavone

Isoflavones analyses are performed on Kerandang dry beans and Kerandang non-dairy milk. Isoflavone content of Kerandang beans presented in Table 2. Kerandang beans were containing glucoside isoflavones (daidzin and genistine) greater than aglycone isoflavones (daidzein and genistein). The largest components of glucoside isoflavones in the kerandang beans is daidzin (105.13 mg/g dry seeds) followed by daidzein (58.35 mg/g dry beans). Similarly, in soy beans, daizin is the largest component of glycoside isoflavones. According to Albulescu and Popovici (2007), glucoside isoflavones daidzin and genistin are the largest component on plant tissue. The similar results were also reported by Lee et al., (2004) that aglycone isoflavone contain in soybean more less 2% while 25% are glucoside isoflavone, 5% are 6"-*O*-acetyl-β-glukosida and 70 - 80% are 6"-*O*-malonyl-β-glucoside component.

Based on the data in Table 2, can be conclude that kerandang beans could potentially use as a food that has a functional value because it contains isoflavones. Even isoflavones content in kerandang beans greater than Checkpea (*Cicer arietenum*).

Table 2. Isoflavones content in kerandang seeds compare with soybeans and Checkpea beans

Isoflavone	Kerandang (*Canavalia virosa*) (µg/g dry beans)	Soybean KS1[a] (µg/g dry beans)	Checkpea (*Cicer arietenum*)[b] (µg/g dry beans)	Kerandang non-dairy milk (µg/100 ml)
Glucosides				
Daidzin	105.13 ± 5.42	656.82 ± 7.19	16.93	102.49 ± 4.58
Genistin	29.61 ± 0.11	106.71 ± 20.0	Nd	28.96 ± 0.93
Aglycones				
Daidzein	58.35 ± 2.19	10.26 ± 0.58	4.54	20.19 ± 2.70
Genistein	1.93 ± 0.06	19.71 ± 2.53	39.29	1.27 ± 0.15
Total isoflavones	195.02	793.50	60.76	152.91

[a] Lin and Lai (2006); [b] Koh and Perera (2012); nd = not detected

Kerandang non-dairy milk contains isoflavones is equal to 152.91 µg/100 ml Kerandang non-dairy milk. During processing of Kerandang non-dairy milk, a decline in isoflavones content, both glucoside and aglycone isoflavones caused by the nature properties of isoflavones that are not resistant to heat treatment. According to Xu et al., (2002), the heat treatment for 3 min at 185 °C, the loss daidzin and genistin respectively by 26% and 27%, but the stability of the warming of daidzin is greater than genistin. Eisen et al., (2003) also reported that during the heating and storage of soymilk at 70-90 °C, occurring reduced in the content of genistin and daidzin while relatively more stable to heat and during storage soymilk. In fact, according to Mathias et al., (2006), the heat treatment at 80-100 °C and pH 2-10 caused the loss daidzin and genistin but lost daidzin more significant than genistin.

Conclusions

In conclusion, the soaking and boiling treatment used in our study were effective reducing the anti-nutrition factors in Kerandang beans, namely HCN and oligosaccharides. In generally, the anti-nutrition content in Kerandang beans can decrease by soaking and boiling treatment. From the result of this study, the ideal pre-treatment to Kerandang non-dairy milk processing is soaking for 3 h and boiling for 10 min of the peeled Kerandang beans because the cyanide, raffinose and stachyose was reduced, respectively 41.04%; 5.20% and 29.0%. Kerandang beans and Kerandang non-dairy milk were containing the isoflavones that either consumed as healthy food.

Conflict of Interest

All the authors declare that they have no conflict of interest.

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