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Effect of soaking time and fermentation on the nutrient and antinutrients composition of *Canavalia ensiformis* (Kacang Koro)

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Abstract. The increasing demand in developing countries for alternative protein sources, coupled with the relatively high cost of importing protein, has led to the search for alternatives, particularly for novel legumes native to the tropics. *Canavalia ensiformis* or Jack Bean (Kacang Koro) could provide adequate protein sources for human consumption if the presence of various antinutrients can be reduced. The most cost-effective processing technique for the detoxifying method is the soaking process. Therefore, the aim of this study was to determine the effect on the nutritional, antinutritional and mineral content of *C. ensiformis* of soaking time and fermentation. The samples were treated by soaking in 1% sodium bicarbonates (NaHCO_3) solutions for 12 h, 24 h and 36 h (at chilled temperature, 10 °C) and fermented (40 °C). The treated samples were analysed for their proximate values and antinutrient factors (hydrogen cyanide (HCN), phytic acid, tannin, saponin and oxalate content). The results showed that proximate values were not significantly affected by soaking treatment and fermentation. After 36 h of soaking in 1% of NaHCO_3 solution, the HCN level in *C. ensiformis* and fermentation process, the HCN level to be reduced to 7.43 ± 0.76 mg/kg while phytic acid was reduced to 0.64 ± 0.00 , saponin to 1.27 ± 0.01 , tannins to 0.01 ± 0.001 and oxalate to 39.96 ± 5.85 . These results suggest good prospects for substituting *C. ensiformis* for any existing protein source as the antinutrient factors could be reduced to an acceptable level. The soaking of *C. ensiformis* in 1% NaHCO_3 coupled with fermentation would also allow the use of *C. ensiformis* in food production.

1. Introduction

The food industry needs an innovative approach to deliver new materials, technologies and fresh, nutritious, sustainable food products [1]. *Canavalia ensiformis* or Jack Bean (Kacang Koro) is a tropical legume with a protein content of more than 26% belonging to the Fabaceae family [2]. *C. ensiformis* could serve as a potential alternative to providing adequate protein to large populations in developing countries suffering from low nutritional deficiencies as high protein rich foods such as



milk, meat, fish and others are rare or expensive to obtain and this improves in the worldwide variety of protein sources for human consumption [3]. Development of products made from *C. ensiformis* is expected to substitute, reduce or even later can replace soy protein, so that the need and dependence on soybeans can be controlled [4].

Increased protein consumption in developing countries, with the relatively high cost of protein imports, led to the search for alternative sources of cheaper and abundant proteins [5]. Due to its excellent germination and initial growth, *C. ensiformis* has great potential as an economic crop, making it relatively easy to establish [6]. The use of untreated (raw) *C. ensiformis* in diets is restricted by the presence of various antinutrients [7] [8]. Soaking is the most cost-effective treatment for the detoxifying method [9]. Therefore, properly soaking processes *C. ensiformis* have great potential for inclusion in diets [10]. Lack of some essential minerals can lead to severe metabolic disorders and can endanger the body's health, as the presence of phytate in the human diet has a negative effect on the absorption of minerals such as zinc, iron, calcium, magnesium, manganese and copper [11].

Domestic processing techniques have shown that exogenous and endogenous enzymes produced during processing significantly reduce the levels of phytates and tannins. Reductions of these anti-nutritional factors have been reported by processing methods such as gamma irradiation, dehulling, soaking, sprouting, boiling, malting and fermentation [12]. Soaking and fermentation as the most economic method to reduce the HCN toxic before fermentation [8]. Soaking treatment makes a significant amount of soluble cyanide leach out of the soaking water and the rate of reduction of cyanide depends on the soaking time and the form of soaking solution [13]; [14]. Many processing methods, such as soaking, germination, boiling, autoclaving, fermentation, genetic manipulation and other processing methods, could eliminate most of the toxic and anti-nutrient effects of these compounds in *C. ensiformis* [15]. Several studies about the *C. ensiformis* soaking in salt and urea solution have effectively removed the HCN but there is less study regarding sodium bicarbonate (NaHCO_3) solution soaking method. Therefore, the purpose of this study was to determine the effect of soaking time and fermentation on the nutritional, antinutritional content and mineral content of *C. ensiformis*.

2. Materials and Methods

2.1. Raw materials

The mature *Canavalia ensiformis* sp. (kacang koro) were collected from Kampung Pagar Besi, Kuala Nerus, Terengganu.

2.1.1. Soaking process

C. ensiformis seeds were washed with tap water and chilled soaked ($10\text{ }^{\circ}\text{C}$) in 1 % of sodium bicarbonate (NaHCO_3) solution at a different time duration (12h, 24h and 36h).

2.1.2. Fermentation process

The sample was dehulled, boiled, drained and air-dried. Then, 0.02 g of Rhizopus mold was inoculated for every 100 g cooked *C. ensiformis*, and packed into plastic bags, then incubated for 26 hrs at $40\text{ }^{\circ}\text{C}$.

2.2. Proximate composition

Proximate analysis was analysed using the techniques developed by AOAC [16] which included moisture, ash, protein, crude fat, crude fibre and carbohydrate.

2.3. Determination of hydrogen cyanide (HCN)

Twenty grams of soaked *C. ensiformis* were blended into small mashed and soaked in 200ml distilled water for 4 h for maceration then proceeded into distillation of samples. NaOH solution will accommodate HCN acid vapour that is released from beans. After 4 h of steaming, the content of HCN in the sample was vaporized and collected into another flask with 20 ml of 2.5% of NaOH. HCN analysis 20ml of distillate solution was added with 8 mL 6 N NH_4OH and 2 mL 5% KI. The sample was then titrated (AgNO_3 solution) until a permanent turbidity due to the formation of AgI was

observed. Concentration of CN⁻ was calculated using the relation that 1 mL of 0.02 M AgNO₃ containing 1.08 mg of HCN [17].

2.4. Determination of phytic acid

Two grams of the sample were weighed and soaked in 100 ml of 2% HCl for 3 h and filtered (Whatman No. 4). In a separate conical flask, a further 25 ml aliquot of the filtrate was added and 5 ml of 0.3 % NH₄SCN solution was added. Precisely 53.5 ml of deionized distilled water was added and then titrated with the normal solution of iron (III) chloride until 5 min of brownish-yellow colour prevailed [18].

2.5. Determination of tannin content

One millilitre of the sample was added to 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu's phenol reagent, 1 ml of 35 % Na₂CO₃ solution and dilute to 10 ml with distilled water. The mixture was then thoroughly mixed (shaken) and allowed to stand at room temperature for 30 min. The sample and standard solutions were then measured at 725 nm with a visible spectrophotometer [19].

2.6. Determination of saponin content

Two grams of the sample was mixed with 100ml of 20% aqueous ethanol solution and incubation for 12 h at 55 °C with constant agitation filtered through Whatman (No. 42) filter paper. The residue was re-extracted for 30 min with 50ml of ethanol solution and weighed along with the extracts. After mixing, the partition was discarded, and the upper layer was removed while the lower aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with a drop-wise addition of NaOH solution. Saponin in the extract was taken up in successive extraction with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin was then dried in an air oven (Gallenkamp) at 60 °C (to remove any residual solvent), cooled in a desiccator and re-weighed [16].

2.7. Determination of oxalate content

One gram of sample was mixed with 75 ml of 3M H₂SO₄. Then 25 ml of the filtrate aliquot was collected and heated to 80 °C – 90 °C. The filtrate was always kept above 70 °C. The hot aliquot was then titrated against 0.05 M KMnO₄ oxide until the extremely faint pale pink colour persisted for 15-30 s. The oxalate content was then calculated by taking 1 ml of 0.05 M KMnO₄ oxide equivalent to 2.2 mg oxalate [20].

2.8. Statistical analysis

The data were analysed using Minitab 14 software and all data obtained were presented at mean ± standard deviation. One-Way analysis of variance (ANOVA) with Fisher's Least Significant Difference (LSD) test was used as the post-hoc test to determine the significant difference between the mean of samples [21].

3. Results and Discussion

3.1. Proximate composition

Table 1. Proximate analysis for raw and five different treatments *Canavalia ensiformis*.

Nutrients (%)	Sample A	Sample B	Sample C	Sample D	Sample E
Moisture content	8.26±0.01 ^b	8.07 ± 0.21 ^c	7.76 ± 0.97 ^c	8.24 ± 0.19 ^b	8.77 ± 0.49 ^a
Ash content	2.17±0.23 ^a	2.15 ± 0.02 ^a	1.75 ± 0.04 ^b	1.45 ± 0.18 ^c	1.40 ± 0.06 ^c
Crude protein	31.54±0.01 ^b	32.26 ± 0.16 ^{ab}	33.87± 1.03 ^a	31.76± 0.34 ^b	32.12± 0.97 ^{ab}

Crude lipid	8.82±0.03 ^a	8.17 ± 0.09 ^b	8.32 ± 0.08 ^b	7.46 ± 0.13 ^c	9.22 ± 0.17 ^a
Crude fibre	3.54±0.01 ^a	2.33 ± 0.07 ^b	2.40 ± 0.09 ^{ab}	3.33 ± 0.13 ^a	3.62 ± 0.06 ^a
Carbohydrate	45.67±3.51 ^c	47.02 ± 2.14 ^b	45.91± 2.42 ^b	47.76± 2.22 ^a	44.87± 1.99 ^a
energy value (KJ/g)	1487.52±11.4 1 ^a	1475.43±14.64 ^a	1467.28±10.7 4 ^{ab}	1429.28±13.8 8 ^b	1315.82±13.2 2 ^b

Note: Means are reported from 3 replications (n = 3). The different superscripts within the same row are significantly different (p<0.05).

A – None soaking treatment

B –12 h soaking treatment with 1% of NaHCO₃

C –24 h soaking treatment with 1% of NaHCO₃

D –36 h soaking treatment with 1% of NaHCO₃

E – *C. ensiformis* fermented after 36 h soaking with 1% of NaHCO₃

Table 1 shows the moisture content in all samples increased due to the soaking treatment and fermented samples. The higher water retention of *C. ensiformis* is most likely due to the *C. ensiformis* has higher water-holding capacity as it contains more crude fiber [22] and the lowest moisture contained in sample C which also contain low value of ash (1.75 ± 0.04). Table 1 also shows that the ash content of the five treatments decreased as the time of the treatments were prolonged. The process of boiling, soaking in water and dehulling could lead to a loss of 41% ash which may residue in the hull of the bean or leached during processing [23]. Besides, the decreased trend of ash content after fermentation was similar to the study by Gabriel and Akharaiyi [24] who reported that the ash content of the fermented *C. ensiformis* was between 1.15-2.75% while the raw unfermented sample was 1.40%. On the other hand, Haron and Raob [25] reported that there was no significant difference in ash content between cooked soybean and fermented soybean. Premarani and Chhetry [26] also reported that showed decreased ash content but no significance of ash content between fermented and unfermented soybean. Therefore, reduction of ash in soaked *C. ensiformis* could be due to the soaking and boiling process only.

Table 1 also shows a significant decrease from highest protein content in sample C (33.87 ± 1.03) to sample A (31.54 ± 0.01). Prolonging the soaking process resulted in significant decrease in protein content. Furthermore, the resulted in significant decrease in protein content of *C. ensiformis* after soaking could be due to the liberation of ammonia from amino acid deamination during leaching [27]. Fermented product such as tempeh has been found to have improved protein quality to better quality of amino acids [28] increased after fermentation and better digestibility of the product due to mold rapidly degraded these proteins, and utilizes amino acids and low-molecular-weight peptides for its own growth [29]. The loss of fibre content in *C. ensiformis* after soaking may due to the process of boiling and dehulling process that cause the loss of fibre content by 61% since the hull of soybean contain 2.8% of crude fibre and hull of this legume contain 1.4% [30].

The reduction of fat content of raw *C. ensiformis* 8.82 ± 0.03 % was reduced to 7.46 ± 0.13 in the sample soaked after 36 h. Similarly, the raw soybean content was 10.6% fat and the value reduced after soaking process 4.48% and boiling process 4.66% [25]. The decrease of fats content may be due to lipase activity and the production of free fatty acids occurred from the earliest stages of fermentation [31]. The results also showed that the carbohydrates content did not change significantly which could be due to germination or radiation treatment up to 36 h of germination in the samples, ranging between 44.87 ± 1.99 to 47.76 ± 2.22 % [32]. During the 1% NaHCO₃ soaking process, a large range of water-soluble high molecular weight oligosaccharides are liberated by degradation of polysaccharides. Compared to other legumes, *C. ensiformis* had lower levels of carbohydrates content, (35–35%), respectively and there was no change due to soaking treatment.

Table 2. Effect of soaking on anti-nutritional content in *C. ensiformis*.

Soaking time (h)	HCN (mg/kg)	Phytic acid %	Saponin	Tanins (%)	Oxalate content (mg/kg)
A	52.78 ± 1.34 ^a	4.12±0.09 ^a	3.78 ^a ±0.01	0.03±0.001 ^a	400.07± 25.23 ^a
B	42.12 ± 0.76 ^b	3.24±0.07 ^b	3.05 ^b ±0.01	0.03±0.001 ^a	389.83± 16.84 ^a
C	37.40 ± 1.34 ^c	2.86±0.02 ^b	2.45 ^b ±0.01	0.03±0.001 ^a	400.20± 34.13 ^a
D	24.71 ± 0.95 ^d	2.11±0.05 ^b	2.51 ^b ±0.01	0.02±0.001 ^b	375.59± 23.92 ^a
E	7.43 ± 0.57 ^e	0.64±0.001 ^c	1.27 ^c ±0.01	0.01±0.001 ^c	39.96± 5.85 ^b

Note: Means are reported from 3 replications (n = 3). The different superscripts within the same column are significantly different (p<0.05).

A – None soaking treatment

B –12 h soaking treatment with 1% of NaHCO₃

C –24 h soaking treatment with 1% of NaHCO₃

D –36 h soaking treatment with 1% of NaHCO₃

E – *C. ensiformis* fermented after 36 h soaking with 1% of NaHCO₃

3.2. Hydrogen cyanide (HCN) content

C. ensiformis HCN levels were found to be substantially decreased (p<0.05) by pre-treatment of 36 h of soaking and 1% of NaHCO₃ solution (52.78 ± 1.34 to 24.71 ± 0.95 mg/kg) (Table 2), which was as safe for use under the EPA recommended dose range of 50-90 mg/kg of cyanide. Fermentation process continued to reduce the HCN level to (7.43 ± 0.57 mg/kg) which was safe for consumption and that was under the WHO suggestion limit of 10 mg/kg. Chilled soaking method was used to eliminate the overgrowth of microorganism during soaking process [33].

3.3. Phytic acid content

Changes in the concentration of phytic acid in treated *C. ensiformis* seeds after soaking to varying hydration levels are shown in Table 2. The lowest phytic acid content was observed in the 1% NaHCO₃ soaking-fermented treatment, which was 0.64±0.001 %, while the highest phytic acid content in raw *C. ensiformis* (sample A) (4.12±0.09 %). The trend shows that the percentage of phytic acid decreases after going through soaking pre-treatments. The percentage reduction in phytic acid content decreased with increasing hydration levels (Table 2). The raw *C. ensiformis* seed contains 4.12% of phytic acid, while phytic acid found in the soaking-boiling process was 0.64%, suggesting a reduction of 85%. A similar reduction of 9.7% was reported for *Sesbania rostrata* after soaking [34]. However, soaking did not change the concentration of phytic acid in *Sesbania aculeata* [34]. This data is in close agreement with [35] who cited that the reduction of phytic acid in maize is high when using pre-treatment.

3.4. Tannin content

Table 2 also depicts the raw *C. ensiformis* seeds containing up to 0.03% tannins. It can be observed that the tannin content in all of the pre-treatment slightly reduced after going through the soaking pre-treatment. It is interesting to note that the lowest tannin content was observed when the sample was fermented. This finding is in close agreement with a study performed by Agume et al. (2017) [36], who used soybean flour as a sample and demonstrated that the tannin content decreased generally with pre-treatment soaking-roasting. Agume et al [37] also reported a similar finding, citing the use of 48 h of maize soaking and the tannin content decreased by 22%. The tannin content is found to be decreased by the use of pre-treatment soaking, which may be attributed to the diffusion of the ANFs in the soaking water.

3.5. Saponin content

There were general decreases in the amounts of saponin as the *C. ensiformis* samples were soaked for 36 h (Table 2). The largest reduction was found in sample 36 h of soaking + fermentation (3.78-1.27%). After soaking and fermentation, the increased saponin reduction may mainly be because saponins are water soluble [38], and consequently leach into the liquid medium (water) [39].

3.6. Oxalate content

Table 2 also reveals that there were no significant differences in the oxalate content of the pre-treatment soaking samples, except for the fermented sample. The trend shows that the oxalate content of *C. ensiformis* samples was reduced as the time for soaking treatments increased. The data shows that oxalate content of the untreated sample (sample A) is 400.07 ± 25.23 mg/kg while the soaking pre-treatment after 36 h is 375.59 ± 23.92 mg/kg. Similarly, Adekanni and co-workers [40] reported that the soaking pre-treatment could reduce oxalate content in Tigernut up to 37 - 58%.

4. Conclusion

The study showed significant impacts on the nutrient and antinutrient compositions in *C. ensiformis* undergone pre-treatment (soaked in 1% NaHCO₃ at different length of time and fermentation) of the final products. The addition of 1% NaHCO₃ in soaked water reduces anti-nutrients such as HCN, phytic acid, saponin, tannins and oxalate content in the analysed samples. The soaking of *C. ensiformis* in 1% NaHCO₃ coupled with fermentation would also allow the use of *C. ensiformis* in food production.

Conflict of interest

The authors declare that there is no conflict of interest in conducting this study.

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