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Degradation of Antinutrient Factors in Lima Bean (*Phaseolus lunatus)* Seed by Thermal and Nonthermal Treatment

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Lima bean (*Phaseolus lunatus)* seed is among the underutilised legumes widely available and thrive well in lowland tropical rain forest areas. Lima bean seed contains both nutritional components and antinutritional factors. These antinutritional factors like tannin, phytate, oxalate, trypsin inhibitor reduce food intake and nutrient utilization. Therefore, our research focused on the decomposition of tannin, phytate, oxalate, trypsin inhibitor by thermal and nonthermal treatment. Our results revealed that boiling at 100^{o}C for 30 minutes in liquid-solid ratio of 5:1; germination for 60 days could significantly degrade these antinutrient factors. Germination was superior to boiling in eliminating these harmful substances.

Keywords: Phaseolus lunatus, boiling, germination, tannin, phytate, oxalate, trypsin inhibitor

INTRODUCTION

Lima bean *(Phaseolus vulgaris)* is a herbaceous plant grown for its edible bean (Emmanuel et al., 2012). It is an annual or short-lived perennial species adaptably survive in poor soils (Akinmutimi, 2001). Lima bean seeds contain high proteins, antioxidants, vitamins, minerals, and plant sterols but low in fat and crude fibre beneficial for human alimentation. However, it has anti-nutritional factors as well. Food intake and nutrient utilization will be lower in the existing of these harmful elements (Kehinde et al., 2014). Oxalate, phytate, tannin and trypsin inhibitor are major antinutritional factors in legums (Ojiako and Igwe, 2008). Tannin had cross-link with proteins and caused a reduction protein digestion of beans (Jacob et al., 2015; Inyang et al., 2015; Ajibola et al., 2016). It might be insoluble or generally inaccessible under physiologic conditions (Cheryan, Munir, 1980). The inhibition of digestive enzymes increased excretion of endogenous protein affecting to digestive tract (Parmar et al., 2017). Tannin can be decreased through dousing or different types of preparing (Yimer, 2019). Phytate is an antinutrient factor normally existed in legume seeds. It is an antioxidant possiblly binding to dietary minerals, interfering with their availability in the digestive tract resulting to mineral deficiency (Tan et a., 1983; Bello et al., 2008). Oxalate is a great concern because of its negative effect on mineral availability. High oxalate feeding can increase the risk of renal calcium absorption implicating as a source of kidney stones (Chai and Liebman, 2004). Oxalate causes gastrointestinal tract aggravation, blockage of the renal tubules by calcium oxalate precious stones, improvement of urinary calculi and hypocalcemia (Oke OL, 1969). Trypsin inhibitor upsets the stomach related process in some physiological responses. The presence of trypsin inhibitor in uncooked animal feed has long been known to cause diminished growth in rats, chickens and other experimental animals. Typsin inhibitor is heat labile and can be inactivated by thermal treatment such as steaming, boiling, baking, roasting, extrusion cooking (Liener, 1994)

or even nonthermal treatment like germination, maturation, draining (Megat and Azrina, 2012; Yimer, 2019).

Thermal treatment is widely applied as an effective means of inactivating the thermo-labile antinutritional factors of legume grains (Osungbade et al., 2016) while improving the nutritive quality of groundnuts (Olaposi et al., 2017). Thermal process inactivates anti-physiological factors particularly trypsin inhibitors and haemagglutinins by unfolding the protein structure, thus making them more susceptible to attack by digestive enzymes (Olaposi et al., 2017). Non-thermal treatment like germination is naturally happened in the seed growth period (Sangronis et al., 2006). In this process, basic matrices are metabolized for respiration and synthesis of new cells prior to forming embryo (Vidal et al., 2002). The process begins with the absorption of moisture by the quiescent dry seed and stop with the appearance of the embryonic axis (Megat and Azrina, 2012). Germination has been demonstrated to increase bioavailability of nutritional components like protein, vitamin, dietary fiber, mineral while reducing antinutritional factors like tannin and phytic acid content (El-Adawy, 2002; Ghavidel and Prakash, 2006; Kaushik et al., 2010). Objective of our study focused on degradation of tannin, phytate, oxalate, trypsin inhibitor by thermal and nonthermal treatment.

MATERIALS AND METHODS

Material

Raw *Phaseolus vulgaris* seedswere collected from super market in SocTrang province, Vietnam. After collecting, they must be kept in dry place before boiling or germination. Chemical reagents such as HCl, NH_4OH, methyl red, $CaCl_2$, H_2SO_4, $KMNO_4$, methanol, vanillin hydrochloric acid, $Fe(NO_3)_3$, NaOH, standard trypsin, benzoyl-dl-arginine-pnitroamide were all analytical grade. The main equipment was spectrophotometer.

Researching method

Experiment #1: Effect of liquid-solid ratio in boiling

Phaseolus vulgaris seeds were washed with clean water before being soaked for 8 hours at ambient temperature. Samples were boiled at 100^{o}C for 30 minutes in different solid: liquid ratio (2:1, 3:1, 4:1, 5:1, 6:1 v/v). The treated samples would then be analyzed tannin (mg/g), phytate (mg/g), oxalate (mg/100g), tripsin inhibitor (TIU/g).

Experiment #2: Effect of incubation time in germination

Phaseolus vulgaris seeds were washed with clean water before being soaked for 8 hours at ambient temperature. Samples were kept under wet muslin cloth and left incubated for different durations (24, 36, 48, 60, 72 hours) at ambient temperature without direct contact with sun light. The treated samples would then be analyzed tannin (mg/g), phytate (mg/g), oxalate (mg/100g), tripsin inhibitor (TIU/g).

Chemical analysis

Tannin (mg/g) was determined by the method of Adegunwa et al. (2011). Phytate (mg/g) was estimated by the method of Wheeler and Ferrel (1971). Oxalate (mg/100g) was examined by the method of Falade et al. (2004). Trypsin inhibitor (TIU/g) was determined by the method ofMbata et al. (2009).

Statistical analysis

The experiments were run in triplicate with three different lots of samples. The data were presented as mean ± standard deviation. Statistical analysis was performed by the Statgraphics Centurion version XVI.

RESULTS AND DISCUSSION

Effect of liquid-solid ratio in boiling to degradation of antinutrient factors in lima bean (*Phaseolus vulgaris)* seeds

Result shown in table 1 revealed that the tannin (mg/g), phytate (mg/g), oxalate (mg/100g), tripsin inhibitor (TIU/g) would be significantly decreased when increasing the liquid-solid ratio in boiling. There was not significant difference at 5:1 and 6:1 of liquid-solid ratio. Mercedes et al. (1999) indicated that antinutritional factors in *Phaseolus vulgaris* were strongly affected by both environmental and genetics factors. Olaposi et al. (2017) proved that soaking and boiling significantly reduced the anti-nutritional factors of the groundnut samples and the effect increased as processing time was elongated. The anti-nutrient content of alkaloid, phytate, saponin, tannin, trypsin inhibitor, oxalate in the lima bean flours were significantly reduced by autoclaving and boiling compared to roasting (Oraka et al., 2017).

Table 1: Effect of liquid-solid ratio (v/v) in boiling to degradation of antinutrient factors

Liquid-solid ratio (v/v)	2:1	3:1	4:1	5:1	6:1
Tannin (mg/g)	3.41±0.01^{a}	3.25±0.00^{ab}	3.11±0.00^{ab}	2.96±0.01^{b}	2.94±0.03^{b}
Phytate (mg/g)	7.25±0.00^{a}	7.06±0.02^{ab}	6.84±0.01^{ab}	6.53±0.00^{b}	6.50±0.02^{b}
Oxalate (mg/100g)	0.65±0.02^{a}	0.47±0.01^{ab}	0.32±0.02^{b}	0.17±0.03^{bc}	0.08±0.01^{c}
Tripsin inhibitor (TIU/g)	18.69±0.03^{a}	18.62±0.03^{a}	18.04±0.01^{ab}	17.36±0.02^{b}	15.05±0.00^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Table 2: Effect of incubation time (hours) in boiling to degradation of antinutrient factors

Incubation time (hours)	24	36	48	**60**	72
Tannin (mg/g)	2.45±0.02^{a}	2.31±0.02^{ab}	2.14±0.00^{b}	2.05±0.02^{bc}	1.87±0.00^{c}
Phytate (mg/g)	5.07±0.01^{a}	4.92±0.00^{ab}	4.87±0.01^{ab}	4.75±0.01^{b}	3.84±0.01^{c}
Oxalate (mg/100g)	0.34±0.03^{a}	0.26±0.03^{ab}	0.19±0.02^{b}	0.11±0.00^{bc}	0.04±0.02^{c}
Tripsin inhibitor (TIU/g)	12.64±0.00^{a}	10.97±0.01^{ab}	8.23±0.01^{b}	6.57±0.02^{bc}	3.69±0.00^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

3.2 Effect of incubation time in germination to degradation of antinutrient factors in lima bean (*Phaseolus vulgaris)* seeds

Result shown in table 2 revealed that tannin (mg/g), phytate (mg/g), oxalate (mg/100g), tripsin inhibitor (TIU/g) would be gradually decreased when extending the incubation time to 60 or 72 hours. Kehinde et al. (2014) investigated the influence of fermentation of ground-cooked Lima of bean seeds by *Bacillus subtilis* and *B. pumilus* in its anti-nutrient contents. Oxalate, phytic acid were reduced in naturally fermented sample.

CONCLUSION

Anti-nutrients in lima bean seeds can be significantly degraded by various processing methods like boiling, roasting, autoclaving, blanching, fermentation, soaking and germination. In this research, we have investigated the liquid-solid ratio in boiling and incubation time in germination to the degradation of tannin, phytate, oxalate and trypsin inhibitor in lima bean seed. Germination is highly prefered to reduce these harmful substances to safe levels. Food intake and nutrient utilization will be better when anti-nutrients are efficiently eliminated.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Nguyen Phuoc Minh arranged the experiments and also wrote the manuscript.

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