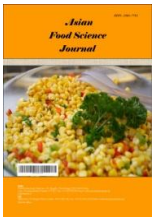

Asian Food Science Journal

20(1): 1-9, 2021; Article no.AFSJ.64312
ISSN: 2581-7752

In-vitro Analysis of Trypsin and Alpha - Amylase Inhibitory Activities in Selected Legume Varieties in Sri Lanka

A. M. C. N. Abeykoon[1], H. M. T. Herath[2*] and M. A. Jayasinghe[1]

[1]Department of Food Science and Technology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Gangodawila, Nugegoda, Sri Lanka.
[2]Food Technology Section (FTS), Modern Research and Development Complex, Industrial Technology Institute (ITI), 503A, Halbarawa Gardens, Malabe, Sri Lanka.

Authors' contributions

This work was carried out in collaboration among all authors. Author AMCNA designed analysis strategies, performed basic laboratory experiments, data analysis and wrote the manuscript. Author HMTH received the research funds and designed analysis strategies and revised manuscript. Author MAJ supervised a student's work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AFSJ/2021/v20i130248
Editor(s):
(1) Dr. Uttara Singh, Panjab University, India.
Reviewers:
(1) Alixelhe Pacheco Damascena, Universidade Federal do Espírito Santo, Brazil.
(2) Ameel Mohammed Al-Mayah, University of Baghdad, Iraq.
Complete Peer review History: http://www.sdiarticle4.com/review-history/64312

Original Research Article

Received 01 November 2020
Accepted 06 January 2021
Published 27 January 2021

ABSTRACT

Aim: To quantify the Trypsin Inhibitory Activity (TIA) and Alpha - Amylase Inhibitory Activity (AIA) in legume varieties with effect of cooking.
Study Design: Seeds of twelve legume varieties grown in Complete Randomized Block Design (CRBD) in experimental field conditions were used and data analysis was performed using one-way ANOVA at 95% confidence interval using MINITAB statistical software.
Place and Duration of Study: Grain Legumes and Oil Crops Research and Development Centre (GLOCRDC), Angunakolapalessa and Industrial Technology Institute (ITI), Colombo, Sri Lanka between June 2019 and Dec 2019.
Methods: Ethanolic (80%) extracts of raw and cooked grain legumes were used. In determining TIA, N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) is used as a synthetic substrate for trypsin enzyme and the rate of hydrolysis was measured by intensity of colour released by p-

**Corresponding author: Email: theja@iti.lk;*

nitroaniline. AIA was carried out determining the maltose content which was released by hydrolysis of starch in the presence of amylase enzyme using reduction of 3, 5-dinitrosalicylic acid.
Results: TIA in raw legumes ranged from 0.65±0.02 mg/g (ANK- Brown) to 1.52±0.01 mg/g (ANKCP1) while in cooked legumes ranged from -0.11±0.1 mg/g (ANK-Black) to 0.61±0.02 mg/g (MI 5). In pressure cooking (120^{o}C, 10 min) considerable reduction in TIA of 53.74% (MI6) to 100% (ANK-Black, Bombay) was observed. A significant difference ($p<0.05$) in TIA among the varieties as well as among cooked form of varieties were observed (one- way ANOVA). Further a significant difference ($p<0.05$) in TIA was observed between cooked and raw form in each legume variety (Paired T-Test).
AIA of cooked samples was ranged from - 11.61% (MI 6) - 23.05% (MISB1) and there was no significant difference ($p<0.05$) in AIA among the most of the legume varieties.
Conclusion: A significant reduction of TIA among the legumes varieties was observed in the pressure cooking process while a significant activity of alpha- amylase was not seen in cooked legumes.

Keywords: Leguminosae; anti-nutritional factors; protease inhibitors; pressure cooking; starch blockers.

1. INTRODUCTION

Grain legumes are annual crops belong to family Leguminosae. They are an important source of protein, minerals, fiber and energy requirement in a diet [1,2]. Next to the cereals legumes provide a range of essential nutrients and contribute to address the under nutrition in the most of the developing countries [3]. Since they are rich source of protein more recently received a particular attention as viable alternative to the meat based diets.

Legume seeds are consumed widely in different areas of the world. Cowpea and yard long bean (*Vigna unguiculata*), green gram (*Vigna radiata*), black gram (*Vigna mungo*), horse gram (*Macrotyloma uniflorum*), soy bean (*Glycine max*), ground nut (*Arachis hypogaea*), beans (*Phaseolus vulgaris*), winged bean (*Psopocarpus tetragonolobus*) have been recognized as economically important varieties cultivated especially in South Asia region [4].

Although legumes provide a variety of health benefits their use in human diet is limited than expected due to the presence of anti-nutritional factors. Anti-nutritional factors (ANFs) are those biological substances present in legumes that reduce nutrient utilization thereby contributing to decrease gastrointestinal and metabolic performance. Legumes contain several anti-nutritional and toxic factors which are either heat labile or heat stable. Phytic acid, saponins, polyphenols, lathyrogens, alpha--galactosides, protease inhibitors, alpha--amylase inhibitors and lecithins which are found in grain legumes [5]. Trypsin is a proteolytic enzyme which involved in the protein digestion in humans. Trypsin inhibitors, one of the major anti-nutritional factors present in legumes bind with trypsin enzyme and reduce the protein digestibility and absorption [6].

Alpha--amylase is a prominent enzyme found in the pancreatic juice which breaks down large insoluble starch molecules into absorbable small molecules. Alpha- amylase inhibitors are also called as starch blockers since it prevents or slows down the absorption of starch in to the body by mainly blocking the hydrolysis of 1, 4 - glycosidic linkages of starch and other oligosaccharides into maltose and other simple sugars. These alpha- amylase inhibitors prevent dietary starches from being digested and absorbed by the body lowering risk of diabetes mellitus type-II.

During the cooking process some of those factors are partly removed or inactivated [7]. In the present study some selected legumes (mung bean, cowpea, soybean and horse gram) were evaluated for the changes in Trypsin inhibitory activity and alpha-amylase inhibitory activity in states of their raw and after cooking process.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Twelve legume varieties were grown in Complete Randomized Block Design (CRBD) in experimental field conditions at Grain Legumes and Oil Crops Research and Development Centre (GLOCRDC), Angunakolapalessa, Sri Lanka were used for screening purpose. Mature seeds of those legume varieties six cowpea

(*Vigna unguiculata*) varieties (Bombay, Dhawala, Waruni, MICP 1, ANKCP 1, and ANKCP 2), two mung bean (*Vigna radiata*) varieties (MI 5, MI 6), two soy bean (*Glycine max*) varieties (Pb 1, MISB 1) and two horse gram (*Macrotyloma uniflorum*) varieties (ANK-Black, ANK-Brown) were used for screening process.

For the raw legume samples the seeds were milled to pass through a 0.5 mm sieve. For cooking treatment, the seeds (25 g) were soaked in distilled water in ratio of 1:10 (seed: water; w/v) overnight at room temperature (25±2°C). After decanting water the soaked seeds (seeds: water 1:5 w/v) were subjected autoclaving for 10 min at 120°C. Soon after decanting the liquid the autoclaved seeds were freeze-dried. The freeze-dried seed samples were ground to fine powder to pass through a 0.5mm sieve and stored at 4^{o}C till analysis [8].

2.2 Sample Extraction

Accurately weighed (around 1.000 g) ground seed powder was extracted with 50 mL of 0.01M sodium hydroxide at room temperature for 3 h. After filtration the pH of the extract was adjusted between 8.4 -10.0 and diluted to 100 mL with distilled water and used for the Trypsin inhibitory activity.

The ground grain powder (2.5 g) was shaken overnight at room temperature (25±2°C) with 20 times of the sample weight of 70% ethanol. Extracts were then centrifuged at 3000 rpm for 15 min. evaporated under vacuum in a rotary evaporator and resulted solution was freeze dried for the Alpha- amylase inhibitor activity [9].

2.3 Determination of Trypsin Inhibitory Activity

Trypsin inhibitory activity of grain legumes was determined according to the method described by Hamerstrand *et al*. 1981 in accordance with AACC International method [10,11]. Initially 0.25 mL of the suspension was mixed with 0.5 mL of trypsin solution (1.00 mg in 50 mL of 0.001N HCL) and incubated for 10 minutes at room temperature (37^{o}C). Then 1.25 mL of 0.04% N-α-benzoyl-DL-arginine-p-nitroanilide hydrochloride (BAPA) substrate was added and further incubated for 10 minutes at room temperature (37^{o}C). Reaction was terminated by adding 0.25 mL of 30% acetic acid and the content was mixed and filtered using 0.5 µm micro filters. The trypsin standard was prepared by adding only trypsin and distilled water without adding the sample. A sample blank was prepared in which trypsin solution was added after termination of the reaction. Absorbance was measured at 410 nm against a sample blank using 96 well micro plate reader (SpectraMax Plus 384, Molecular Devices, USA) and TIA was calculated using following equation. All measurements were done in triplicate.

$$\text{Trypsin Inhibitory Activity (TIA} = \frac{(A)\,(B)*100}{(C)\,(1000)\,(W)\,(V)}$$

Where,

A = Absorbance of trypsin standard – Absorbance of test sample, corrected for absorbance sample blank, B = Dilution factor, C = TU factor, 0.019 based on the fact that 1µg of pure trypsin produced 1.9 TU under conditions of the assay, W = Sample weight (g), V = Sample volume (mL).

2.4 Determination of Alpha-Amylase Inhibitory Activity

Alpha-amylase inhibition of grain legumes was determined according to the method described by Bernfeld, 1955 with slight modifications [12]. Initially 50 µL of the suspension was mixed with 40 µL of 1% starch solution. Then 860 µL of 100 mM sodium acetate buffer was added to it and incubated at 40^{o}C for 10 minutes. Then 50 µL of alpha-- amylase enzyme (porcine pancreas) was added and further incubated at 40^{o}C for 15 minutes. After that 500 µL of 3, 5-dinitrosalicylic acid (DNS) reagent was added and placed in a boiling water bath for 5 minutes and allowed to cool in a water bath containing ice. Control experiments were carried out in an identical way replacing sample extracts with 100 mM sodium acetate buffer. A sample blank was prepared by replacing the sample extracts, starch and enzyme solution with 100mM sodium acetate buffer. Absorbance was measured at 540 nm against a sample blank using 96 well micro plate reader (SpectrMax Plus 384, Molecular Devices, USA) and alpha-- amylase inhibition % was calculated. All measurements were done in triplicates.

$$\text{Inhibition (\%)} = [A_c - (A_s - A_b / A_c]*100$$

Where A_c is the absorbance of the control, A_b is the absorbance of the blank (sample blank) and A_s is the absorbance in the presence of sample.

2.5 Statistical Analysis

Statistical analysis of data was done using one-way ANOVA to identify the significant differences among the samples at 95% confidence interval

using MINITAB statistical software and Tukey Pair wise comparisons to identify whether there is a significant difference among raw and cooked forms of each variety.

3. RESULTS AND DISCUSSION

Trypsin Inhibitory Activity content of grain legumes mainly depends on the extracting solvent and extracting time. Further the factors involved in protein solubility and recovery depend on the ratio of content of protein and type of solvent, particle size of flour, temperature, length of extraction time, pH, ionic strength and concentration of extractant as well as the hydration properties of proteins [13].

The aqueous alkali has been popular among other solvents due to high capability for protein solubilisation. Therefore, in this study trypsin inhibitors were extracted using alkaline media.

3.1 Trypsin Inhibitory Activity of Raw Legumes

TIA of raw and processed legume samples is shown in the Table 1.

According to the results obtained, TIA of raw legumes ranged from 0.65 ± 0.02 mg/g (horse gram variety; ANK BROWN) - 1.52 ± 0.01 mg/g (Cowpea variety; ANKCP1) on dry basis. A significant difference ($p< 0.05$) in TIA among the varieties was observed. Varieties of MICP 1 (1.39 ± 0.05 mg/g), MI5 (1.37 ± 0.02 mg/g), Waruni (1.35 ±0.03 mg/g) and Bombay (1.34 ± 0.02 mg/g) showed the second highest TIA without significant ($p<0.05$) differences among each other. Considerable differences within the species were observed in mung beans, cowpea and soybeans except among the species of horse gram.

TIA of cowpea exhibited maximum value for ANKCP1 (1.52±0.01 mg/g) and minimum value was found for ANKCP2 (0.90 ± 0.002 mg/g) in raw form. The values for MICP 1(1.39 ± 0.05 mg/g), Waruni (1.35 ± 0.03 mg/g) and Bombay (1.34 ± 0.02 mg/g) were not significantly different and had significantly higher than those for Dhawala (1.11 ± 0.03 mg/g) and ANKCP2 (0.90 ± 0.002 mg/g). In comparison of mean values of TIA in mung bean varieties, MI5 (1.37 ± 0.02 mg/g) is significantly higher ($p<0.05$) than MI6 (1.18 ± 0.06mg/g. A significant difference ($p<0.05$) was found between two soybean varieties MISB 1 (0.83±0.05 mg/g) and Pb 1 (0.98±0.03 mg/g). TIA of Pb 1 is significantly higher than TIA of MISB1. Su and Chang 2002 had reported that soybean contains the highest amount of trypsin inhibitors that accounts for two to six percent of whole soybean protein [14]. Further, other legumes contain trypsin inhibitors in significant quantities but the soybean inhibitor quantities were found to be the largest by far [14]. In the present study, TIA of soybean was not observed in significantly ($p<0.05$) higher amounts. The reason for the variation in TIA may be due to the differences in genetic and environmental conditions [15].

Table 1. Trypsin inhibitory activity in raw and cooked form of legume varieties

Legume	Variety	Trypsin inhibitory activity		
		Raw (mg/g) Mean ± SD	Cooked (mg/g) Mean ± SD	Percentage reduction
Horse Gram	*Macrotylomauniflorum*(ANK-Brown)	0.65±0.02^{h}	0.04±0.12^{def}	93%
	Macrotylomauniflorum(ANK-Black)	0.69±0.01^{h}	-0.11±0.1^{g}	116%
Soya bean	*Glycine max* (MISB 1)	0.83±0.05^{g}	0.35±0.04^{b}	57.28%
	Glycine max (Pb 1)	0.98±0.03^{e}	0.35±0.04^{b}	63.79%
Mung bean	*Vigna radiata* (MI 6)	1.18±0.06^{c}	0.55±0.02^{a}	53.74%
	Vigna radiata (MI 5)	1.37±0.02^{b}	0.61±0.02^{a}	55.86%
Cowpea	*Vignaunguiculata* (ANKCP 2)	0.90±0.002^{f}	0.02±0.02^{ef}	98.01%
	Vignaunguiculata (ANKCP 1)	1.52±0.01^{a}	0.09±0.05^{cde}	94.31%
	Vignaunguiculata (MICP 1)	1.39±0.05^{b}	0.32±0.02^{b}	77.33%
	Vignaunguiculata (Waruni)	1.35±0.03^{b}	0.16±0.05^{c}	88.42%
	Vignaunguiculata (Dhawala)	1.11±0.03^{d}	0.13±0.06^{cd}	88.23%
	Vignaunguiculata (Bombay)	1.34±0.02^{b}	-0.04±0.08^{fg}	103%

Data presented as mean ± SE (n=3). Mean values in a column superscripted by different letters are significantly different (p<0.05)

3.2 Trypsin Inhibitory Activity of Cooked Legumes

In pressure cooked samples, TIA ranged from - 0.11 ±0.1mg/g (horse gram variety: ANK Black) - 0.61±0.02 mg/g (mung bean; MI5) on dry basis. A significant difference ($p<0.05$) among varieties was observed in cooked form. Mung bean variety MI 5 showed the highest TIA. Pb 1, MISB1, MI 6 and MICPI showed the second highest TIA. Considerable differences in TIA among the varieties were observed in cowpea and horse gram while significantly difference ($p<0.05$) was not observed within the species of mung bean and soybean.

Comparison between cooked and raw form of each legume variety using Paired t-Test was shown that there is a significant difference ($p<0.05$) in TIA. Legume seeds exhibited considerable reduction in TIA which is ranging from 53.74% (MI 6) to 100% (ANK-Black).

Mung bean varieties had shown low percentage reduction of TIA in cooking showing MI 6 53.74% and MI 5 55.86%. This result is deviate to the literature stated by Grewal and Jood 2006, where percentage of inhibition ranged between 65-70% in mung bean [16]. It has been further observed that temperatures above 80°C can damage some important nutrients, such as lysine, sulfur amino acids and heat-labile vitamins. According to the information published for mung beans, autoclaving or pressure-cooking represents the best option for home cooking since this method preserves the highest amount of minerals in the seed [17].

The percentage of reduction of TIA in cowpea varieties ranged from 77.33%- 100%. That range is within the range stated by Khattab [7] and it was higher compared to reduction in soybean varieties (57.28%- 63.79%). Percentage reduction of horse gram varieties ranged from 93%- 100%. Results of the present study revealed that pressure cooking at 120^{o}C for10 mins had significant effects on TIA (Fig. 1).

The Trypsin Inhibitory Activity is found to be significantly ($p<0.05$) higher in raw samples when compared to the cooked samples. Pressure cooking brought a total removal of trypsin inhibitory activity in horse gram variety ANK Black and cowpea variety Bombay. A relatively high reduction was observed in cowpea and horse gram varieties, with lower reductions for Mung bean and Soy bean varieties. A complete removal of trypsin inhibitory activity due to cooking has been reported previously for legumes by many researchers for common beans [18,19,20]. As all the food legumes are heat treated before consumption by humans the destruction of those inhibitors is expected. However some residual activity can be found when proper conditions were not achieved. Since the TIA is time and temperature dependent in the present processing conditions could be effectively used to increase the nutritional quality of legumes by destroying most of the trypsin inhibitors and other anti-nutrients. According to the present results, for complete inactivation of TIA it may be longer duration of cooking required. While extending the time periods for thermal treatment can inactivate TIA effectively denaturing of essential proteins resulting in amino acid degradation, browning reaction and other deteriorative reactions were occurred [21]. According the present study, autoclaving 10 min may not be sufficient enough to totally deactivation of TIA. Therefore the longer time duration (around 15 or 20 min) could be achieved for better results.

3.3 Alpha- Amylase Inhibitory Activity

Alpha- amylase catalyzes the hydrolysis of glycosidic linkages in the starch and releases the hydrolyzed products, which constitute the first step in the enzymatic degradation of this polymer. Maltose released from starch is measured by the reduction of 3, 5-dinitrosalicylic acid. The intensity change in colour was measured spectrophotometrically. Since high amylase inhibition results in many harmful side effects in human beings, low level of alpha - amylase inhibitors from natural fruits, vegetables and legume grains are reported to offer a good strategy to control postprandial hyperglycemia over the synthetic drugs [22]. Alpha- amylase inhibitory activity of grain legumes is presented in Table 2.

According to the results, percentage inhibition of legume varieties varied from 11.61% -23.5%. A significant difference ($p<0.05$) in AIA was not observed among the legume varieties. All the varieties showed percentage inhibitory activities below 50% at concentration of 100 µg/ml. The highest percentage of inhibition was observed in soybean variety MISB 1 (23.5%) and the lowest was observed from mung bean variety MI 6 (11.61%).

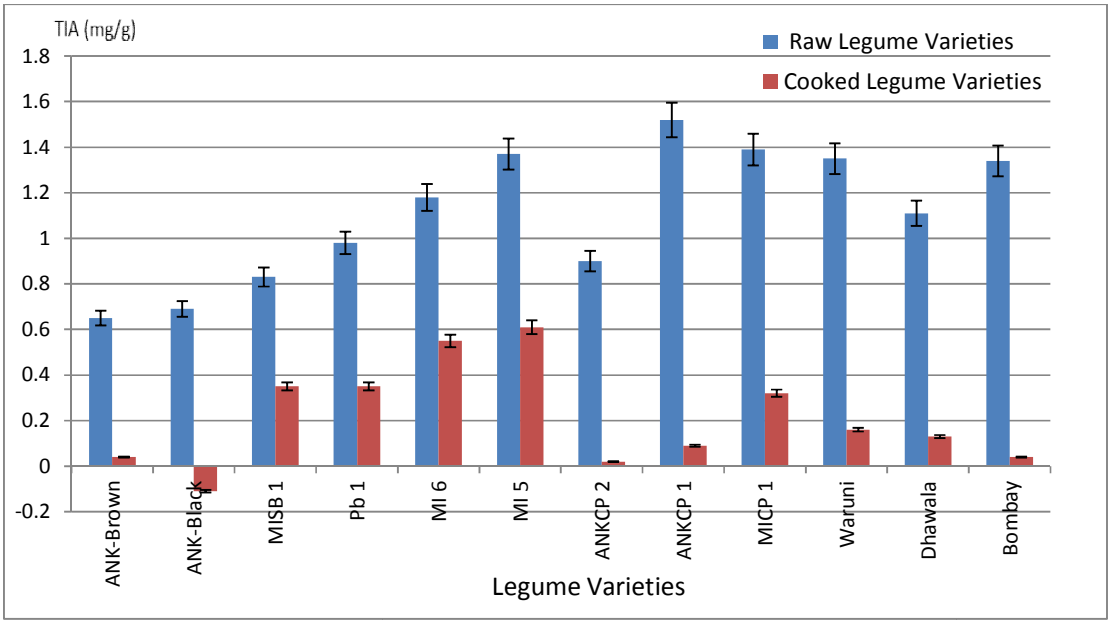

Fig. 1. Trypsin Inhibitory activity in raw and cooked legume varieties

Table 2. Percentage Alpha- amylase inhibition in cooked legume seeds

Legume	Variety	Alpha- amylase inhibition % Mean ± SD
Horse gram	*Macrotylomauniflorum* (ANK-Brown)	19.05± 2.43[a]
	Macrotylomauniflorum (ANK-Black)	20.26 ± 2.83[a]
Soybean	*Glycine max* (MISB 1)	23.50 ± 1.53[a]
	Glycine max (Pb 1)	16.80 ± 1.78[ab]
Mung bean	*Vigna radiata* (MI 6)	11.61 ±2.90[b]
	Vigna radiata (MI 5)	19.70 ± 3.35[a]
Cowpea	*Vignaunguiculata* (ANKCP 2)	22.49 ± 2.77[a]
	Vignaunguiculata (ANKCP 1)	18.41 ± 2.93[ab]
	Vignaunguiculata (MICP 1)	22.65 ± 1.88[a]
	Vignaunguiculata (Waruni)	18.25 ±1.86[ab]
	Vignaunguiculata (Dhawala)	17.91 ± 1.75[ab]
	Vignaunguiculata (Bombay)	20.26 ± 0.28[a]

Data presented as mean ± SE (n=3). Mean values in a column superscripted by different letters are significantly different ($p<0.05$)

In comparison of AIA values in varietal wise no significant difference ($p<0.05$) was observed within the cowpea variety. The highest percentage of inhibition was observed in MICP 1 (22.65%) followed by ANKCP 2 (22.49%), Bombay (20.26%), ANKCP 1 (18.41%), Waruni (18.25%), Dhawala (17.91%) respectively. Olusegun and Emmanuel, 2019 had reported the percentage inhibitory activities above 50% at concentrations of 0.20 mg/ml to 1.0 mg/ml in cowpea [23]. In mung bean varieties, a significant difference ($p<0.05$) in alpha- amylase inhibition was observed between MI 5 and MI 6. Within the variety the highest inhibition was observed in MI 5 (19.70%) and the lowest was observed from MI 6 (11.61%). In soybean, there was no significant difference ($p<0.05$) between the two varieties MISB 1 and Pb 1. Within the variety highest inhibition percentage was observed in MISB 1 (23.50%) and the lowest was

observed in Pb 1 (16.80%). Minor information is available in the nature, whether heat-resistant or heat-labile amylase inhibitor present in legume seeds. Singh [24] stated that the amylase inhibitors of chickpea and pigeonpea as heat-labile. Irshad & Sharma [25] reported the study on purification of heat-resistant amylase inhibitor from peanut. Likewise a study on presence of amylase inhibitors with different heat-stabilities has been revealed in bean seeds [26].

Lan Shi1 and Kaiwen Mul [27] reported that approximately 80–93% reduction of AIA and a complete inactivation of alpha- amylase in soybean. According to the previous literature amylase inhibitory activity of common beans was comparatively higher. This may be due to the differences in cultivars, climatic conditions, location, soil type and crop year. The cellular structure of the seed and the concentration of the solvent also affect the removal of alpha- amylase inhibitors from legumes. The factors which refer to alpha- amylase inhibitor activity are the reaction temperature, incubation period and presence of certain ion. The optimal reaction pH value for the inhibitor is 4.5–5.5 and optimal temperature is 22–37°C and it becomes inactivated completely after 10 minutes of cooking.

According to the results obtained in the present study there was no significant difference between the two horse gram varieties. Highest percentage inhibition was observed in ANK-Black (20.26%) and lowest in ANK-Brown (19.05%).

4. CONCLUSION

TIA of raw legumes ranged from 0.65±0.02 mg/g (Horse gram variety; ANK BROWN) - 1.52±0.01mg/g (Cowpea variety; ANKCP1). In pressure cooked samples, TIA ranged from - 0.11 ± 0.1mg/g (Horse gram variety: ANK Black) - 0.61±0.02 mg/g (mung bean; MI5). A significant difference ($p<0.05$) in TIA among the raw form of seeds as well as a significant difference ($p<0.05$) in TIA among cooked form of seeds were observed (one- way ANOVA). Further a significant difference ($p<0.05$) in TIA was observed in comparison (Paired T-Test) between cooked and raw form in each legume variety. According to the results legume seeds exhibited considerable reduction in TIA 53.74%-100% when cooking. AIA was ranged from 11.61% (MI 6)-23.05% (MISB1) at concentration of 100 µg/ml. There was no significant difference ($p<0.05$) in AIA among most of the legume varieties. Conclusively, the study revealed that thermal processing methods can be used to increase the nutritional quality of legumes by destroying most of the protease inhibitors or the anti-nutrients.

FUNDING

This research project was financially supported by the Government of Sri Lanka as a treasury grant (TG18/147) to Industrial Technology Institute (ITI).

ACKNOWLEDGEMENTS

The authors acknowledged the Grain Legumes and Oil Crops Research and Development Centre, Angunakolapelessa, Sri Lanka for supplying samples for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Iqbal A, Khalil I, Ateeq N, Sayyar KM. Nutritional quality of important food legumes. Food Chemistry. 2006;97(2):331-335.
2. Joshi A, Liu, Kadam SS, Deshpande S, Jambhale N. Handbook of world food legumes: Nutritional Chemistry, Processsing Technology, and Utilization, Boca Raton: CRC press. 1989;1:23-50.
3. Mubarak AE. Nutritional composition and Anti-nutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chemistry. 2005;9(4):489-495.
4. AgStat. Socio Economics and Planning Centre, Department of Agriculture, Sri Lanka; 2015.
5. Khokhar S, Apenten RKO. Anti-nutritional factors in food legumes and effects of processing. In The role of food, agriculture, forestry and fisheries in human nutrition, Encyclopedia of Life Support Systems (EOLSS). Publishers Co Ltd., Oxford, UK. 2003;4:82-116.
6. Tarade K, Rekha S, Aniruddha PA. Study on Degradation Kinetics of Trypsin Inhibitors in Horse Gram

(Dolichosbiflours). International Journal of Food Engineering. 2007;3(3):1-12.

7. Khattab RY, Arntfield SD. Nutritional Quality of Legume Seeds As Affected by Some Physical Treatments 2. Anti-nutritional Factors. LWT—Food Science and Technology. 2009;42:1113-1118.
8. Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata*) seed extracts. Food Chemistry. 2007;101: 10-19.
9. Abeysekara WKSM, Jayawardana SAS, Abeysekara WPKM, Yathursan S, Premakumara GAS, Ranasinghe P. Antioxidant potential of selected whole grain cereals consumed by Sri Lankans: A comparative *in-vitro* study. Sri Lankan Journal of Biology. 2017;2(2):12-24.
10. Hamerstrand GE, Black LT. and Glover JD. Tripsin Inhibitors in soy products; modification of the standard analytical procedure. Cereal Chemistry. 1981;58(1): 42.
11. Kakade ML, Rackis JJ, Mcghee JE, Puski G. Determination of Tripsin Inhibitor Activity of Soy products: Collaborative Analysis of an Improved Procedure. American Association of Cereal Chemists. Inc. 3340, St. Paul, Minnesota, U.S.A. 1974;51:376-382.
12. Bernfeld P. Amylases, alpha- and beta. In: Methods in Enzymology, S.P Colowick, N.O. Kaplan, (eds.). New York Academic Press. 1955;149-158.
13. Benjakul S, Visessanguan W, Thummaratwasik P. Isolation and characterization of trypsin inhibitors from some Thai legume seeds. Journal of Food Biochemistry. 2000;24(2):107-127.
14. Su G, Chang KC. Trypsin inhibitory Activity *In-Vitro* Digestibility and Sensory Quality of Meat-Like Yuba Products as Affected by Processing. Journal of Food Science. 2002;67:1260-1266.
15. Bacon JR, Lambert N, Matthews P, Arthur AE, Duchene C. Genotype, environment and genotype environment effects on Trypsin inhibitory in peas. Journal of the Science of Food and Agriculture. 1995;67(1):101-108.
16. Grewal A, Jood S. Effect of processing treatments on nutritional and Anti-nutritional contents of green gram. Journal of Food Biochemistry. 2006;30(5):535-546.
17. Avilés-Gaxiola S, Chuck-Hernández C, Serna Saldivar SO. Inactivation methods of trypsin inhibitor in legumes: a review. Journal of Food Science. 2018;83(1):17-29.
18. Alonso R, Aguirre A, Marzo F. Effects of extrusion and traditional processing methods on anti nutrients and *in-vitro* digestibility of protein and starch in faba and kidney beans. Food chemistry. 2000;68(2):159-165.
19. Deshpande SS, Sathe SK, Salunkhe DK, Cornforth DP. Effects of dehulling on phytic acid, polyphenols and enzyme inhibitors of dry beans (Phaseolus vulgaris L.). Journal of Food Science. 1982;47(6): 1846-1850.
20. Singh U, Jambunathan R. Studies on desi and kabull chickpea (*Cicerarietinum* L.) cultivars: levels of protease inhibitors, levels of polyphenolic compounds and *in-vitro* protein digestibility. Journal of Food Science. 1981;46(5):1364-1367.
21. Vagadia BH, Vanga SK, Singh A, Gariepy Y, Raghavan V. Comparison of conventional and microwave treatment on soymilk for inactivation of Trypsin inhibitors and *in vitro* protein digestibility. Foods. 2018;7(1):6.
22. Kwon YII, Vattem DA, Shetty K, Evaluation of clonal herbs of Lamiaceae species for management of diabetes and hypertension. Asia pacific Journal of Clinical Nutrition. 2006;15(1):107-118.
23. Olusegun OA, Emmanuel EO. Alpha--Amylase–Inhibitory Properties and in vitro Antioxidant Potentials of Cowpea Seed Protein Hydrolysates. American Association for Science and Technology. 2019;6(1):1-12.
24. Singh U. Anti-nutritional factors of chickpea and pigeonpea and their removal by processing. Plant Foods for Human Nutrition. 1988;38(3):251-261.
25. Irshad M, Sharma CB. Purification and properties of alpha--amylase protein-inhibitor from Arachishypogaea seeds. Biochimica et Biophysica Acta (BBA)-Enzymology. 1981;659(2):326-333.
26. Iguti AM, Lajolo FM, Occurrence and purification of. alpha-.-amylase inhibitors in bean (*Phaseolus vulgaris L.*) varieties.

Journal of Agricultural and Food Chemistry. 1991;39(12):2131–2136.

27. Lan Shi, Kaiwen Mu S D, Nickerson MT. Changes in levels of enzyme inhibitors during soaking and cooking for pulses available in Canada. J Food Sci Technol. 2017;54(4):1014–1022.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/64312