The Effect of Common Pre-treatment and Cooking Techniques on the Antioxidant Capacity of Chickpea *(Cicer arietinum)*, Cowpea *(Vigna unguiculata)* and Green Gram *(Vigna radiata)*

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Abstract *- Legumes are rich in antioxidants which are beneficial in reducing the oxidative stress in human body. Antioxidants present in legumes could be changed by common pre-treatments and cooking techniques practiced in Sri Lanka. Therefore, the aim of this study was to compare the antioxidant capacity of chickpea (Cicer arietinum), cowpea (Vigna unguiculata) and green gram (Vigna radiata) after subjecting to combinations of pre-treatments (soaking and germination) and cooking techniques (cooking in a clay pot, aluminum pot, pressure cooker and microwave oven). The untreated legumes were used as the control. Antioxidant capacity (AC) was determined using 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay. Values are expressed as μmol trolox equivalent antioxidant capacity (TEAC) in dry weight (DW) basis. In green gram, microwave cooking of raw green gram resulted significantly ($p<0.05$) higher AC (2031.48 ± 200.23 μmol TEAC/100g DW) compared to control. In chickpea and cowpea, germination followed by aluminum pot cooking showed the highest AC over the other treatment (1756.70 ± 122.20 and 1403.82 ± 74.67 μmol TEAC/100g DW respectively) which were significantly ($p<0.05$) low compared to the control. According to the results, germination followed by aluminum pot cooking could be recommended to preserve antioxidants in white chickpea. Raw, germinated and soaked cowpea subjected to different cooking techniques (except microwave cooking of soaked, germinated and raw cowpea, pressure cooking of soaked and aluminum pot cooking of raw cowpea) could be recommended to preserve antioxidants. In green gram, microwave cooking and clay pot cooking of raw green gram, germination and soaking followed by aluminum pot cooking could be recommended to preserve antioxidants.*

Keywords: Antioxidant capacity, Cooking techniques, Legumes, Pre-treatments

I. INTRODUCTION

Long term exposure to free radicals is one of the major reasons for many chronic non-communicable diseases (Sen Ray, 2015).

Free radicals are atoms or molecules containing odd or unpaired electrons. Usually, free radicals are highly reactive and unstable (Phaniendra *et al.,* 2015). However, antioxidants reduce the harmful effect of free radicals by neutralizing or reducing their production (Sen Ray, 2015). Legumes are one of the major food commodity which is widely grown and consumed all over the world. Human attention has been increased towards legumes due to its beneficial effect on human health. Legumes provide carbohydrate, proteins, fiber, minerals, B vitamins, low amount of fat and it has a low glycemic index (GI) (Polak, 2015).

Legumes should be processed before consumption to improve its nutritional and sensory properties. Since legumes are hard to cook, it should be subjected to soaking and germination before cooking to reduce cooking time. Cooking reduces the anti-nutritional substances and increases the bioavailability of some nutrients. Improved digestibility and palatability of legumes also resulting from cooking (Amarakoon, 2009).

Chickpea *(Cicer arietinum),* cowpea *(Vigna unguiculata)* and green gram *(Vigna radiata)* are commonly consumed legumes in Sri Lanka. Different pre-treatments (soaking and germination) and different cooking techniques

(cooking using clay pot, cooking using aluminum pot, pressure cooker and microwave oven) are used to process those legumes. Both pre-treatments and cooking techniques effect on antioxidant capacity of legumes. However, the level of the effect varies according to the method. Therefore, the present study was planned to compare antioxidant capacity of legumes after subjecting to combinations of pre-treatment and cooking techniques using chickpea, cowpea and green gram available in Sri Lanka.

II. METHODOLOGY

A. Plant materials

White chickpea, cowpea (*Waruni*) and green gram (*MI5*) used in the study were collected as bulk samples from the local supermarket, Anuradhapura and field crop research institute, Mahailuppallama respectively.

B. Sample preparation

Seeds were well cleaned prior to the treatments. Soaked seeds were prepared by overnight soaking with 1:10 grain to water ratio. Mineral water was used as the water source.

Germinated seeds were prepared as below,

Overnight soaked seeds were lined with wet cotton cloth and allowed to germinate in dark at 24 h for green gram, 48 h for cowpea and chickpea. Then sprouts were washed using mineral water.

Then raw, soaked and germinated seed samples were cooked until seeds become soft using clay pot, aluminum pot, pressure cooker (BR-10L, India) and microwave oven (LG MH7046S, India).

Softness of the cooked seeds was identified at the point when grains split easily and not showed white inner core after it pressed between two glass slides (Yu-Wei and Wang, 2015).

C. Reagents

2, 2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), Potassium persulfate ($K_2S_2O_8$) were obtained from Sigma Company (St. Louis, USA).

D. Preparation of sample extracts

Treated samples were dried in an oven at 40^oC for 3-4 days. Both treated and untreated samples were powdered and sieved and stored in airtight containers under refrigerated condition until further analysis. The flour sample (1.5 g) was dissolved in phosphate saline buffer (pH 7.4) and mixed vigorously. Then the samples were filtered using Whatman no.1 filter paper and used for analysis.

E. Moisture content determination

Moisture content of flour samples was determined using the oven dried method at 105^oC and calculated in dry basis (n=4) (AOAC, 1990).

F. Antioxidant capacity determination

Antioxidant capacity of treated and untreated samples was determined using 2, 2'-Azino-bis-(3-ethylbenzo thiasolin-6-sulfonic acid assay (ABTS assay) specified by Re, *et al.* (1999) with slight modifications. ABTS 10 mg tablet was dissolved with 2.6 ml of phosphate saline buffer (pH 7.4) and it was mixed with 11.5 ml of potassium persulphate ($K_2S_2O_8$- 2.45 mmol). Then it was kept in dark at room temperature for 12-16 h to facilitate free radical formation.

ABTS free radical cation solution was diluted with distilled water until the absorbance reached 0.70 $\pm$ 0.02 at 620 nm. Diluted $ABTS^+$ solution (290 μl), legume flour extract (1.5 μl) and phosphate saline buffer (8.5 μl) were introduced to the ELISA plate reader (Thermo Scientific Multiskan, Ex primary EIA v.2.3, Finland) and absorbance was measured at 620 nm. Distilled water was used as the blank. Absorbance readings were corrected by multiplying it from path length correction factor of micro plate reader. Then standard curve equation y = 0.0367x + 0.0405 (R^2 = 0.9967) was applied and antioxidant capacity was expressed as (μmol/100g) trolox equivalent antioxidant capacity (TEAC) in dry basis.

G. *Statistical design*

Statistical design of the study was two factor factorial completely randomized design (CRD). The mean values were separated using Tukey's studentized range test at 95% confidence interval. Data were analyzed using SAS (version 9.0, SAS Institute Inc. Cary, USA).

IV. RESULTS AND DISCUSSION

Antioxidant capacities of untreated and treated samples are stated in Table 1. Antioxidant capacity of untreated and treated green gram ranged from 209.80 to 2031.48 μmol TEAC/ 100g DW. Microwave cooking of raw green gram showed the highest TEAC value (2031.48 μmol TEAC/ 100g DW), which is significantly higher (p<0.05)

compared to the control. This result was compatible with the *in vitro* antioxidant capacity conducted by Chakraborty and Bhattacharyya (2014) on microwave cooked lentils, green gram, Bengal gram, red kidney beans and soybean. It could be due to the formation of new bioactive compounds (Ilyasoglu and Burnaz, 2015) and releasing the bound phenolic compounds having antioxidant properties during cooking due to heat treatments (Jegon, 2004).

Antioxidant capacity of untreated and treated cowpea was ranged from 136.75 to 3434.61 μmol TEAC/100g DW. Pre-treatment and cooking technique combinations significantly (p<0.05) reduce the antioxidant capacity of cowpea compared to control. Highest TEAC was showed by germination followed by aluminum pot cooking (1403.82 μmol TEAC/100g DW).

Antioxidant capacity of untreated and treated white chickpea was ranged from 147.63 to 2983.03 μmol TEAC/100g DW. Similar trend of significant reduction of antioxidant capacity compared to control also showed white chickpea as cowpea. Highest antioxidant capacity of white chickpea was showed by germination followed by aluminum pot cooking (1756.70 μmol TEAC/100g DW). Reduction of antioxidant capacity compared to untreated samples (control) could be due to destruction of thermo liable antioxidant compounds during cooking as a result of exposure to high temperatures. Similar kind of results of decreased antioxidant capacity were also showed for boiled green gram (Chandrasiri *et al.*, 2016), pressure cooked cowpea (Barros *et al.*, (2017) and pressure cooked germinated and ungerminated green gram (Gujral *et al.*, 2011).

V. CONCLUSION

In white chickpea, germination followed by aluminum pot cooking could be recommended to preserve antioxidants.

Raw, germinated and soaked cowpea subjected to different cooking techniques (except microwave cooking of soaked, germinated and raw cowpea, pressure cooking of soaked and aluminum pot cooking of raw cowpea) could be recommended to preserve antioxidants.

In green gram, microwave cooking and clay pot cooking of raw green gram, germination and soaking followed by aluminum pot cooking could be recommended to preserve antioxidants.

Table 1: Antioxidant capacity of untreated and treated white chickpea, cowpea and green gram

Processing Techniques	**TEAC μmol/100g (DW)**		
	Green gram	**Cowpea**	**White chickpea**
Untreated	1201.78 ± 257.22^{bcd}	3434.61 ± 140.73^{a}	2983.03 ± 269.62^{a}
Soaking followed by clay pot cooking	841.88 ± 64.60^{cdef}	616.57 ± 147.84^{bcd}	147.63 ± 66.96^{c}
Soaking followed by aluminum pot cooking	1421.27 ± 139.76^{abc}	593.31 ± 98.69^{bcd}	545.23 ± 214.68^{c}
Soaking followed by pressure cooking	375.39 ± 164.46^{ef}	403.95 ± 100.84^{cd}	463.61 ± 50.19^{c}
Soaking followed by microwave cooking	460.93 ± 146.01^{def}	386.31 ± 86.67^{d}	671.05 ± 106.25^{c}
Germination followed by clay pot cooking	616.63 ± 122.92^{cdef}	585.35 ± 215.11^{bcd}	437.02 ± 80.04^{c}
Germination followed by aluminum pot cooking	1824.64 ± 129.33^{ab}	1403.82 ± 74.67^{b}	1756.70 ± 122.20^{b}
Germination followed by	355.02 ± 159.11^{ef}	1260.94 ± 290.16^{b}	413.96 ± 113.42^{c}

pressure cooking			
Germination followed by microwave cooking	209.80 ± 15.35^{f}	305.27 ± 107.03^{d}	797.28 ± 153.47^{c}
Clay pot cooking of raw legumes	1264.78 ± 115.01^{abcd}	745.55 ± 30.33^{bcd}	825.74 ± 36.25^{c}
Aluminum pot cooking of raw legumes	1006.56 ± 316.12^{bcdef}	420.60 ± 350.55^{cd}	511.89 ± 104.84^{c}
Pressure cooking of raw legumes	1095.31 ± 67.05^{bcde}	1226.68 ± 124.15^{bc}	582.38 ± 183.30^{c}
Microwave cooking of raw legumes	2031.48 ± 200.23^{a}	136.75 ± 16.17^{d}	311.10 ± 23.07^{c}

(n = 4)
Values are given as mean ± SEM
(Different letters within the column indicate the significant difference at p<0.05)

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ACKNOWLEDGEMENT

Support given by the two departments of Rajarata University of Sri Lanka: Department of Biochemistry, Faculty of Medicine and Allied Sciences and the Department of Animal and Food Sciences, Faculty of Agriculture is gratefully acknowledged.