

Influence of Pre-treatment on Secondary Metabolites and Hypo-Glycemic Activity of Custard Apple (*Annona squamosa*) Peel

Nilam Roy & Sasikala S*

Department of Food Process Engineering, School of Bioengineering, Faculty of Engineering and Technology, SRM University, Kattankulathur, 603203, India

ABSTRACT

Introduction: Plant secondary metabolites, present in the outer layers of fruits and vegetables in higher amounts, are structurally diverse and comprise different classes of phyto-constituents that have a number of health-promoting properties. Blanching is an important pre-treatment that is used to inactivate the enzymatic oxidation. **Methods:** This study was designed to investigate the effects of steam blanching (3, 5 and 7 min) and drying (50°C) of custard apple (*Annona squamosa*) peel on the quantification of secondary metabolites and hypo-glycemic activity. Unblanched sample dried at 50°C served as control. Quantitative tests for alkaloids, tannins, saponins and flavonoids were carried out for all samples and *in vitro* alpha amylase inhibition test was done to confirm the hypo-glycemic properties. All analyses were done in triplicates. **Results:** Quantitative results for alkaloids, tannins, saponins showed a significant reduction ($p < 0.05$) as the blanching time increased, whereas for the unblanched sample, it was not statistically significant ($p > 0.05$). The exception was observed in the flavonoid content which showed a significant increase for 3 min and 5 min blanched samples, while the 7 min blanched sample showed a reduction in flavonoid content over the unblanched and fresh samples. Alpha amylase inhibition test similarly showed a decreasing trend for blanched samples ranging from IC_{50} value of 3.31 to 5.53 $\mu\text{g}/\text{mL}$ compared to the unblanched with IC_{50} value of 4.92 $\mu\text{g}/\text{mL}$ and fresh sample with IC_{50} value of 6.37 $\mu\text{g}/\text{mL}$. **Conclusion:** From the study, it is inferred that steam blanching and drying have a significant impact on the quantification of secondary metabolites and subsequently on its hypo-glycemic activity. A steam blanching time of 5 min is the optimum for processing of custard apple peel.

Key words: Alpha amylase inhibition, custard apple peel, drying, secondary metabolites, steam blanching

INTRODUCTION

Health is a condition of complete physical, mental and social well-being and not just the absence of disease or weakness. In humans, it is the ability of individuals or a population to adjust and self-manage

when confronted with physical, mental or social challenges. Health and nutrition are the most important interlinked factors for maintenance of physical well-being. Globally, chronic diseases such as cardiovascular, obesity, cancer and diabetes have taken

* Correspondence: Sasikala Shanmugam; Email: sasishan.shiva@gmail.com

an immense toll on the health of individuals partly due to behavioural aspects such as smoking, ingesting saturated fatty acids in the form of fried snacks, consumption of alcohol, etc. Relatively, these diseases are also outcomes of oxidative stress triggered by the free radical mechanism in the human body. It has been inferred that an increased intake of a diet rich in fruits and vegetables, comprising mainly dietary fibre and antioxidants, can reduce oxidative stress and other chronic diseases. Diabetes, cardiovascular diseases and cancer are the major health problems and continue to be one of the primary causes of death in the world. Western medicines employ numerous therapeutic agents to treat these diseases but they are expensive and may have adverse side effects. Thus, it is essential for pharmacological investigations to identify new compounds or lead structures for the enhancement and development of novel agents to treat these diseases (Savithramma, Rao & Ankanna, 2011). Natural products are obtained mainly from medicinal and food plants or their parts which are used as a prime source in health care for ages. The beneficial effects of these products are due to the blend of high molecular weight such as dietary fibre and low-molecular weight such as secondary metabolites. These components are chemically heterogeneous and comprise different classes of phyto-constituents (essential oils, alkaloids, acids, steroids, tannins, saponins, polyphenols, etc.) and are directly associated with a number of health-promoting properties such as being anti-thrombotic, anti-carcinogenic, anti-inflammatory anti-diabetic; they may also have vaso-protective activities. Generally, these bioactive components are present in the outer layers of fruits and vegetables in higher amounts (Pandey & Rizvi, 2009).

Annona squamosa belongs to the family of Annonaceae and is popularly known as 'Sharefa' or 'Sitaphal' in Hindi and 'Custard apple' or 'Sugar apple' in English. It is an evergreen tree or shrub that stems from

tropical lowland climates without any irrigation (Parvin *et al.*, 2003). The other closely related species of Annonaceae family include: *Annona cherimola*, *Annona reticulate*, *Annona muricata*.

Numerous folklore literature claim that custard apple and its related species are effectively valuable against several ailments. The palatable portion of the fruit is rich in polyphenolic compounds, magnesium, copper, potassium, calcium, minerals, protein, carbohydrates and dietary fibre. Fruit pulp extracts have been reported to possess anti-diabetic properties (Kaleem *et al.*, 2006). Researchers have claimed that each and every part of the tree bark, stem, leaf, stem and seed possess therapeutic properties i.e. anti-diabetic, anti-helminthic, anti-microbial, anti-inflammatory and anti-carcinogenic (Pomer *et al.*, 2009). Ayurvedic or herbal drugs developed from seeds of custard apple have been found to be 300 times more effective than an anti-cancer drug (Parthasaradhi *et al.*, 2004). According to the study conducted by Gupta *et al.* (2005) and Nandagaon & Kulkarni (2012), ethanolic and methanolic extracts of *Annona squamosa* leaf exhibited good anti-diabetic properties in type 1 and type 2 diabetes. The roots of the plant are beneficial in treating acute dysentery, spinal marrow diseases and depression while the leaves have been utilised in cases of sores, prolapse of the anus and swelling (Chao-Ming *et al.*, 1997). An *in vivo* study carried out on the peel extracts of custard apple exhibited anti-hyperglycemic effects on streptozotocin diabetes-induced mice (Sharma *et al.*, 2013).

Fresh custard apples when peeled are vulnerable to oxidation reactions due to the presence of polyphenols which act as the substrate for enzymatic oxidation (Kamble & Soni, 2010). The same can be observed in the white pith of the peel. Enzymatic oxidation reactions are triggered by the action of polyphenoloxidase enzyme. Hence, pre-treatment before processing can be beneficial in inhibiting any considerable changes

in phytochemicals. Blanching is one such technique to arrest the action of the enzymes (Larrauri, 1999). The process essentially uses steam or hot water as a heat transfer medium. Generally, steam blanching is preferred over water blanching because water blanching causes leaching of many essential constituents during its process. The time and temperature applied during blanching brings about the desired inactivation, which also depends on the nature of the product being blanched. Apart from the pre-treatment, drying is also a form of preservation to prolong and protect the perishable fruits and vegetables. Dehydration through drying preserves food by the removal of water which disables the microbe's ability to grow and survive. Microorganisms thrive well only when there is ample moisture content in its surroundings and ambient temperature for its survival. Thus, drying any food of perishable nature reduces moisture content which results in inhibition of growth and development of microbes.

Significant amounts of fruits and vegetables wastes/by-products are generated by the food processing industries. In general, studies show that by-products contain a variety of biologically active compounds that are mostly discarded as wastes (Peschel *et al.* 2006). Thus, this not only wastes a potentially valuable resource but also aggravates a serious disposal problem. The use of these wastes or residues can contribute to lower production costs in the food industry and create alternative functional food ingredients for human consumption.

Thus, the present study delves into the influence of pre-treatment on the secondary metabolites (phyto constituents) of the custard fruit peel extracts and its alpha amylase inhibition potential.

METHODS

Sample preparation

Fresh custard apple fruits were purchased from the nearby markets in Chennai. The fruits were thoroughly washed and skinned. The peel was collected and steam blanched at 100°C for 3 min, 5 min and 7 min to inactivate the polyphenoloxidase enzyme. The blanched and unblanched peels were then dried at 50°C in a tray drier until they attained constant weight. The unblanched peel sample served as the control. The dried peel were ground into powder and sealed in aluminium pouches for subsequent use.

Experimental design

The present study was designed with steam blanching at 100°C for 3 min, 5 min and 7 min and drying of the blanched samples at 50°C. Custard apple peel blanched-dried for 3 min at 50°C (B-TD I), 5 min at 50°C (B-TD II) and 7 min at 50°C (B-TD-III) were the samples to be evaluated. Unblanched samples dried at 50°C (UB-TD) served as control. All the results were compared against ethanolic extract of fresh custard apple peel, blanched and unblanched peel powders. All the analyses were done in triplicates and reported as means with \pm Standard Deviation (SD). Statistical Analy-

Product	Independent Variables	Dependent Variables
Custard apple peel powder	Steam blanching at 100°C for 3, 5 and 7 min	Colour values Alkaloid content Tannin content Flavonoid content Saponin content Hypoglycemic activity (<i>in vitro</i> alpha amylase inhibition test)

sis was carried out using two-way ANOVA with SPSS software version 20 for the following experimental design. Three experimental trials were conducted for each sample and the statistical significance was set at $p < 0.05$.

Determination of colour of custard apple peel powder

The colour index of the blanched and unblanched dried custard apple peel powder was measured using a -Colour Quest XE Hunter Colour Meter, based on the $L^* a^* b^*$ colour system. L^* denotes lightness on a 0 - 100 scale from black to white while a^* and b^* denote the hues which represent two colour axes with a^* denoting redness (+) or greenness (-) and b^* denoting yellowness (+) or blueness (-). The equipment was calibrated using a white tile and the samples were measured in three replicates with the average being reported.

Quantification of secondary metabolites

Quantification of alkaloid content

The alkaloid content of the samples was determined as per the method described by Oseni & Okoye (2013) and Harborne (1973). Five gram of the sample was weighed into a 250 ml beaker, added with 200 ml of 10% acetic acid in ethanol, covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, which was dried and weighed.

$$\% \text{ Alkaloid} = \frac{\text{Weight of precipitate (mg)}}{\text{Weight of sample (mg)}} \times 100 \dots (1)$$

Quantification of tannins

The total tannin content was assessed by the standard protocol of Keerthana, Kalaivani

& Sumathy (2013) with little modifications as follows: 0.5 mL of the sample extract was diluted with 80% ethanol. From the diluted sample, 0.1 mL was added to 2 mL of Folin-Ciocalteu reagent. After 8 min, 7.5 mL of 7% sodium carbonate solution was added and incubated for 2 h. The absorbance was measured at 760 nm and the tannin content was estimated using tannic acid curve as the standard.

Quantification of flavonoids

The ethanolic extract was treated with 0.1 ml of 10% aluminum chloride and 0.1 ml of 1 M sodium acetate. The absorbance of the reaction mixture was measured at 415 nm after incubation at room temperature for 30 min. From the standard graph, the flavonoid content in the extract was determined using catechin as standard (Keerthana *et al.*, 2013).

Determination of saponin content

Saponins were quantified in accordance to the method described by Famurewa, Olatujoye & Ajibode (2014). Sample powder weighing 20 g was put into each conical flask and 100 ml of 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. To the aqueous layer, 60 mL of n-butanol was added. The combined extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remnant solution was heated in a water bath. After evaporation, the samples were dried in the oven to attain constant weight and the saponin content was calculated as a percentage.

Determination of hypo-glycemic activity*In-vitro* Alpha amylase inhibitory assay

Alpha amylase inhibitory activity was performed in accordance to method described by Maeda, Kakabayashi & Matsubara (1985). Alpha amylase was evaluated with 0.5% starch as substrate in 0.1 M sodium phosphate buffer of pH 7.0. The ethanolic extract of custard apple at various concentrations (10-100 µg/ml) was mixed with the substrate in phosphate buffer at 37°C for 5 min to start the reaction and terminated by the addition of 2 ml of DNS reagent (1% 3,5-dinitrosalicylic acid and 12% sodium potassium tartrate in 0.4 M NaOH). The reaction mixture was heated for 15 min at 100° C and then diluted with 10 ml of distilled water in an ice bath. The control reaction was carried out without the extract. The absorbance was measured at 540 nm. The percentage of inhibition was calculated by the following formula:

Alpha Amylase Inhibition %

$$= \frac{\text{Absorbance Control} - \text{Absorbance sample} \times 100}{\text{Absorbance Control}} \dots \dots (2)$$

Acarbose was used as the standard and IC50 values were determined by the standard calibration curve of α-amylase enzyme inhibition against concentration of extracts.

Statistical analysis

Data were expressed as means ± standard deviation (SD) of three replications and sta-

tistical analysis (ANOVA) was done using SPSS program (version 19.0 SPSS Inc.). The values were considered to be significantly different when $p < 0.05$.

RESULTS**Quantification of secondary metabolites**

The quantitative analyses for the screening of secondary metabolites are shown in Table 1. The test revealed that the unblanched tray dried (UB-TD) sample showed an increase in the level of tannins, saponins compared to the fresh samples, whereas the for blanched tray dried (B-TD(I-III)) samples there was a significant reduction of alkaloids, tannins and saponins. Thus, it can be inferred that B-TD I and B-TD II retained phyto-constituents close to fresh sample. The increase in the levels of flavonoids was higher in the B-TD (I-II) (26.79 ± 0.3 and 26.90 ± 0.04) than in the fresh (23.87 ± 0.3) and UB-TD (22.89 ± 0.04) samples; owing to the inhibition of enzymatic degradation of phenolic constituents, there was a reduction in flavonoid content in B-TD III. This suggests that steam blanching (100°C) at 7 min leads to the maximum degradation of heat sensitive phenolic components beyond a certain time.

In-vitro alpha amylase inhibitory assay

In this study, alpha amylase inhibitory assay was quantified at five concentrations (10, 25, 50, 100 and 200 µg/mL) of ethano-

Table 1. Quantification of secondary metabolites (mean ± SD, n=3)

Secondary metabolite analysis	Treatments				
	Fresh	UB-TD	B-TD		
			I-3min	II-5min	III-7min
Alkaloid (%)	15.78 ± 0.1a	15.77 ± 0.2a	14.98 ± 0.3b	14.65 ± 0.03c	13.26 ± 0.01d
Tannin (%)	20.05 ± 0.03b	20.52 ± 0.03a	19.65 ± 0.03c	19.60 ± 0.04c	16.89 ± 0.02c
Saponin (%)	5.86 ± 0.02b	5.90 ± 0.1a	5.82 ± 0.3b	5.56 ± 0.5c	5.07 ± 0.03d
Flavonoid (%)	23.87 ± 0.3e	22.89 ± 0.04d	26.79 ± 0.3b	26.90 ± 0.04a	26.02 ± 0.05c

Data represent the mean ± standard deviation of triplicate readings; values with the same lowercase superscript letter along the same row are not significantly different ($P > 0.05$).

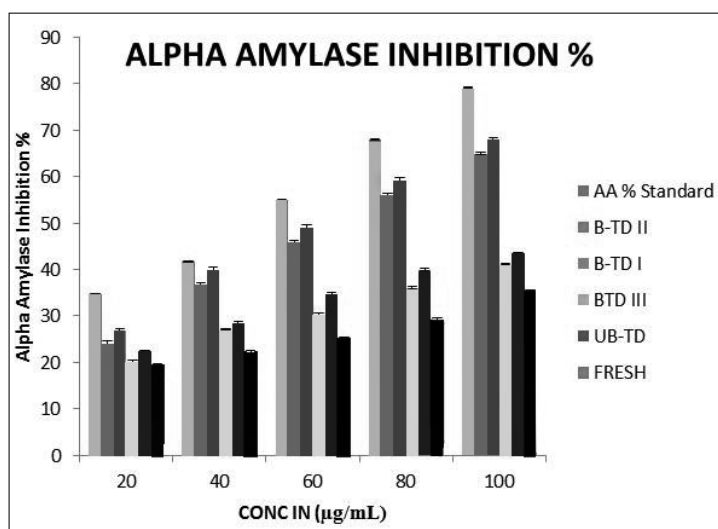


Figure 1. % alpha amylase inhibition for samples at all concentrations with \pm SD.

Table 2. IC_{50} value in ($\mu\text{g/mL}$) of alpha amylase inhibition assay for all evaluated samples

Alpha amylase Inhibition ($\mu\text{g/mL}$)	Treatments				
	Fresh	UB-TD	B-TD		
			I-3min	II-5min	III-7min
IC_{50} value in ($\mu\text{g/mL}$)	6.37 ± 0.4	4.92 ± 0.3	3.317 ± 0.05	3.51 ± 0.2	5.35 ± 0.1

Data represent the mean \pm standard deviation of triplicate readings

lic extract of custard apple peel subjected to drying and different blanching times. Inhibition was observed at all concentrations (Figure 1). Acarbose was used as a standard and the solution without the extract served as control. From the assay, the IC_{50} value, that is, the concentration of the extracts (comprising the alpha-amylase inhibitor) that inhibited 50% of the alpha amylase enzyme activity is tabulated in Table 2.

DISCUSSION

Determination of colour of custard apple peel powder

Colour analysis of the samples was done using Hunter colour meter to evaluate the

blanching technique used to inhibit the polyphenol oxidase enzyme. Before the quantification of secondary metabolites, a visual appearance study was conducted by a set of panel members to show the effect of blanching on the colour of the powdered sample. It was observed that based on the results of Hunter Colour Meter, B-TD-II had a good colour ($L^* = 69.93 \pm 0.4$, $a^* = -3.47 \pm 0.015$ and $b^* = 20.05 \pm 0.02$; where 'a' denotes redness/greenness, 'b' denotes yellowness/ blueness) compared to the other blanched and unblanched samples. This suggests that the blanching time of 5 min was able to successfully inhibit the enzyme polyphenol peroxidase and had a better visual appealing colour. A similar kind of

study was carried out to assess the effect of drying methods on the colour of *Portula caoleracea* L. leaves (Youseef & Mokhtar, 2014). In their study, the authors reported an increase in lightness of the dried leaves at all drying methods, but a higher L* and better b* values of the dried leaves were observed in the freeze drying method compared to the hot-air drying method. Hot air drying at 70°C led to a higher a*(redness) value, thus, impairing the visual appearance of the powder. Another study conducted by Porntewabanha & Siriwongwilaichat (2010) reported that drying of fresh lettuce leaves led to undesirable colour changes, while drying of leaves after pre-treatment (blanching) resulted in better retention of green colour.

Quantification of secondary metabolites

The assay carried out to quantify tannins, alkaloids and saponins for both B-TD and UB-TB revealed that blanching time did have a significant difference ($p < 0.05$) on the plant secondary metabolites content (Table 1). A previous study conducted by Famurewa *et al.* (2014) showed that the tannin, oxalate, phytates and saponin moderately decreased with an increase in drying temperatures but the decrease was not statistically significant. In our study, the blanched-dried samples at different blanching times and drying temperature combinations had significant difference ($p < 0.05$). In another research, Irondi, Anokam & Ndidi (2013) noted an increase in alkaloid, saponin and tannin content of *Carica papaya* seeds at 50°C of oven drying, whereas there was a decrease in the flavonoid content compared to the other drying techniques in the study. This is in accordance with the report that tannins, saponins and oxalates decline with thermal processing (Adegunwa *et al.*, 2011). Similar findings were reported in the study of blanching effects on the nutrient and anti-nutrient content of pumpkin leaves, where a significant reduction was observed in

the anti-nutrient contents of the blanched-dried pumpkin leaves (Fadupin, Osuoji & Ariyo, 2014). According to the study conducted by Ahmed & Ali (2013) on bioactive compounds of fresh and processed cauliflower, it was observed that steam blanching had the greatest retention of total flavonoids and water blanching had the greatest loss of flavonoid content.

In-vitro alpha amylase inhibitory assay

Numerous fruits, vegetables and plants are a tremendous repository of bioactive compounds possessing a multitude of therapeutic effects (Okoli & Iroegbu, 2005). Many fruit residue extracts have been reported to have an alpha amylase inhibitory effect. This study is the first to report the influence of pre-treatment on the secondary metabolites or constituents and its effect on the alpha amylase inhibitory effect of the custard peel. The results showed that the extracts possessed significant activity, least being the B-TD III and fresh peel extract. Among all the evaluated conditions, UB-TD showed an increase in alpha amylase inhibition activity ($IC_{50} = 4.92 \mu\text{g/mL}$) and B-TD I showed the highest activity ($IC_{50} = 3.31 \mu\text{g/mL}$) followed by B-TD II ($IC_{50} = 3.51 \mu\text{g/mL}$) (B-TD I > B-TD II > UB-TD > B-TD-III > FRESH). Similarly, IC_{50} value of acorbose was found to be $2.85 \mu\text{g/mL}$. These results correlate with the quantitative test of secondary metabolites that showed a decreasing trend with blanching conditions, while a significant difference was not observed for samples that were only subjected to drying temperatures. Studies carried out to investigate the various phyto constituents of plants claim that tannins, saponins and alkaloids are the responsible bioactive components for various therapeutic activities, that is, anti-inflammatory, antimicrobial, antispasmodic, antidiabetic, anticarcinogenic, anticonvulsant and cytotoxic activities (Supradip *et al.*, 2010). In a previous study Ashok *et al.* (2013) carried out preliminary phytochemical screening

of shade-dried custard apple peel and reported the presence of alkaloids, saponins, flavonoids, carbohydrates and amino acids in the alcoholic extract of the shade-dried peel. All previous studies are based on the qualitative analysis of these bioactive components of the studied sample. No studies exist for the quantitative analysis of custard apple peel. Recent studies on plant alkaloids have thrown light on the pharmacological properties such as antidiabetic and hypo-lipidemic activities (Stévigny, Bailly & Quetin-Leclercq, 2005; Singh *et al.*, 2010). A study by Ashok *et al.* (2013) has reported on the antidiabetic and hypo-lipidemic activity of the shade dried custard apple peel. According to this study, the alcoholic extract of the shade dried peel powder was found to show significant decrease in blood glucose and lipid profile of streptozotocin-induced diabetic rats, thus supporting this study's recent quantification of secondary metabolites and *in vitro* alpha amylase inhibition assay.

CONCLUSION

The present research revealed that a drying temperature of 50°C and blanching times of 3 min (B-TD I) and 5 min (B-TD II) are the optimum processing conditions for processing custard apple peel. However, in terms of visual appearance, the powdered peel sample B-TD II ($L^* - 69.93 \pm 0.4$, $a^* - 3.47 \pm 0.015$ and $b^* - 20.05 \pm 0.02$) had higher lightness (L^*) and less redness (a^*) than the B-TD I. Quantitative tests revealed that B-TD II also retained better flavonoid content (26.90 ± 0.04) and other secondary metabolites (alkaloids -14.65 ± 0.03 , tannins -19.60 ± 0.04 and saponins 5.56 ± 0.5) compared with the B-TD III (flavonoid 26.02 ± 0.05 , alkaloid -13.26 ± 0.01 and saponin -5.07 ± 0.03). Results of an *in vitro* alpha amylase inhibition test carried out to assess the hypoglycemic activity too showed very good inhibition concentration (IC_{50} value) $3.51 \pm 0.2 \mu\text{g/mL}$ in B-TD II powdered peel

extract compared to the unblanched (UB-TD) and B-TD III, correlating with the results of other secondary metabolites. Thus, it can be inferred that pre-treatment (steam blanching) followed by drying treatment is a better way of processing rather than only hot air drying which results in undesirable changes in the visual appearance as well as in the bioactive constituents. Furthermore, the utilisation of these agro-wastes to develop functional foods by incorporating these as nutraceutical ingredients or additives would be of economic as well as therapeutic value.

The present research was carried out with the fresh peel of mature ripened *Annona squamosa* Lin species during its peak season in South India. Also, the findings of the present study may differ with the maturity levels and geographical location of the fruit in other parts of the world

ACKNOWLEDGEMENT

We would like to acknowledge the encouragement and important suggestions put forward by Dr M Vairamani, Dean of Bioengineering Department and Dr KA Athamselvi, Head of the Food Process Department. The technical assistance of colleagues and lab staff is gratefully acknowledged.

REFERENCES

- Ahmed FA & Ali FMR (2013). Bioactive compounds and antioxidant activity of fresh and processed white cauliflower. *Biomed Res Int* 2013: 1-9.
- Adegunwa MO, Alamu EO, Bakare HA & Oyeniyi CO (2011). Proximate and bioactive contents of some selected vegetables in Nigeria: Processing and varietal effects. *Am J Food Nutr* 1(4): 171-177.
- AOAC (2000). Official Methods of Analysis. Association of Official Analytical Chemists, Gaithersburg, MD.
- Ashok S, Ashish KS, Tara C, Manoj K & Suresh A (2013). Preliminary Phytochemical

- Screening of Fruit Peel Extracts of *Annona squamosa* Linn. *Current Pharma Res* 4(1): 1038-1043.
- Chao-Ming L, Ning-Hua T, Hui-Lan Z, Qing M, Xiao-Jing H, Yi-Neng H & Jun Z (1997). Cyclopeptide from the seeds of *Annona muricata*. *Phytochemistry* 45(3): 521-523.
- Fadupin GT, Osuoji U & Ariyo O (2014). Effect of blanching on nutrient and anti-nutrient content of pumpkin (*Cucurbit aepo*) leaves. *West African J Food Nutr* 12(2): 18-24.
- Famurewa JAV, Olatujoye JB & Ajibode A (2014). Drying phenomenon and influence on the anti-nutritional and pasting properties of Cocoa yam (Taro). *J Sci Res & Reports* 3(2): 275-283.
- Gupta RK, Kesari AN, Watal G, Murthy PS, Chandra R, Maithal K & Tandon V (2005). Hypoglycaemic and antidiabetic effect of aqueous extract of leaves of *Annona squamosa* (L.) in experimental animal. *Current Science* 88: 2005.
- Harborne JB, (1973). *Phytochemical Methods*, Chapman and Hall, Ltd., London, pp 49-188.
- Irondi AE, Anokam KK & Ndidi US (2013). Effect of drying method on phytochemicals composition and antioxidant activities of *Carica papaya* seeds. *Intern J Biosci* 3(11): 154-163.
- Kaleem M, Asif M, Ahmad QU & Bano B (2006). Antidiabetic and antioxidant activity of *Annona squamosa* extract in STZ-induced diabetic rats. *Singapore Med J* 47(8): 670-675.
- Kamble KJ & Soni SB (2010). Effect of steam blanching on quality of custard apple pulp. *J Horticulture For* 2: 104-107.
- Keerthana G, Kalaivani MK & Sumathy A (2013). *In vitro* alpha amylase inhibitory and anti-oxidant activities of ethanolic leaf extract of *Croton bonplandianum*. *Asian J Pharm Clin Res* 32-36.
- Larrauri JA. 1999. New approaches in the preparation of high dietary fiber from fruit by-products. *Trends Food Sci Tech* 10: 3-8.
- Maeda K, Kakabayashi S & Matsubara H (1985). Complete amino acid sequence of an alpha-amylase inhibitor in wheat kernel (019-inhibitor). *Biochim Biophys Acta* 828(3): 213-221.
- Nandagaon VS & Kulkarni AR (2012). In vitro antioxidant and cytotoxicity activity of aqueous and alcoholic extracts of *Annona squamosa* Linn. *Res J Pharm Bio Chem Sci* 3: 479-482.
- Okoli S & Iroegbu CU (2005). In vitro antibacterial activity of *Synclisis cabrida* whole root extracts. *Afr J Biotechnol* 4(9): 946-952.
- Oseni OA & Okoye VI (2013). Studies of Phytochemical and antioxidant properties of the fruit of watermelon (*Citrullus lanatus*). *J Pharm Biomed Sci* 27(27): 508-514.
- Pandey KB & Rizvi SI (2009). Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2(5): 270-278.
- Parvin Shahnaj Mst, Islam Ekramul M, Rahman Motiur Md & Haque Ekramul Md (2003). *Pakistan J Biol Sci* 6 (18): 1593-1596.
- Parthasaradhi BV, Reddy M, Ali M & Kumari L (2004). Antitumor activity of *Annona squamosa* seed extract is through the generation of free radicals and induction of apoptosis. *Indian J Biochem Biophys* 41: 167-72.
- Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzía I, Diego Jiménez, Lamuela-Raventos R, Buxaderas S & Codina C (2006). An industrial approach in the search for natural antioxidants from vegetable and fruit wastes. *Food Chem* 97(1): 137-150.
- Pomer KW, Lowe J, Crabtree S & Keller (2009). Identification of *Annona ceousaceto* genins in the ripe fruit of the North American pawpaw (*Asimina triloba*). *J Agric Food Chem* 57: 8339-43.
- Porntewabancha D & Siritwongwilaichat P (2010). Effect of pre-treatments on the drying characteristics and colour of dried lettuce leaves. *As J Food Ag-Ind* 3(06): 580-586.
- Savithramma N, Rao ML & Ankanna S (2011). Screening of traditional medicinal plants for secondary metabolites. *Int J Res Pharm Sci* 2(4): 643-647.
- Sharma A, Chand T, Khardiya M, Yadav KC, Mangal R & Sharma KA (2013). Antidiabetic and antihyperlipidemic activity of *Annona squamosa* fruit peel in streptozotocin-induced diabetic rats. *Inter J Toxicol Pharmacol Res* (5): 15-21.

- Supradip S, Walia S, Kumar J, Dhingra S & Parmar BS (2010). Screening for feeding deterrent and insect growth regulatory activity of triterpenic saponins from *Diploknema butyracea* and *Sapindus mukorossi*. *J Agric Food Chem* 58(1): 434-440.
- Stévigny C, Bailly C & Quetin-Leclercq J (2005). Cytotoxic and antitumor potentialities of aporphinoid alkaloids. *Curr Med Chem Anticancer Agents* 5(2): 173-182.
- Singh A, Duggal S, Kaur N & Singh J (2013). Berberine: alkaloid with wide spectrum of pharmacological activities. *J Nat Prod* 3: 64-75.
- Youseef KM & Mokhtar SM (2014). Effect of drying methods on the antioxidant capacity, color and phytochemicals of *Portulacaoleracea* L. Leaves. *J Nutri Food Sci* 4(6): 1-6.