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RESEARCH ARTICLE

A PRELIMINARY STUDY ON ESTIMATED GLYCAEMIC INDEX AND MICROSTRUCTURE OF STARCH, IN BOILED *CUCURBITA MOSCHATA* (SQUASH) FOUND IN SRI LANKA.

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Abstract

There is an increased tendency to use non-pharmacological strategies such as dietary interventions in health related problems. Especially in the case of chronic illnesses, dietary interventions are used along with pharmacological treatment for proper management of patients. Dietary interventions are helpful not only in the management but also in prevention of most of the long term illnesses. In Sri Lanka, squash are commonly consumed as a soup or with major meals usually after traditional cooking. Starchy vegetables with high glycemic index lead to rapidly elevated blood glucose levels, which is associated with risk of obesity and diabetes mellitus. Therefore identification of thermal processing methods that can be used as dietary interventions will help to improve quality of life. The aim of this study was to evaluate the estimated glycaemic index and microstructure of starch in boiled preparation of squash found in Sri Lanka. Estimated glycaemic index was determined using *in vitro* digestion procedures. Brightfield florescence microscopy was used to observe the changes in microstructure. The estimated glycaemic index of boiled squash was 13.1 ± 4.1 and microscopy showed a high degree of cell disruption and release of starch out of cells in thermally processed preparation when compared to raw sample. Foods with a low glycaemic index (<55), help slow absorption of carbohydrates and prevent extreme blood glucose fluctuations. Therefore the results of this study conclude that boiled squash is safe for obese and diabetic population. Further studies with other cooking methods will be needed to provide more knowledge.

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Introduction:-

Starch is the main carbohydrate in human nutrition and is a major component in plant foods. It is the most common digestible polysaccharide found in plants. Common sources of starch include grains, potatoes, legumes and other vegetables. Starch is found in all parts of plants (leaves, stems, roots, tubers, seeds). Starch is a complex carbohydrate made up of two components, amylose and amylopectin. The digestion of starch begins in the mouth with salivary α-amylase which hydrolyzes amylose and amylopectin, forming dextrins; short chain polysaccharides.

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Salivary amylase action continues in the stomach until gastric acid lowers the pH and inactivates the enzyme. Dextrins are further digested in the small intestine when pancreatic α-amylase is secreted. Dextrins are broken down by pancreatic α-amylase into disaccharides such as maltose, isomaltose. By the action of several disaccharidases such as isomaltase, maltase, theses disaccharides are further breakdown into monosaccharides such as glucose which will be absorbed into the body (Champe et al. 2008).

Carbohydrates provide a significant fraction of energy in the diet of most of the organisms including humans. Glycemic Index (GI) ranks carbohydrate-containing foods in a 0 to 100 scale on how quickly and how much they elevate blood glucose levels. Foods can be classified as having a low (<55), intermediate (55-70), or high (>70) GI. GI can be estimated by *in vitro* rate and extent of starch digestibility, which is called Estimated Glycemic Index (EGI) (Lemlioglu-austin 2012). Foods with a low GI produce slower and lower rise in blood glucose level compared to foods with high GI (Nutrition Education Materials Online 2014). Foods with low GI also tend to create a sense of satiety over a long period of time and may be helpful in limiting caloric intake (Champe et al. 2008). Therefore foods with low GI are beneficial in controlling and reducing the risk of diabetes mellitus and obesity.

Many studies have revealed that processing methods of starchy vegetables can affect the microstructure and GI. An *in vivo* study done on some commonly eaten West Indian carbohydrate-rich vegetables such as yams and potatoes has shown that foods processed by roasting or baking may result in higher GI whereas boiling of foods may contribute to a lower GI diet (Bahado-Singh et al. 2006).

Effect of thermal processing on microstructure has been investigated previously. Some nutrients found in plants are protected in nature against degradation inside cells by attaching to membranes, occluding inside cell organelles, or binding to cell walls, but this natural protection lowers bioavailability. Thermal and physical processing, mastication, and to a limited extent digestion break down the cell walls, making the release of nutrients from the food matrix easier and rendering them available for absorption in the intestine (Parada and Aguilera 2007). According to degree of the heat treatment applied, tissue and cell disruption increase and there is more release of nutrients from cells which increase bioaccessibility of those nutrients. Thermal properties of pumpkin and squash starch has been studied in China and the results indicate that swelling and solubility of squash starch increases with rise in temperature (Yin and Wang 2016).

Studies on GI have been done using both *in vivo* and *in vitro* methods on different types of natural and processed foods. The *in vitro* methods have improved for reliable determination of bioavailability of genuine *in vivo* metabolites, as they are rapid, cheap, and circumvent ethical issues related to the use of humans or animals (Parada and Aguilera 2007). Concern for development and utilization of *in vitro* methods has been increased lately and several studies also have been done to find EGI using *in vitro* methods. Methods to determine total starch content in foods and *in vitro* digestion procedures to determine EGI have been developed and they gave similar results to *in vivo* GI methods (McCleary et al. 1997) (Goñi et al. 1997). Using these methods, studies have been done for different preparations of vegetables such as sweet potatoes (Allen et al. 2012). Studies have also been done to see whether *in vivo* GI values can be predicted using EGI values obtained from *in vitro* procedures and it has been concluded that *in vivo* and *in vitro* GI values are much identical (Vera et al. 2002, Monro and Mishra 2010). For example Goni and Garcia-Alonso has proved in their study on processed potatoes that the Estimated Glycaemic Index (EGI) obtained from starch digestibility procedure was in accordance with the reported GI values, for potatoes processed in the same way (Garcia-Alonso and Goni 2000). Hence it is fair to apply *in vitro* GI results for living beings.

Studies on GI of vegetables such as cassava (staple foods of African population) have been reported mostly from African countries. An *in vivo* study shows that cassava paste gives relatively high GI (GI= 86) and another study report from Tanzania tells that cassava flour has a relatively low GI (GI=49.84) and is good for treatment and management of diabetes (Kouamé et al. 2014, Ruhembe et al. 2014). These studies and another *in vivo* study on Jamaican sweet potatoes show that method of food processing has a significant impact on GI (Bahado-Singh et al. 2011).

There are limited studies reported on glycemic index of fruits of Cucurbitaceae family such as pumpkin and squash. But studies on important nutrients such as carotenoids in those vegetables have been reported. One such *in vitro* study done in Sri Lanka has revealed that squash curry contains 44.6 ± 12.3 µg/g (dry weight, n=6) β-carotene and only 6.3 ± 1.4 µg/g (dry weight, n=6), (14%) is bioaccessible. In the same study, it has been found that the pumpkin curry had more bioaccessibility (32.3 %) when compared to boiled pumpkin (18.7 %) since the degree of heat treatment is higher in the preparation of curry. Other than the degree of heat treatment, addition of coconut milk (fat) has made an hydrophobic environment which increased bioaccessibility of carotenoids (Priyadarshani and Chandrika 2007).

In Sri Lanka, traditional preparations such as curry (boiled with coconut milk) and boiled preparations of squash are common. Squash in boiled form is a commonly consumed preparation. Hence, examining the effect of boiling on EGI and microstructure of Squash is pertinent to encourage consumption of foods low in starch by obese and diabetic subjects. There are varieties of some non-leafy vegetables in Sri Lanka where investigations for starch have not yet been done. There are various kinds of cooking methods in Sri Lanka for these vegetables and the effect of them on the microstructure of starch and GI are not known. Hence, there is a need for a more complete database on above parameters of these vegetables in terms of food as eaten. This study will provide a platform and encouragement for further studies.

Methodology:-

Sampling:-

Squash (*Cucurbita moschata*) were purchased at random from three different places in Colombo district. A sample from each (100 g × 3) was taken for analysis.

Thermal processing:-

Thermal processing (boiling) was done in different houses at different days randomly. The 100 g samples were boiled separately. Squash was washed thoroughly and cut into pieces of $1.5 \times 1.5 \times 1.5$ cm^3 in size and allowed to boil in salty water for about 15 minutes in an uncovered pan.

Determination of total starch in boiled squash:-

The procedure was conducted according to method established by McCleary et al (McCleary et al. 1997).
From homogenously crushed samples of boiled squash 50.0 mg were accurately weighed and 3 mL of distilled water was added carefully to moisten the sample. An amount of 3 mL of 4 M KOH was added to it. It was shaken at room temperature for 30 minutes using a vortex. An amount of 3 mL of 0.4 M sodium acetate buffer (pH 4.75) was added to it. pH was adjusted to 4.75 with 1 M and 2 M HCl. An amount of 80 µL of amyloglucosidase from *Aspergillus niger* (Sigma-Aldrich A7095) was added to it. It was mixed well and incubated at 60 °C for 45 minutes in a shaking incubator. The mixture was centrifuged at 3000 ×g for 15 min. The supernatant liquid was collected into a 500 mL volumetric flask using a pipette. Volume was made up to 500 mL by adding distilled water. Glucose content for each sample was determined using Glucose Oxidase - Peroxidase kit (BioSystems REF 11538). Procedure was done in triplicate for each sample. Same procedure was carried out for three samples from fresh white bread as reference samples.

Determination of EGI of boiled squash by *in vitro* digestion procedure:-

The procedure was conducted according to method established by Goni et al (Goñi et al. 1997).

From the homogenously crushed samples of boiled squash, 50.0 mg was accurately weighed and were homogenized in 5 mL of HCl-KCl mixture (pH 1.5) for 1 minute using a homogenizer with controlled speed (level 4). The samples were incubated at 40 ^{o}C for 60 minutes in a shaking incubator with 0.1 mL of pepsin (Himedia RM 084) solution (0.2 mL of HCl-KCl mixture containing 1 mg of pepsin from porcine gastric mucosa). An amount of 7.5 mL of Tris-malate buffer was added to make pH to 6.9. An amount of 2.5 mL alpha amylase solution (5 mL of Tris-Malate buffer containing 2.6 UI of alpha amylase from porcine pancreas) was added. The flasks were placed in a shaking incubator at 37 ^{o}C.

Aliquots (0.1 mL) were taken every 30 minutes from 0 to 3 hour. Alpha amylase was inactivated by immediately placing the tubes in a boiling water bath for 10 minutes with vigorous shaking for every 30 seconds. Then 1 mL of 0.4 M sodium acetate buffer (pH= 4.75) and 30 µL of amyloglucosidase were added. The samples were incubated at 60 ^{o}C for 45 minutes to hydrolyze the starch into glucose. Finally, the glucose concentration was measured using a Glucose Oxidase-Peroxidase kit.

Amount of starch in mg was calculated as glucose in mg × 0.9. The digestion curves were prepared according to the following non-linear equation established by Goni et al (Goñi et al. 1997).

$$C = C\infty(1 - e^{-kt})$$

Where C is the percentage of starch hydrolyzed at time t (min); $C\infty$ is the equilibrium percentage of starch hydrolyzed after 180 min; and k is the kinetic constant. The variables $C\infty$ and k were estimated for each sample using SPSS for windows 21.

The area under the hydrolysis curve (AUC) was calculated for each sample using the equation;

$$AUC = C\infty(tf - to) - (C\infty/k)[1 - \exp[-k(tf - to)]$$

Where tf is the final time (180 min) and to is the initial time (0 min).
Hydrolysis Index (HI) was obtained by dividing the AUC of each sample by corresponding AUC of reference sample (fresh white bread, GI=100). Finally EGI was predicted with the formula;

$$EGI = 39.71 + (0.549 \times HI)$$

Procedure was done in triplicate for each boiled squash sample. Same procedure was carried out for three samples from fresh white bread as reference samples.

Microscopy of starch granules:-

Brightfield microscopy was done using FSX 100 Olympus microscope equipped with FSX-BSW software. Brightfield microscopy images at ×20 and ×40 magnification were obtained for raw and boiled samples of Squash. For visualization of starch granules, slides were stained with iodine and they were examined and photographed.

Determination of Moisture Content:-

Moisture contents of raw and boiled squash were determined by drying amounts from each sample in triplicate in an oven at 60 °C until a constant weight was obtained. The percentage moisture for each sample was calculated based on the average weight of the three dried samples.

Results:-

EGI of boiled squash:-

The EGI of boiled squash was 13.05 ± 4.10 (n=3) with a moisture content of 92.68 %.

Effect of boiling on microstructure:-

Brightfield microscopy images of starch in YFM preparations are shown in Figure 1 below. Under microscopy iodine stained starch granules were observed in blue-purple colour. Boiling of squash has caused cell separation, disruption as well as starch gelatinization where starch granules swell and rupture and starch become solubilized (Ratnayake and Jackson 2009).

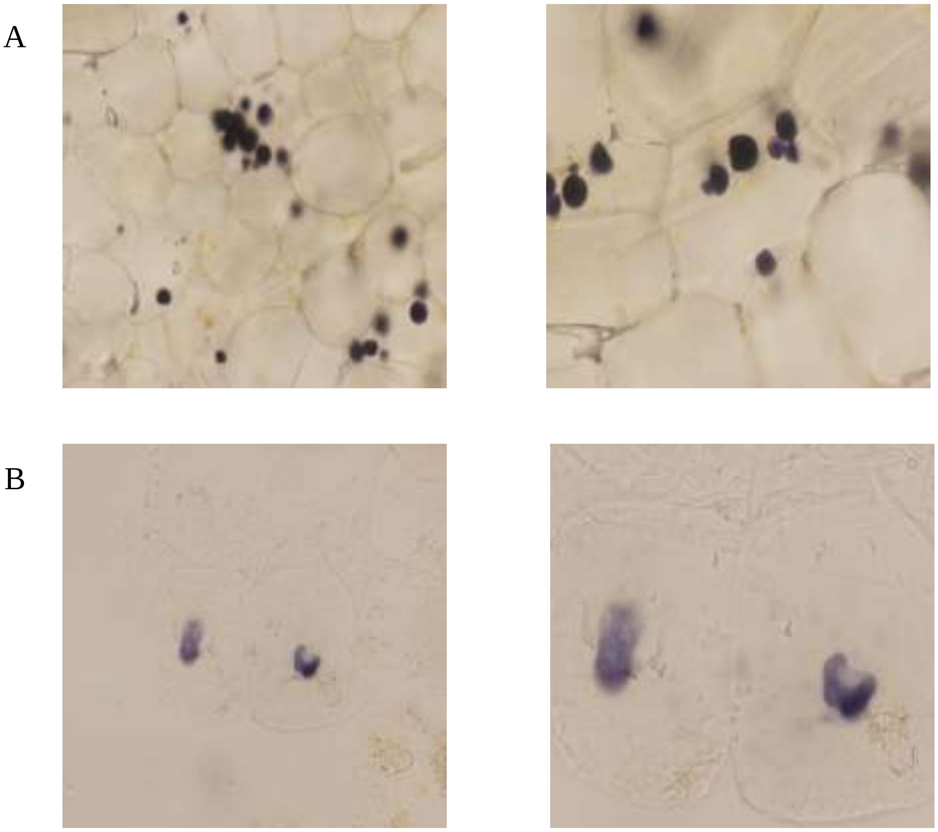

Figure 1:-Brightfield microscopy images of starch granules in squash

(A) Raw squash sample shows intact cells with cell walls which are closely bound to each other. Starch present as globules in some cells in limited numbers. (B) Boiled squash sample shows two cells with less prominent cell walls containing one large starch cluster per each cell. (Left column shows brightfield images in ×20 magnification and right column shows same corresponding images in ×40 magnification)

Discussion:-

The principle aim of the present study was to investigate the effect of boiling on the EGI and microstructure of starch in squash (*Cucurbita moschata*) found in Sri Lanka. Brightfield microscopy was carried out to visualize starch. EGI were determined by *in vitro* digestion procedures that simulated the human gastrointestinal digestion. Starch in plant foods is generally organized into granules inside amyloplasts and can be visualized using staining techniques (Brackmann et al. 2011). Changes in microstructure such as starch gelatinization, cell separation, plasmolysis etc. can be result due to thermal processing (Parada and Aguilera 2007).

This study shows that boiled squash has a GI of 13.05. According to GI indexing system of 0-100 boiled squash is a low GI food. Changes in microstructure as described above were evident in boiled squash when compared to raw squash.

Similar results have been reported from several studies which conducted in similar manner to this study but on different kind of vegetables. A study of EGI values of boiled and fried forms of hausa potato (*Solenostemon rotundifolius poir*) shows that they have intermediate GI and safe for patients with diabetes mellitus (Eleazu et al. 2017) In a comparative study of yellow fleshed manioc in boiled and curry form has revealed that boiled preparation has lower EGI compared to curry form and boiled form is safe for people with diabetes mellitus (Wickramasinghe et al. 2016). Another *in vitro* study on evaluating effect of processing on starch in potatoes shows that starch digestibility is improved after processing and it is also affected by the processing methods. For example boiled and mashed potatoes showed the highest rate of digestion but raw potato was hardly digested (Garcia-Alonso and Goni 2000).

Findings on starch contents in plant foods provide solutions for some of the major nutrient related diseases such as diabetes and obesity. Hence the main concern is to find ways to process foods in a way that give low amounts of glucose to body for prevention and management of Diabetes and obesity. Further studies on other preparation methods of squash and also on other Sri Lankan non leafy vegetables are needed to provide more knowledge on this topic. Future studies can use this study as a platform and generate more data which will help improve health.

The EGI of boiled squash is lower than the EGI of vegetables such as boiled yellow fleshed manioc (24.6) in Sri Lanka (Wickramasinghe et al. 2016).

Results of this study clearly show that boiling has an effect of disrupting cell membranes and solubilizing starch thereby facilitating the release of starch from cells during digestion. According to the EGI value boiled squash is a low GI food and safe for people with diabetes and obesity.

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