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Estimation of glycemic carbohydrates from commonly consumed foods using modified anthrone method

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ABSTRACT *The aim of the present study is to estimate the glycemic carbohydrates from commonly consumed foods using modified anthrone method. The existing anthrone method is a chemical method, which is widely used for estimation of starch and soluble sugars in foods. In the present study, we have modified the extraction procedure by enzymatic digestion rather chemical extraction, where in the glycemic carbohydrates were extracted in-vitro, mimicking the in-vivo condition by using enzyme (α-amylase, protease and amyloglucosidase) digestion followed by anthrone estimation. The estimate of the present method on glycemic carbohydrates in rice varieties ranges from 69.84 to 73.21%, in vegetables from 1.72 to 9.42%, and in legumes from 45.82 to 53.12 % which is in accord with the HPLC analysis wherein 74.01 to 80.02% in rice, 1.24 to 8.24% in vegetable and 42.16 to 49.97% in legumes respectively. This method can be more applicable than the present method for the estimation of glycemic carbohydrates in all kinds of foods. Furthermore, it is cost effectiveness and less laboriousness and advantageous.*

Introduction

Carbohydrates play a major role in human nutrition, meeting about 40-85% of energy intake. Their most important nutritional property is their easy digestibility in the small intestine. In terms of their physiological and nutritional role, they are often classified as available and unavailable carbohydrates (FAO/WHO, 1998). The glycemic carbohydrates provide carbohydrates to body cells, mainly in the form of glucose. In general, only monosaccharides are absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and it is continued in the small intestine by pancreatic amylase (Vasankari, 2006). Numerous studies have shown that high intake of carbohydrate rich foods including rice significantly increase the risk of obesity, type 2 diabetes and chronic diseases such as cardiovascular and some cancers (Jankin, et al., 2000; Gross, et al., 2004; Salmeron, et al., 1997; Liu, et al., 2000; Franceschi and Maso, 2001; Augustin and Maso, 2001.)

In research on macronutrients to date, the role of dietary carbohydrates in human nutrition has been less extensively studied than those of protein and fat. The main reason for this has been the absence of sound and rapid methodologies (FAO, 2003). The old habits die hard and value for carbohydrate content of foods has long been derived by "difference", rather than analyzed directly (FAO, 2003). Under this approach, the other constituents in the food (protein, fat, water, alcohol, ash) are determined individually, summed and subtracted from the total weight of food (FAO, 2003). It is not clear that carbohydrate estimated in this fashion whether includes fiber, as well as some non carbohydrate components such as organic acids (Marrill, 1973). Therefore the traditional method of expressing carbohydrate by "difference" is inaccurate because it includes a number of non-carbohydrate components, such as lignins, organic acids, tannins, waxes etc. (Nantel, 2007).

Chemical analysis, on the other hand, reports the constituent that will lead to an elevated blood glucose response once consumed and digested in the human body on each individual carbohydrate constituent such as sugars, starches, dietary fibre, sugar alcohols, etc. (Nantel, 2007). Shaw (1997) has made an extensive compilation of techniques used for sugar analysis; Southgate (2007) has provided an exhaustive review of the same. Further studies have also emphasized that carbohydrate determinations should describe chemical composition accurately, and provide information of nutritional relevance (Englyst, 2007).

The available techniques like HPLC and GC can also be used for the estimation of glycemic carbohydrates, but these methods are highly expensive and time consuming. Accurate methods for the estimation of glycemic carbohydrates in foods are currently gaining a great interest in nutrition research and are essential for computing correct energy intake. It is therefore important for consumers to be aware of the exact levels of each element contained in foodstuffs. For this reason, carbohydrates have to be determined only by direct analysis (http.//www.csir.co.za/enews/2011.jan/11.html.). The enzymatic method is considered to be an important analytical method for glycemic carbohydrate estimation as it is substrate specific.

The existing anthrone method is widely used for the determination of starch and soluble sugars in plant materials. Generally sugars and carbohydrates are extracted from dried and ground plant material. First soluble sugars are extracted with aqueous ethanol; later starch is extracted with an acid. In the present study we have modified the extraction procedure of existing anthrone method by enzymatic digestion rather chemical method, the glycemic carbohydrates were extracted by in-vitro digestion using a cocktail of α-amylase, protease and amyloglucosidase, which mimic in-vivo digestion and then followed by anthrone estimation.

In the present study the protease enzyme is used to extract the protein embedded carbohydrates. It is assumed that some of the sugars were probably associated with protein and cannot be isolated simply by extracting with other two enzymes, α-amylase and amyloglucosidase. Using the modified anthrone method we determined, the amounts of glycemic carbohydrate content from commonly consumed foods, which are collected from local markets of the twin cities of Hyderabad and Secunderabad, Andhra Pradesh State, India.

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Materials

Samples: Samples of commonly consumed foods, including vegetables, cereals such as rice varieties, and legumes were procured from the local markets of twin cities of Hyderabad and Secunderabad, Andhra Pradesh, India.

Replicate values of different fractions of glycemic carbohydrate content of these food samples were determined. The grains and vegetables were dried and then milled to flour and passed through a 250 µm sieve and different fractions of sugars were determined by following the modified Anthrone method (Thimmaiah, 1999).

Method

Sample preparation and sugar extraction

Duplicate test portions of cereals (rice), legumes and vegetables were treated with heat-stable α-amylase, protease, and amyloglucosidase in order to hydrolyze proteins embedded carbohydrates and starch, under laboratory conditions, as given in the following.

Food samples (100 mg) were taken in to 16 X 125 mm tubes with screw caps in duplicate. Five milliliters of (0.08M) phosphate buffer pH 6.0 were added to the tubes. The tubes were stored at 4°C for 12 h for hydration of the matrix. The sample was subjected to enzyme hydrolysis to degrade soluble starch. α-amylase solution (50 µL) was added, and the tubes were placed in a water bath at 95°C for 30 min (Daihan Labtech Co., Ltd. Korea). After 30 min, the tubes were removed and cooled to 60°C and adjusted to pH 7.5 with 1 milliliter of 0.275 M NaOH. Protease solution (50 µL) was added to the tubes, and then incubated at 60°C for 30 min. After that 1 milliliter of 0.325M HCl was added to the tubes to decrease the pH to 4.5. After adjusting the pH, amyloglucosidase solution (150 µL) was added and then the tubes were incubated at 60°C for 30 min. The residue was separated by centrifugation. The liquid portion was placed to 100 milliliter volumetric flask and made up to the mark with de ionized water.

The amount of glycemic sugars in the supernatant was determined by using anthrone reagent. Different volumes of supernatant, 0.2–1 milliliter in to a series of test tubes were taken and the volume was made up to 1 milliliter with distilled water to each tube. Four milliliter of anthrone reagent was added and the tubes were placed in boiling water bath for 8 min and cooled rapidly under running tap water. The optical density of green to dark green was measured at 630 nm against blank and the concentration of glycemic carbohydrate was calculated using standard curve. The standard curve was constructed using glucose as a standard. The analysis of glycemic carbohydrates were also carried out by HPLC using refractive index detector as described by Casterlin et al.,(1999).

Results and Discussion

This procedure provides quantification of food extract containing glycemic carbohydrates present in the food as digestible carbohydrates. Cereals like rice varieties showed very narrow variation of total sugars, soluble and insoluble starches. Table 1 indicates the fractions of glycemic carbohydrates in the branded rice after enzymatic treatment. The values of glycemic carbohydrates ranged from 69.84% in Nookalu sambar to 73.21% in Sona masoori old. In general, cereals like rice were rich source of carbohydrates and the results are very much comparable to the values obtained by HPLC methods. Table 2 indicates the glycemic carbohydrates in vegetables and legumes after enzymatic treatment. Among vegetables and legumes analyzed, the glycemic carbohydrates ranged from 1.72 in Ladyfinger to 9.42% in Carrot and 45.82% in Green gram to 53.12 % in White pea and these results are also similar to that of HPLC estimation. The glycemic carbohydrates are slightly varying in the rice, vegetables and legumes analyzed. The variation is may be due to varietal differences.

To our knowledge, this is the first study to report that, the analysis of glycemic carbohydrates in foods, which are digestible in the human gastrointestinal tract by using enzymes that mimic the human system. Southgate (1969) have suggested that any analytical procedure for glycemic carbohydrates must of necessity represent a compromise between the "ideal" procedure based on the known properties of the carbohydrates and a practical laboratory procedure. Vitaladasa and Belavady (1980) have shown the importance of glycemic carbohydrates in normal and therapeutic diets. Babu Jaisingh & Ramesh (1987) have shown that the determination of fructose in the presence of certain proteins by a modified anthrone-sulfuric acid method. Casterline et al., (1999) have reported that the total carbohydrates of 78.4 % to 81.4 % in rice cocoa by treating with the same enzymes and analyzed by HPLC using refractive index detector.

Costa et al., (2010) have reported glycemic carbohydrates and total carbohydrate in different foods of Australia, Belgium, Bulgaria, Germany, Greece, Iceland, Italy, Lithuani, Poland, Portugal, Spain and Turkey by following the method of by difference. Barreira et al. (2010) have shown that sugars profile of different Chestnut and Almond cultivars by HPLC-RI. Miguez Bernardez et al. [24] have reported that HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain). Ellingson et al., (2010) have developed a method for the direct determination of glycemic carbohydrates in low-carbohydrate products using high-performance anion exchange chromatography.

Conclusion

This study demonstrates that modified anthron method is applicable to determine the glycemic carbohydrates in different varieties of foods. This method can be used for routine analysis of all kinds of foods to generate glycemic carbohydrates content. This method is simple, rapid, and sensitive, and gives reproducible results. The reagent is inexpensive and stable, and a given solution requires only one standard curve. The color produced is permanent and it is unnecessary to pay special attention to the control of the conditions. It is therefore important for consumers to be aware of the exact levels of each element contained in foodstuffs. For this reason, carbohydrates have to be determined only by direct analysis. Furthermore, it is a cost effective method requiring no relatively expensive equipment and analytical material which are used in HPLC method.

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Table 1. Carbohydrate content of different branded rice samples [(a)]

Name	Available carbohydrates by modified Anthrone method	Available carbohydrates by HPLC method
Three roses	70.58±0.47	74.01±0.36
Warangal sona masoori	72.88±0.54	77.84±0.48
Masoor sambar	72.81± 0.46	76.16±0.39
Hansa nookalu	71.50±0.42	77.28±0.66
Asha Jyothi kurnool	72.44±0.31	76.86±0.33
Sona masoori kurnool	71.97±0.24	79.91±0.17
Sona masoori old	73.21±0.24	80.02±0.12
Hansa old	71.34± 0.91	76.99±0.27
Nookalu sambar	69.84±0.56	79.79±0.15
King kurnool	73.17± 0.80	79.56±0.47

[a]In g/100g product ± standard deviation; n = 3

Table 2. Available carbohydrate content of different vegetable and legume samples [(a)]

Name	Available carbohydrates by modified Anthrone method	Available carbohydrates by HPLC method
Vegetables		
Bitter guard	1.85±0.07	1.45±0.03
Brinjal	2.32±0.14	1.92±0.11
Carrot	9.42±0.32	8.24±0.21
Lady finger	1.72±0.12	1.24±0.05
Onion	8.61±0.19	7.30±0.31
Legumes		
Horse gram	48.56±0.30	45.07±0.22
Green gram	45.82±0.28	42.16±0.23
Red gram	50.16±0.36	48.11±0.29
Cowpea	47.45±0.47	45.91±0.41
White pea	53.12±0.52	49.93±0.48

[a]In g/100g product ± standard deviation; n = 3

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