Indian J.Sci.Res. 7(1) : 1-9, 2016 ISSN : 0976-2876 (Print)
ISSN : 2250-0138 (Online)

ANALYSIS OF AVAILABLE CORBOHYDRATE FRACTIONS FROM INDIAN FOODS BY USING A MODIFIED AOAC TOTAL DIETARY FIBER METHOD

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ABSTRACT

The carbohydrate content of foods has long been derived by "difference", rather than analyzed directly. The aim of the present study is to estimate the sugars and starch that are digestible in the human gastrointestinal tract by using enzymes that mimic the human system under laboratory conditions by using a modified AOAC total dietary fiber method in foods. Among the foods analyzed, the rice varieties were resulted in the total soluble sugars, ranging from 5.65% to 9.54%, vegetables 0.05% to 4.34% and the legumes from 0.46% to1.01%. Soluble starches in rice samples ranged from 09.03% to 16.87%, in vegetables from 0.18% to 1.06% and in legumes from 26.44% to 32.44%. Insoluble starches in rice samples were observed to be bracketed in between 55.07% to 59.08%, where as in vegetables from 0.38% to 4.15% and legumes 14.66 to 20.23. Concluding our observation, the total amount of starches and total sugars in rice fell in between 74.01% to 80.02%, in vegetables from 1.24% to 8.24%, and in legumes from 42.16% to 48.11. This analytical method can be used for routine analysis of all kinds of foods to generate their content of digestible starches and sugars.

KEYWORDS : Carbohydrates, Starches, Sugars, Rice, Vegetables, Legumes

Rice, a staple food for more than half of the humanity is a very rich source of carbohydrate contributing about 85 percent of energy. According to the Association of Japanese Agricultural Scientific Society every continent on the planet produces rice except Antarctica (Nogakkai, 1975). The major rice growing countries are China, India, Indonesia, Bangladesh, Thailand, Burma, Vietnam, Japan and the Philippines. Rice is staple food for 65% of the population of India. It is also cultivated by the majority of farmers. Nutritional quality of rice has received more attention in the developing countries as monotonous consumption of rice may lead to deficiencies of essential minerals, vitamins, and other nutritional components (Bouis, 2003) In India, the grain legumes are mostly consumed in the form of dhal (decorticated split cotyledons), after dehusking and cooking them in water to the desirable degree of softness . The immature seeds of the gram are used as a vegetable in India; generally, large seeded cultivars are preferred for this purpose. Grain legumes are a rich source of protein, vitamins, especially the B-complex, and minerals such as calcium and iron (Meiner et al., 1976). In India, like in many other developing countries, vegetables, and fruits constitute the main dietary source of pro-vitamin A.

Carbohydrates play a major role in human diet, comprising 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 (Meiner et al., 1976). Numerous studies have shown that carbohydrate- rich foods including rice significantly increase the risk of obesity, type 2 diabetes and chronic diseases such as cardiovascular and some cancers (Gross, 2004).

` 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. But old habits die hard and, since a value for carbohydrate content of foods has long been derived by difference, rather than analyzed directly. 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 should be clear that carbohydrate estimated in this fashion includes fiber, as well as some components that are not strictly speaking carbohydrate, e.g. organic acids. Total carbohydrate can also be calculated from the sum of the weights of individual carbohydrates and fiber after each has been directly analyzed.

However, the traditional method of expressing carbohydrate by difference is problematic because it includes a number of non-carbohydrate components, such as lignins, organic acids, tannins, waxes and some malliard products (Nantel, 2007). High - performance liquid chromatography (HPLC) with refractive index (RI)

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detection is a powerful technique for quantification of various types of sugars and was chosen for this study. Shaw has made an extensive compilation of techniques used for sugar analysis (Shaw, 1998) and Southgate has provided an exhaustive review of the same (Southgate, 1991)

Available and accurate methods development for the estimation of dietary carbohydrates in foods is currently gaining a great interest in nutrition research and is essential for computing the correct energy intake. Digestible carbohydrates such as starch are important components of foods such as cereals. Sugars such as sucrose are also important because they are often added to foods during processing. Therefore, the present study was carried out for the analysis of carbohydrates that are digestible in the human gastrointestinal tract by using enzymes that mimic the human system under laboratory conditions specified in AOAC Method 985.29 for total dietary fiber. We have also determined the amount of individual sugars such as fructose, glucose, and sucrose from commonly consumed foods collected from local markets in the twin cities of Hyderabad and Secunderabad, Telangana State, India.

MATERIALS AND METHODS

a. Sugars

Fructose, glucose, sucrose, maltose and lactose (>99.5% purity; Sigma Chemical Co.,St.Louis, MO) used in this study.

b. Enzymes

Total Dietary Fiber Kit (Sigma, TDF-100A) was used. This kit includes 10 mL heat-stable α-amylase, 500 mg protease, and 30 mL amyloglucosidase.

c. Phosphate buffer

0.08M, pH 6.0. Dissolve 1.400 g anhydrous dibasic sodium (Na_2HPO_4) and 9.68 g monobasic sodium phosphate monohydrate ($NaH_2PO_4.H_2O$) in 1 L water. Check pH and adjust if necessary.

d. NaOH

0.275N. Dissolve 11.00 g NaOH in L water

e. HCL

0.325M. Dilute 325 mL 1M HCL to 1L with water.

f. Samples

Samples of commonly consumed foods, including vegetables, cereals such as rice, and legumes were procured from the local markets of twin cities of Hyderabad and Secunderabad, Telangana, India. All the food samples were defatted with petroleum ether, before sugar extraction.

Replicate values of different fractions of 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 defatted with petroleum ether. The different fractions of sugars were determined by following the method of Casterlin et al., 1999.

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 and starch under laboratory conditions, as given in the following steps.

Step I

Samples were taken in to 16 X 125 mm tubes with screw caps in duplicate. Ten milliliters of pH 6.0 phosphate buffer (0.08M) were added to the tubes. The tubes were stored at 4°C for 12 hr for hydration of the matrix. The tubes were centrifuged to separate particles (Eppendorf AG 5810R 22331 Hamburg) at 4°C and 3500 g. A 5 ml of the aqueous portion from each tube was filtered through 0.45 μm syringe filter (What man International Ltd Maid stone England) into another 16 X 125 mm tube for analysis through steps II and III. The remaining 5 ml slurry was used for analysis in Step IV.

Step II

Two milliliters of filtered portion was pipetted in to a test tube and 2 mL acetonitrile was added. After 12 hr of precipitation, the residue was separated by centrifugation. The aqueous portion was cleaned by passing through an auto vial syringe of mesh less 0.45 μm (Whatman International Ltd Maidstone England) and LC-NH2 SPE. The resulting filtrate was then analyzed by HPLC for sugars.

Step III

Another 3 ml of the 5 ml filtered aqueous portion from step I was subjected to enzyme hydrolysis to degrade soluble starch. α-Amylase solution (50 μL) was added, and the tubes were placed in a 95°C water bath (Daihan Labtech

Co., Ltd. Korea). After 30 min, the tubes were removed and cooled to 60°C and adjusted to pH 7.5 with 0.4 ml of 0.275N NaOH. Protease solution was added to the tubes which were incubated at 60°C for 30 min. Now 0.4 mL 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. After the tubes had cooled, 3 mL of acetonitrile was added. After allowing for overnight precipitation, the residue was separated by centrifugation. The liquid portion was filtered through a 0.45µm (Whatman International Ltd Maidstone England) and then cleaned by SPE. The filtrate was analyzed by HPLC using the operating conditions as mentioned in step II.

Step IV

The insoluble residue slurry from step I was subjected to enzyme hydrolysis in the same way as described for Step III, except that 1mL of 0.275N NaOH, 1 mL of 0.325M HCl, and 7 mL of acetonitrile were used.

HPLC Separation of Carbohydrates

HPLC analysis of carbohydrates was carried out by the procedure described by Casterlin et al 1999. The chromatographic system consisted of a Shimadzu (model LC6A) chromatograph equipped with system controller, SCL6A, RID-10A RI detector, an integrator C-R3A chromate pack and stainless steel LC-NH_2 25 cm X 4.6 mm column preceded by a Supel-guard column containing LC-NH_2 packing (Supelcosil, 5µm particle size). By injecting 10 µl of the sample extract in to the HPLC column, isocratic separation of carbohydrate fractions was accomplished, with a mobile phase consisting of acetonitrile: water (80:20 (v/v)), at a flow rate of 1 ml per min. Standards of fructose, glucose, sucrose, maltose and lactose were purchased from Fluca Chemicals (USA). The HPLC was calibrated daily by injecting 10 µl standard mixtures, the concentration of each sugar ranging from 4.5 to 9.6 mg/ml. Peak identification was based on retention times and confirmed using standards.

CALCULATION

Calculation of Sugars: Sugars were calculated with the following formula;

$$\text{Sugars, } \mu g/g = \frac{\text{Peak area} \times \text{std factor} \times \text{volume factor}}{\text{Test portion weight, g}}$$

Where peak area = chromatographic peak area of sugar, std factor = conversion factor peak area to sugar value in micrograms based on sugar standard curve (slope), and volume factor =10.0 for Step II, 15.125 for Step III, or 14.25 for Step IV.

Calculation of Soluble Starch

The amount of individual sugars in Step III is the amount remaining after subtraction of the amount of the corresponding sugar determined in Step II. Because of hydrolysis by enzymes used in Step III, the amount of glucose derived from maltose is subtracted from the amount of glucose in Step III. A maltose-to-glucose conversion factor of 0.9 is used in this case.

Calculation of Insoluble Starch

In Step IV, the amount of individual sugars is the amount remaining after subtraction of the amount of the corresponding sugar determined in Step III and of the amount of glucose derived from maltose determined in Step II. The amounts of soluble starches are obtained by multiplying the increased amount of glucose from hydrolysis of soluble material by 0.9. The amounts of insoluble starches are obtained by conversion of the increased amount of glucose in the insoluble material. The amount of glucose derived from maltose is not included in this determination.

Statistical Analysis

The mean and standard deviation for each fraction of carbohydrates in each foodstuff was calculated. The differences in mean values between foodstuffs were tested using one-way analysis of variance.

RESULTS

The analytical steps shown in the procedure allowed the measurement of total carbohydrates in food samples. This procedure provided a food extract containing all sugars present in the food as simple sugars and digestible carbohydrates. The use of enzymes in step III and Step IV led to an increase in the amount of glucose resulting from the hydrolysis of starches. Figure1 shows typical HP liquid chromatograms of standard fructose, glucose, sucrose,

Table 1 : Carbohydrate Analysis of Different Branded Rice Sample

Carbohydrate, g/100g						
Rice	**Step: Material, treatment**	**Fructose**	**Glucose**	**Sucrose**	**Maltose**	**Lactose**
Three roses	II: Soluble, w/o enzyme	0	0.38±0.04	6.16±0.26	0	0
	III: Soluble, with enzyme	0	10.03±0.26	3.00±0.10	0	0
	IV: Insoluble, with enzyme	0	61.60±0.35	0	0	0
Warangle sona masoori	II: Soluble, w/o enzyme	0	1.10±0.01	6.94±0.21	0	0
	III: Soluble, with enzyme	0	11.13±0.01	1.09±0.01	0	0
	IV: Insoluble, with enzyme`	0	65.21±0.40	0	0	0
Masoor sambar	II: Soluble, w/o enzyme	0	1.09±0.00	6.79±0.00	0	0
	III: Soluble, with enzyme	0	12.10±0.07	1.05±0.01	0	0
	IV: Insoluble, with enzyme	0	62.60±0.37	0	0	0
Hansa nookalu	II: Soluble, w/o enzyme	0	1.11±0.01	7.10±0.01	0	0
	III: Soluble, with enzyme	0	12.25±0.04	1.06±0.01	0	0
	IV: Insoluble, with enzyme	0	63.32±0.13	0	0	0
Ashajyoti Kurnool	II: Soluble, w/o enzyme	0	1.05±0.01	3.23±0.11	0	0
	III: Soluble, with enzyme	0	13.55±0.18	2.03±0.02	0	0
	IV: Insoluble, with enzyme	0	64.84±0.15	0	0	0
Sonamasoori Kurnool	II: Soluble, w/o enzyme	0	1.02±0.11	5.06±0.01	0	0
	III: Soluble, with enzyme	0	15.75±0.18	0.58±0.01	0	0
	IV: Insoluble, with enzyme	0	65.64±0.25	0	0	0
Sona masoori old	II: Soluble, w/o enzyme	0	1.10±0.01	4.99±0.12	0	0
	III: Soluble, with enzyme	0	16.00±0.12	0.58±0.01	0	0
	IV: Insoluble, with enzyme	0	65.50±0.46	0	0	0
Hansa old	II: Soluble, w/o enzyme	0	1.05±0.01	4.68±0.15	0	0
	III: Soluble, with enzyme	0	16.05±0.04	0.54±0.05	0	0
	IV: Insoluble, with enzyme	0	62.53±0.08	0	0	0
Nukaloo sambar	II: Soluble, w/o enzyme	0	1.12±0.03	2.37±0.09	0	0
	III: Soluble, with enzyme	0	17.25±0.25	2.16±0.07	0	0
	IV: Insoluble, with enzyme	0	65.13±0.16	0	0	0
King Kurnool	II: Soluble, w/o enzyme	0	1.29±0.16	4.49±0.11	0	0
	II: Soluble, with enzyme	0	18.75±0.11	1.83±0.09	0	0
	IV: Insoluble, with enzyme	0	61.19±0.68	0	0	0

Table 1.1 : Carbohydrate Analysis of Different Vegetable and Legume Samples

Carbohydrate, g/100g						
Veg	**Step: Material, treatment**	**Fructose**	**Glucose**	**Sucrose**	**Maltose**	**Lactose**
Vegetables						
Bitter guard	II: Soluble, w/o enzyme	0	0.05±0.01	0	0	0
	III: Soluble, with enzyme	0	0.27±0.01	0	0	0
	IV: Insoluble, with enzyme	0	1.29±0.08	0	0	0
Brinjal	II: Soluble, w/o enzyme	0.08±0.01	0.06±0.01	0	0	0
	III: Soluble, with enzyme	0.03±0.01	0.63±0.01	0	0	0
	IV: Insoluble, with enzyme`	0.19±0.02	1.10±0.04	0	0	0
Carrot	II: Soluble, w/o enzyme	0.30±0.01	0.34±0.01	2.03±0.04	0	0
	III: Soluble, with enzyme	0.05±0.01	1.18±0.02	0.41±0.03	0	0
	IV: Insoluble, with enzyme	0.47±0.11	3.15±0.03	0.74±0.04	0	0
Ladyfinger	II: Soluble, w/o enzyme	0.09±0.00	0.10±0.01	0	0	0
	III: Soluble, with enzyme	0.08±0.01	0.52±0.01	0	0	0
	IV: Insoluble, with enzyme	0.12±0.03	0.42±0.04	0	0	0
Onion	II: Soluble, w/o enzyme	0.14±0.04	0.16±0.04	0.35±0.05	0	0
	III: Soluble, with enzyme	0.19±0.00	0.20±0.04	0	0	0
	IV: Insoluble, with enzyme	1.26±0.08	4.61±0.26	0.87±0.11	0	0
Legumes						
Horse gram	II: Soluble, w/o enzyme	0.16±0.02	0.30±0.01	0	0	0
	III: Soluble, with enzyme	0	32.29±1.61	0	0	0
	IV: Insoluble, with enzyme	0	17.28±1.15	0	0	0
Green gram	II: Soluble, w/o enzyme	0	0	0.19±0.03	0	0
	III: Soluble, with enzyme	0	29.38±0.64	0	0	0
	IV: Insoluble, with enzyme	0	17.25±0.98	0	0	0
Red gram	II: Soluble, w/o enzyme	0	0	1.01±0.04	0	0
	III: Soluble, with enzyme	0	36.04±1.13	0	0	0
	IV: Insoluble, with enzyme	0	16.29±0.62	0	0	0
Cowpea	II: Soluble, w/o enzyme	0	0	0.61±0.02	0	0
	III: Soluble, with enzyme	0	29.96±0.21	0	0	0
	IV: Insoluble, with enzyme	0	20.38±0.79	0	0	0
White pea	II: Soluble, w/o enzyme	0	0	0.80±0.02	0	0
	II: Soluble, with enzyme	0	32.15±0.40	0	0	0
	IV: Insoluble, with enzyme	0	22.48±0.79	0	0	0

Table 2 : Carbohydrate Content of Different Branded Rice Samples (g/100g)[(a)]

Name	Soluble Starch	Insoluble Starch	Total Starch	Total Sugar	Starches and Sugars
Three roses	09.03 ± 0.16	55.44 ± 0.14	64.47 ± 0.30	9.54 ± 0.17	74.01
Warangal Sona Masoori	10.02 ± 0.37	58.69 ± 0.08	68.71 ± 0.45	9.31 ± 0.00	77.84
Masoor Sambar	10.89 ± 0.09	56.34 ± 0.31	67.23 ± 0.41	8.93 ± 0.02	76.16
Hansa nookalu	11.02 ± 0.03	56.99 ± 0.07	68.01 ± 0.11	9.27 ± 0.07	77.28
Asha Jyothi kurnool	12.19 ± 0.90	58.35 ± 0.38	70.55 ± 0.48	6.31 ± 0.05	76.86
Sona Masoori Kurnool	14.17 ± 0.16	59.08 ± 0.22	73.25 ± 0.38	6.66 ± 0.04	79.91
Sona Masoori Old	14.40 ± 0.31	58.95 ± 0.40	73.35 ± 0.72	6.67 ± 0.05	80.02
Hansa Old	14.44 ± 0.16	56.28 ± 0.44	70.72 ± 0.60	6.27 ± 0.07	76.99
Nookalu Sambar	15.52 ± 0.09	58.62 ± 0.61	74.14 ± 0.71	5.65 ± 0.11	79.79
King Kurnool	16.87 ± 0.03	55.07 ± 0.11	71.94 ± 0.15	7.61 ± 0.01	79.56

Values are expressed as mean ± standard deviation; n = 6, [a]DB, dry base

Table. 3 : Carbohydrate Content of Different Vegetable and Legume Samples (g/100g)[(a)]

Name	Soluble Starch	Insoluble Starch	Total Starch	Total Sugar	Starches and Sugars
Vegetables					
Bitter guard	0.24 ± 0.01	1.16 ± 0.07	1.40 ± 0.08	0.05 ± 0.01	1.45
Brinjal	0.57 ± 0.01	0.99 ± 0.04	1.56 ± 0.05	0.36 ± 0.05	1.92
Carrot	1.06 ± 0.02	2.84 ± 0.03	3.90 ± 0.05	4.34 ± 0.25	8.24
Lady finger	0.47 ± 0.01	0.38 ± 0.04	0.85 ± 0.05	0.39 ± 0.05	1.24
Onion	0.18 ± 0.04	4.15 ± 0.23	4.33± 0.27	2.97 ± 0.32	7.30
Legumes					
Horse gram	29.06 ± 1.45	15.55 ± 1.04	44.61 ± 2.49	0.46 ± 0.03	45.07
Green gram	26.44 ± 0.58	15.53 ± 0.88	41.97± 1.46	0.19 ± 0.03	42.16
Red gram	32.44 ± 1.02	14.66 ± 0.56	47.10 ± 1.58	1.01 ± 0.04	48.11
Cowpea	26.96 ± 0.19	18.34 ± 0.71	45.30 ± 0.90	0.61 ± 0.02	45.91
White pea	28.94 ± 0.36	20.23 ± 0.71	49.17± 1.07	0.80 ± 0.02	49.97

Values are expressed as mean ± standard deviation; n = 6
[a]DB, dry base and [a]EF, edible foods for vegetables

maltose, and lactose. And Fig. 2 shows typical HP liquid chromatograms of carrot in step IV.

Table 1 and Table 1.1 represent the individual fractions of sugars in the branded rice, vegetables and legumes before and after enzymatic treatment.

Table 2 indicates the values of total sugars, soluble starch and insoluble starch rice sample tested. The values of total soluble sugars ranged from 5.65% (Nookalu sambar) to 9.62% (Hansa old), soluble starch from 9.03% (Three roses) to 16.87% (King kurnool) and insoluble starches

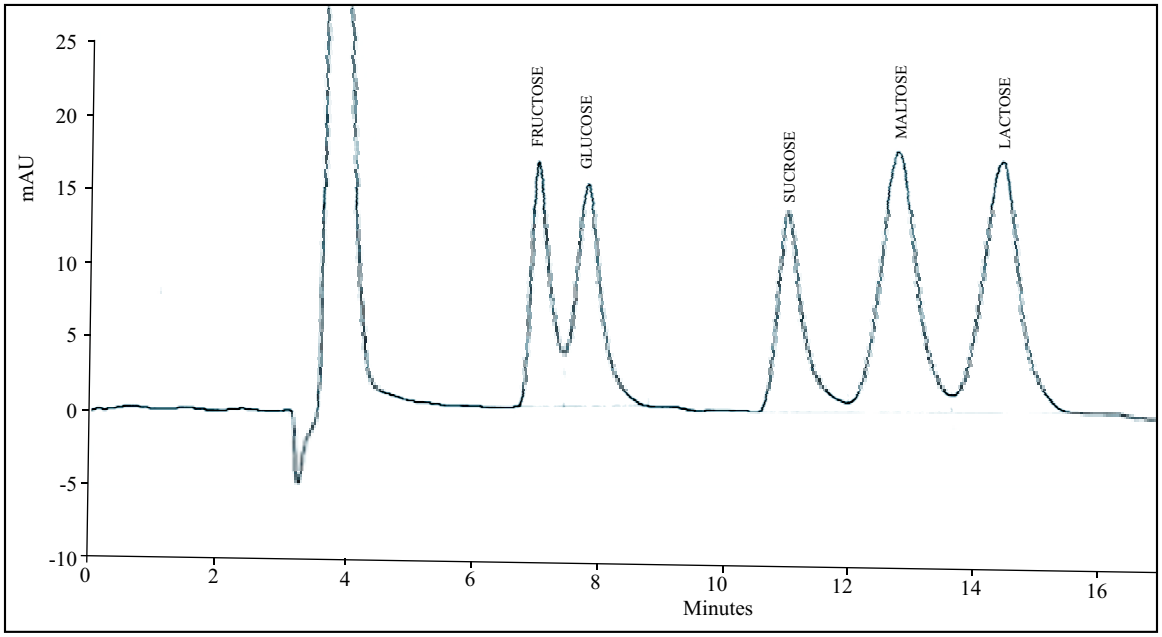

Figure 1. HP Liquid Chromatograms of Reference Standard of Fructose, Glucose, Sucrose, Maltose and Lactose

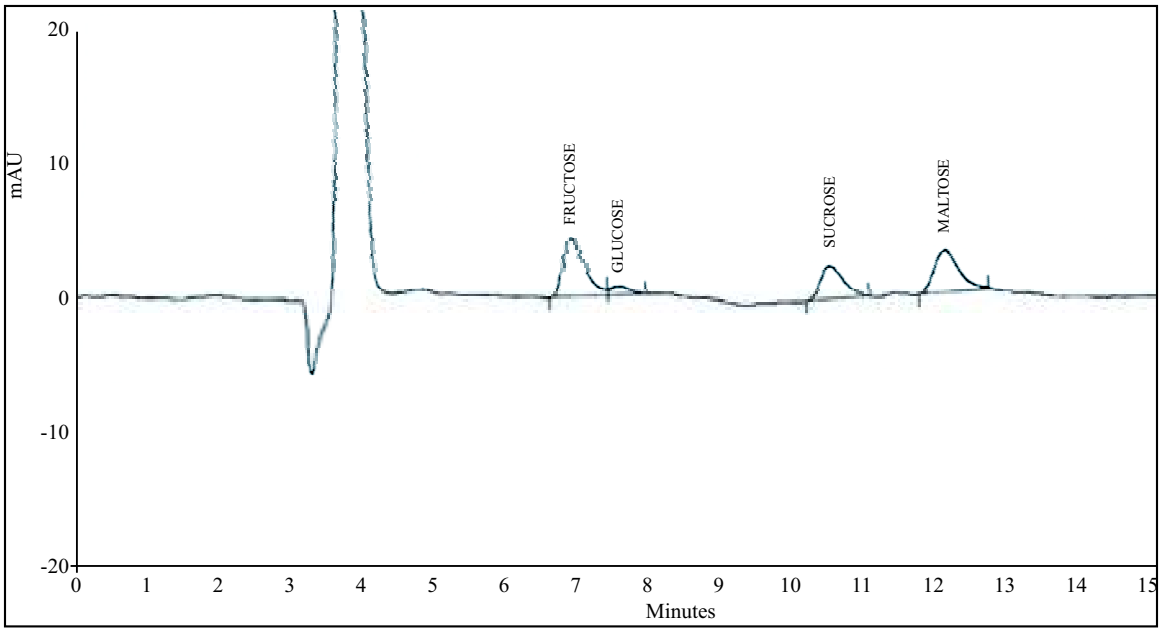

Figure 2 : HP Liquid Chromatograms of Carrot in Step IV

from 55.07% (King kurnool) to 59.08% (Sona masoori). In general rice varieties are rich source of carbohydrates and these varieties were showed very narrow variation of total sugars, soluble and insoluble starches. The total amount of carbohydrate including starches and total sugars among rice varieties fell in between 74.01% (Three roses) to 80.02% (Sona masoori old) (Table 2).

The results of carbohydrate fractions among the vegetables analyzed were showed the total sugars from 0.05% (Bitter guard) to 4.34% (Carrot), soluble starch from 0.24% (Bitter guard) to 1.06% (Carrot) and insoluble starches from 0.38% (Ladyfinger) to 4.15% (Onion). Total carbohydrates, among the vegetable varieties studied, fell in

the range of 1.24% (Lady Finger) to 8.24% in carrot (Table 3).

The results of carbohydrate fractions among the legumes analyzed were showed the total sugars ranging from 0.19% (green gram) to 1.01% (red gram), soluble starch from 26.44% (green gram) to 32.44% (red gram), insoluble starch from 14.66% (red gram), to 20.23% in (white pea) and the amount of total carbohydrates among the pulses ranges from 42.16% (green gram) to 49.97% in (white pea). The results of the carbohydrate fractions like soluble, insoluble starches and total sugars were varied significantly in pulses.

DISCUSSION

Casterline et al., 1999 reported 13.9% of soluble starches, 57.6% of insoluble starches, 6.9% of total sugar and 78.4% of starches and sugars in rice. They have also shown 13.8% of soluble starches, 25.3% of insoluble starches, 42.2% of total sugar and 81.4% of starches and sugars in rice (cocoa). Costa et al (2010) have reported available carbohydrates and total carbohydrate in different foods of Australia, Belgium, Bulgaria, Germany, Greece, Iceland, Italy, Lithuania, Poland, Portugal, Spain, and Turkey by following the method of by difference according to FAO. Casterline et al (1999) have also reported that 1% of soluble starches, zero percent of insoluble starches, 1% of total sugar and 1% of starches and 2% of sugars in green pea sample. Barreira et al (2010) have shown that sugars profile of different Chestnut and Almond cultivars by HPLC-RI. Bernardez et al (2004) have reported that HPLC determination of sugars in varieties of chestnut fruits from Galicia (Spain)

CONCLUSION

The traditional method of expressing carbohydrate by "difference" is problematic because it includes a number of non-carbohydrate components, such as lignins, organic acids, tannins, waxes. This study demonstrates the determination of digestible carbohydrate fractions such as starches and sugars in different varieties of foods by using modified AOAC total dietary method. This analytical method can be used for routine analysis of all kinds of foods to generate their content of digestible starches and sugars in food composition data base. In the present study fructose, maltose and lactose were not detected in all varieties of rice sample tested. The total amounts of sugars and digestible starches slightly vary in the rice varieties, vegetables and legumes because of varietals differences

ACKNOWLEDGEMENT

The author thank to Director In-charge, National Institute of Nutrition, Hyderabad, for his interest and encouragement.

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