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Analysis of Digestible Carbohydrates in Different Varieties of Basmati Rice and Other Popular Cereal Samples by Using HPLC-RI

S. Devindra and T. Longvah

Food Chemistry Division, National Institute of Nutrition, (ICMR) Hyderabad-500 007, India

Abstract: Analysis of digestible carbohydrate such as free sugars and starch are important components of foods like cereals. The objective of this study was to assess the digestible carbohydrates in different branded basmati rice and in other popular cereal samples of local markets of twin cities of Secunderabad and Hyderabad. In the study, we determined carbohydrates that are digestible in the human upper gastrointestinal tract by using enzymes that mimic the human system under laboratory conditions. After enzymatic hydrolysis, free sugars were extracted and determined by HPLC using refractive index detector. The chromatographic separation was achieved using Supelcosil-NH2 column using an isocratic elution with acetonitrile/water (80:20, *v/v*) at a flow rate of 1.0 ml/min. Among the Basmati rice varieties analyzed the total soluble sugars were in the range of 4.42% to 7.00% and other popular cereals are in the range of 14.41% to 36.80%. Soluble starches in basmati rice samples ranged from 10.89% to 17.06% and in other popular cereals from 6.89% to 9.54%. Insoluble starches in basmati rice were observed to be bracketed in between 55.97% to 61.73%, where as in other popular cereals from 28.59% to 44.47%. Concluding our observation, the total amount of starches and total sugars in basmati rice fell in between 73.14% to 80.47% and in other popular cereals from 64.47% to 76.12%.

Key words: Sugar · Starches · α-Amylase, protease · Amyloglucosidase

INTRODUCTION

Basmati rice is an important cash crop of Haryana, Punjab and Western Uttar Pradesh, but Haryana is popular for producing the best quality of basmati rice. Haryana accounts for 33% of total export of basmati rice from the country. The appetizing aroma of basmati rice is highly valued in many parts of the world and is preferred over non aromatic once for after cooking, they[1] exude pleasant aroma and show linear kernel elongation without significant increase in breadth [1]. The nutritive value of rice and rice based diets is basically governed by their chemical composition but information on nutritional evaluation of basmati rice is scanty [1]. Other cereal grains in particular wheat, maize, barley, sorghum, bajra, etc. are an excellent source of carbohydrate, dietary fiber and protein. They are also a good source of B-group vitamins, vitamin E and a number of minerals, notably iron, zinc, magnesium and phosphorus [2].

Carbohydrates are the most prevalent source of food energy in the world. They play a major role in human diet, comprising about 40-75% 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 [3]. Dietary carbohydrates are defined as sugars, oligosaccharides and complex carbohydrates. Sugars include monosaccharides, such as glucose and fructose, disaccharides, such as sucrose, maltose, lactose and polyols and complex carbohydrates (polysaccharides) comprising of starches and non-starch polysaccharides [4]. Numerous studies have been shown that carbohydrate rich foods including rice significantly increase the risk of obesity, type 2 diabetes and chronic diseases such as cardiovascular disease and some cancers [5-10]. 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 based on gas-liquid chromatography, high performance liquid chromatography and enzymatic analysis. But old habits die hard and, since a value for carbohydrate content of foods has long been derived by "difference", with

Corresponding Author: S. Devindra, Food Chemistry Division, National Institute of Nutrition, (ICMR) Hyderabad-500 007, India. Tel: +91-40-27197265, E-mail: devindravs@indiatimes.com.

apparently satisfactory results, there appears to be little incentive to develop more specificity for routine food composition analysis. However, the traditional method of expressing carbohydrate by "difference" is problematic because it includes a number of non-carbohydrate components, such as lignin, organic acids, tannins, waxes and some Mail lard product [11]. High-performance liquid chromatography (HPLC) with refractive index (RI) detector is a powerful technique for quantification of various types of sugars and was chosen for this study. Shaw [12], has made an extensive compilation of techniques used for sugar analysis and Southgate [13] has provided an exhaustive review of the same. To accurately quantify carbohydrates, it was necessary to modify starch and dietary fiber methods so that sugars could be separated from other components such as proteins, fibers and macromolecules which create a back flow, which in turn interferes with the resolution of sugar peak [14]. Determination and quantification of carbohydrate fractions in foods is currently of great interest in nutrition research and essential for computing the correct energy intake.

Therefore the present study was carried out with a view to analyze the correct dietary carbohydrate fractions in different varieties of foods by using enzymes that mimic the human system under laboratory conditions.

MATERIALS AND METHODS

Materials: Different branded variety of basmati rice and some other popular cereal samples were procured from different local markets of twin cities of Hyderabad and Secunderabad, from Andhrapradesh State, India.

Standards and Reagent: Fructose, Glucose, Sucrose, Maltose and Lactose (>99.5% purity; Sigma Chemical Co., St.Louis, MO) were used in this study. Thermo-stable α-amylase, protease and amyloglucosidase were also purchased from Sigma Chemical Co., St.Louis, MO. Acetonitrile 99.9% was of HPLC grade from Merck. All other chemicals were obtained from Sigma Chemical Co. (St.Louis, MO). Water was treated in a Mili-Q water purification system (Millipore, Milli-Q-lement, France).

Preparation of Standard Solution: Solutions of individual sugars (fructose, 4.7 131 mg/ml; glucose, 4.5 mg/ml; sucrose, 4.5 mg/ml; maltose, 9.6 mg/ml; and lactose, 9.5 mg/ml) in acetonitrile-water (1+1) were used as standard. Peak areas were plotted against the corresponding amount of the standard injection in to the HPLC system and linear relationships were obtained from 0 to 160 µg for glucose, fructose, sucrose, maltose and lactose. Food sample extracts were injected and a response that fell within the linear range was used in the determination of concentration.

Samples Preparation: Samples were dried and then milled to flour and passed through a 250 µm sieve and different fractions of sugars were determined by following the method of James *et al*., 1999. Duplicate test portions of rice flour were treated with heat-stable α-amylase, protease and amyloglucosidase to hydrolyze proteins and starch under laboratory conditions as given in the following steps.

Step I: One hundred mg of test samples was taken in to 16 x 125 mm tubes with screw caps in duplicate. Ten milliliters of pH 6.0 phosphate buffer 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 effect to sedimentations of particles and then 5 ml of the aqueous portion from each tube was filtered through 0.45 µm nylon filter 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 filtrate from step I was pipetted into a test tube to which 2 ml of Acetonitrile was added. After 12 hr of precipitation, the residue was separated by centrifugation. The aqueous portion was passed through an auto vial syringe less 0.45 µm nylon filter and LC-NH2 SPE. The resulting filtrate was then analyzed by HPLC for sugars.

Step III: Another 2 ml portion from the 5 ml filtrate 161 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 95°C water bath. After 30 min, it was removed and cooled to 60°C and adjusted to pH 7.5 with 0.4 ml of 0.275N sodium hydroxide solution. To these tubes protease solution was added and incubated at 60 °C for 30 min and then 0.4 mL of 0.325M HCl was added to reduce the pH to 4.5. Amyloglucosidase solution (150 µL) was added and then tubes were incubated at 60°C for 30 mn. After the tubes had cooled to room temperature, 3 mL acetonitrile was added. After overnight precipitation, the residue was separated by centrifugation. The liquid portion was filtered through a 0.45 µm nylon filter and then cleaned by SPE. The filtrate was analyzed by HPLC.

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

Instrumentation and Operating Conditions: Carbohydrate fractions were determined by high performance liquid chromatography coupled to a refraction index (HPLC-RI). The HPLC equipment consisted of Shimadzu system LC-10ATvp pumps, SIL-HTA / HTC auto sampler and RID-10A RI detector and SCL-10AVP system controller using Shimadzu LC- CLASSVP™ data system software. The chromatographic separation was achieved with a Supelcosil LC-NH2 25cm X 4.6 mm column preceded by a Supel-guard column containing LC- NH2 packing operated at ambient temperature. The mobile phase used was acetonitrile-deionized water, 8:2 (*v/v*) at a flow rate of 1 ml/min and the injection volume was 10 µl. The results are expressed in g/100 g of dried weight, calculated by internal standard normalization of the chromatographic 191 peak area. Sugar identification was made by comparing the relative retention times of sample peaks with standards.

Soluble Materials: 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.

Insoluble Material: 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: Sugars extracted were performed in duplicate and each sample was injected 6 times in HPLC-RI. The results are expressed as mean values±standard deviation.

RESULTS AND DISCUSSION

The analytical steps shown above 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. Figure 1 shows typical HP liquid chromatograms of standard fructose, glucose, sucrose, maltose and lactose.

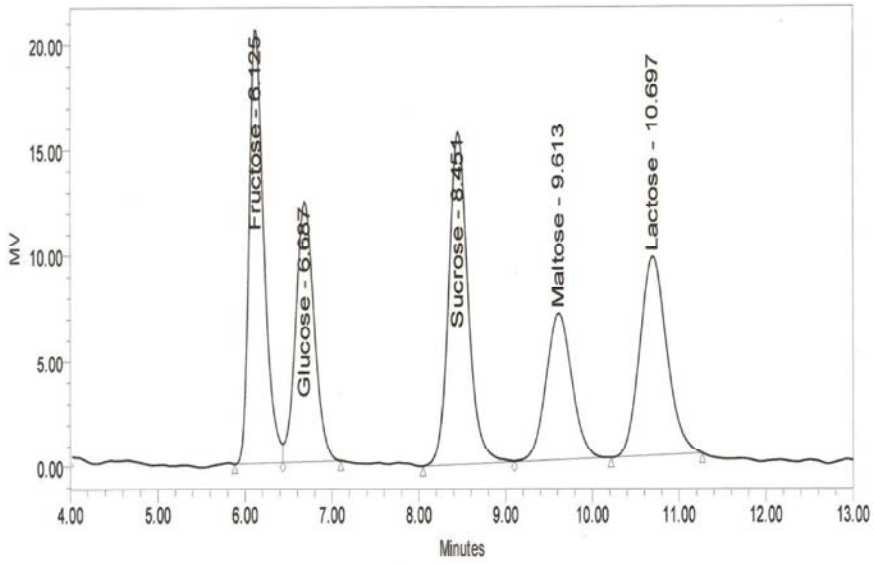

Fig. 1: HP liquid chromatograms of reference standard of fructose, glucose, sucrose, maltose and lactose

Table 1[z2]: Carbohydrate analysis of different branded rice samples[a]

		Carbohydrate, g/100g				
Rice	Step: Material, treatment	Fructose	Glucose	Sucrose	Maltose	Lactose
Basmati Rice						
Davat Basmati	II: Soluble, w/o enzyme	0	1.12±0.00	5.26±0.21	0	0
	III: Soluble, with enzyme	0	13.05±0.18	0.62±0.01	0	0
	IV: Insoluble, with enzyme	0	66.77±0.64	0	0	0
Punjabi Basmati	II: Soluble, w/o enzyme	0	0.57±0.01	3.38±0.06	0	0
	III: Soluble, with enzyme	0	18.95±0.11	0.47±0.00	0	0
	IV: Insoluble, with enzyme	0	62.50±0.35	0	0	0
Kohinoor	II: Soluble, w/o enzyme	0	0.46±0.01	4.53±0.15	0	0
Basmati	III: Soluble, with enzyme	0	14.35±0.11	0.65±0.00	0	0
	IV: Insoluble, with enzyme	0	66.77±0.64	0	0	0
Indiagate	II: Soluble, w/o enzyme	0	0.93±0.01	2.97±0.08	0	0
Long Basmati	III: Soluble, with enzyme	0	15.20±0.14	2.16±0.07	0	0
	IV: Insoluble, with enzyme	0	62.61±0.46	0	0	0
Indiagate	II: Soluble, w/o enzyme	0	0.88±0.04	3.20±0.11	0	0
Super Basmati	III: Soluble, with enzyme	0	13.05±0.18	1.34±0.02	0	0
	IV: Insoluble, with enzyme	0	62.19±0.41	0	0	0
Harati Basmati	II: Soluble, w/o enzyme	0	0.92±0.01	3.33±0.06	0	0
	III: Soluble, with enzyme	0	13.65±0.11	1.79±0.01	0	0
	IV: Insoluble, with enzyme	0	64.90±0.08	0	0	0
Davat elina	II: Soluble, w/o enzyme	0	0.81±0.01	3.57±0.10	0	0
Basmati	III: Soluble, with enzyme	0	12.10±0.14	1.23±0.02	0	0
	IV: Insoluble, with enzyme	0	65.20±0.10	0	0	0
Indiagate	II: Soluble, w/o enzyme	0	0.93±0.01	3.88±0.12	0	0
Classic Basmati	III: Soluble, with enzyme	0	12.80±0.07	1.21±0.02	0	0
	IV: Insoluble, with enzyme	0	64.09±0.10	0	0	0
Neckles	II: Soluble, w/o enzyme	0	0.87±0.06	3.10±0.03	0	0
Basmati	III: Soluble, with enzyme	0	13.70±0.07	1.39±0.04	0	0
	IV: Insoluble, with enzyme	0	62.05±0.21	0	0	0

[a] In g/100g product±standard deviation; n = 6.

Table 2[z3]: Carbohydrate content of different branded rice samples [a]

Name	Soluble starch	Insoluble starch	Total starch	Total sugar	Starches and sugars
Davat Basmati	11.75±0.16	61.73±0.46	73.48±0.53	7.00±0.00	80.47
Panjabi Basmati	17.06±0.33	56.42±0.30	73.48±0.62	4.42±0.03	77.72
Kohinoor Basmati	12.92±0.14	60.09±0.23	73.01±0.48	5.66±0.04	78.66
Indiagate Long Basmati	14.27±0.16	57.09±0.46	71.36±0.60	6.33±0.08	77.69
Indiagate super Basmati	11.75±0.17	55.97±0.56	67.72±0.60	5.43±0.08	73.14
Harti Basmati	12.29±0.15	58.41±0.31	70.70±0.43	6.03±0.06	76.72
Davat elina Basmati	10.89±0.08	58.68±0.17	69.57±0.15	5.61±0.07	75.18
Indiagate classic basmati	11.52±0.15	57.67±0.58	69.19±0.60	6.04±0.18	75.23
Neckles Basmati	12.33±0.25	56.25±0.51	68.58±0.67	5.27±0.24	73.85

[a] In g/100g product±standard deviation; n = 6.

Basmati Rice: Cereals like basmati rice and other popular cereal varieties showed variation of total sugars, soluble and insoluble starches. Tables 1 and 2 indicate fractions of sugars in the branded basmati rice and other popular cereals before and after enzymatic treatment. The values of total sugars ranged from 4.42% (Punjabi basmati) to 7.00% (Davat basmati), soluble starch from 10.89% (Davat elina basmati) to 17.06% (Punjabi long basmati) and insoluble starches from 55.97% (Indiagate super basmati) to 61.73% (Davat basmati) (Table 2). In general, cereals like rice were rich source of carbohydrates. To conclude in rice, the total amount of starches and total sugars in rice fell in between 73.14% (India gate super basmati) to 80.47% (Davat basmati) (Table 2). James *et al*. [14], have 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).

Table 3[Z4]: Carbohydrate analysis of different branded rice samples[a]

Rice	Step: Material, treatment	Carbohydrate, g/100g Fructose	Glucose	Sucrose	Maltose	Lactose
Cereals						
Jawar	II: Soluble, w/o enzyme	0.71±0.02	1.36±0.11	0	0	0
	III: Soluble, with enzyme	1.36±0.11	8.30±0.06	14.60±1.06	0	0
	IV: Insoluble, with enzyme	0	31.77±0.03	11.74±1.45	9.22±0.00	0
Bajra	II: Soluble, w/o enzyme	0	2.18±0.06	0	0	0
	III: Soluble, with enzyme	4.45±0.00	10.61±0.56	14.60±0.21	0	0
	IV: Insoluble, with enzyme	0	44.78±1.05	0	0	0
Wheat	II: Soluble, w/o enzyme	0	0	0.56±0.00	0	0
	III: Soluble, with enzyme	1.54±0.21	9.13±0.34	18.30±0.35	0	0
	IV: Insoluble, with enzyme	0	49.43±0.94	0	0	0
Ragi	II: Soluble, w/o enzyme	0	0	22.70±1.98	0	0
	III: Soluble, with enzyme	0	7.66±0.02	14.10±0.28	0	0
	IV: Insoluble, with enzyme	0	36.03±0.20	0	0	0
Wheat Bambi	II: Soluble, w/o enzyme	0	0	0	1.75±010	0
	III: Soluble, with enzyme	0.84±0.15	8.75±0.22	16.50±0.50	0	0
	IV: Insoluble, with enzyme	0	36.03±0.20	1.17±0.03	0	0
Maiz	II: Soluble, w/o enzyme	0	0	0	0	0
	III: Soluble, with enzyme	1.81±0.25	10.12±0.62	12.60±0.00	0	0
	IV: Insoluble, with enzyme	0	48.28±0.86	0	0	0

[a] In g/100g product±standard deviation; n = 6.

Table 4: Carbohydrate content of different branded rice samples[a]

Name	Soluble starch	Insoluble starch	Total starch	Total sugar	Starches and sugars
Jowar	7.47±0.16	28.59±0.13	36.06±0.54	28.41±0.42	64.47
Bajra	9.54±1.12	40.29±0.09	49.83±1.22	21.24±0.08	71.07
Wheat	8.21±0.03	44.47±0.11	52.70±0.15	20.40±0.01	73.09
Ragi	6.89±0.16	32.42±0.14	39.32±0.30	36.80±0.17	76.12
Wheat bambi	7.87±0.12	41.42±0.34	49.30±0.46	20.26±0.05	69.56
Maize	9.10±0.09	43.44±0.61	52.55±0.71	14.41±0.11	66.96

[a] In g/100g product±standard deviation; n = 6.

Soluble, insoluble starches and total sugars varied significantly in other popular cereals (Table 4). The values of total sugars ranged from 36.06% (Sorghum) to 52.70% (Wheat), soluble starch from 6.89% (Ragi) to 9.52% (Bajra), insoluble starch from 28.59% (Jowar), to 44.47% in (wheat) and the amount of total carbohydrates among the cereals ranges from 64.47% (Jowar) to 76.12% in (Ragi).

CONCLUSION

This study demonstrates that the method is applicable to determine the digestible starches and sugars in different varieties of foods. This analytical method can be used for routine analysis of all kinds of foods to generate their content of digestible starches and sugars. Fructose, maltose and lactose were not detected in all varieties of basmati rice sample tested whereas found in other popular cereals such as Sorghum (Jowar). The total amounts of sugars and digestible starches slightly vary in the basmati rice and other popular cereals are because of varietals differences.

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