



Estimation of Macronutrients and Resistant Starch Content of Commonly Consumed Plant Foods

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ABSTRACT

Background: The present study aims to develop a database on the macronutrients and resistant starch content of commonly consumed plant foods such as cereals, pulses, roots and tubers and vegetables.

Methods: Analysis of macronutrients such as moisture, protein, fat, ash and dietary fiber was carried out by using standard AOAC methods, available carbohydrates by modified Anthrone method and resistant starch by Megazyme assay kit.

Result: The results of the moisture content among the cereals tested ranged from 0.23% to 7.3%, pulses from 5.05% to 7%, roots and tubers from 74.7% to 80.35% and plantain 84.67%, protein in cereals ranged from 6.99% to 11%, pulses from 16.32% to 22.92%, roots and tubers from 7.74% to 11.87% and plantain contains 6.71%, fat content of cereals ranged from 2.17% to 6.06%, pulses from 2.3% to 6.53%, roots and tubers from 0.3% to 0.49% and plantain contains 0.14%, ash content of cereals ranged from 0.44% to 2.16%, pulses from 2.58% to 4.06%, roots and tubers from 1.18% to 1.38% and plantain contains 0.73% and the carbohydrate of cereals ranged from 59.21% to 80.35%, pulses from 19.06% to 47.76%, roots and tubers from 11.86% to 16.72% and plantain contains 11.49%. The resistant starch content of cereals ranged from 0.16% to 5.71%, pulses from 1.86% to 29.4%, roots and tubers from 28.41% to 45.7% and plantain contains 39.88% respectively. The non-resistant starch content of cereals ranged from 49.63% to 73.18%, pulses from 5.05% to 35.98%, roots and tubers from 2.43% to 19.31% and the plantain contains 17.03%. The total starch is the sum of the resistant starch and non-resistant starch. The total starch content of cereals ranged from 50.1% to 74.39%, pulses from 23.97% to 37.22%, roots and tubers from 33.62% to 48.58% and the plantain contain 56.91% respectively.

Key words: Cereals, Plant foods, Pulses, Resistant starch, Root and tubers, Vegetables.

INTRODUCTION

Macronutrients are the nutrients that provide calories or energy and are required for the body in large amounts to carry out daily life activities rhythmically and adequately. In addition to water, humans require four primary macronutrients from their staple foods, including carbohydrates, proteins, fats and dietary fiber, which are often called proximate principles since they are the main bulk of the food (Vijay *et al.*, 2017).

The Food composition database symbolizes a significant role to analyse the dietary pattern and problems while dealing with public health nutrition, where only accuracy in the food composition databases can help in quantifying the nutrients to assess nutritional consumption (Foster and Adamson, 2010). Hence, it is paramount to keep the National food composition databases comprehensive and up-to-date by maintaining the inherent quality for nutritional monitoring (Ocké *et al.*, 2021). Arabic comprehensive food composition database was developed in myfood24 by incorporating parameters, like food identification, cleaning, mapping, translation, allocating portion sizes and quality checking (Bawajeeh *et al.*, 2021). In some way, this kind of database seems less implicit in addressing the actual energy balance.

The importance of carbohydrates in human nutrition has less been explored than those of macronutrients like proteins

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and fats (FAO, 2003). Many food composition databases available in the world used the old and indirect, by “difference” method to analyze the carbohydrate content rather than analyzing it directly (FAO, 2003). Since the possible errors that arise from the individually analyzed macronutrients (protein, fat, water, alcohol and ash) are directly reflected on by “difference” value of carbohydrates, hence sophisticated method for estimating carbohydrates in food samples is the current trend among researchers because of its degree of accuracy in a result which is also necessary for calculating the energy balance (Devindra, 2015).

Starch is a main source of carbohydrate in a variety of foods termed as glycemic and resistant starch. Resistant

starch is the starch portion that cannot be digested in the small intestine but may be fermented in the large intestine forming short-chain fatty acids and generating a range of beneficial changes like increasing stool bulk, giving a mild laxative effect to promote regularity (Englyst *et al.*, 1992; Asp, 1992; Also, 2012). RS is categorized into 4 types *i.e.*, RS1-type of resistant starch is physically inaccessible to digestion due to intact cell walls *eg*: and grains (Phillips and Young, 1995). RS-are native starch granules protected from digestion by the structure of the starch granules *eg*: green bananas, raw potatoes and tuber starch (Englyst *et al.*, 1992). RS3 type of starch is formed when the retrograded starch which makes inaccessible to digestive enzymes *eg*: cooked then cooled potato, rice, or pasta (Sun *et al.*, 2006; Cummings and Stephen, 2007). RS4. These are chemically modified starches, which do not occur naturally but are prepared or modified commercially *eg*: citrate and phosphate starches (Sun *et al.*, 2006). Resistant starch acts as a prebiotic, is hypocholesterolemic, acts against colon cancer and fat accumulation and supports intestinal calcium and iron absorption (Sajilata *et al.*, 2006). The incorporation of resistant starch in the diet will reduce the post-prandial glucose spike and limit the digestion and absorption in the small intestine (Costacou and Mayer-Davis, 2003).

Due to the process of RS3 retrogradation, the starch is cooked and cooled and made inaccessible for the digestive enzymes for the postprandial glucose spike. Application of processes that optimize and stabilize resistant starch and its utilization as an ingredient in functional food product development will greatly contribute to its availability for consumption and there will not be any monotony of foods for the diabetic population. Hence, studies about resistant starch content are limited to only a few foods. Therefore the present study was conducted to develop a database on resistant starch in some commonly consumed plant foods and the database can be of great use for the management of Diabetes and their food choices.

MATERIALS AND METHODS

Procurement of sample

The samples were procured from local markets of twin cities *i.e.*, Secunderabad and Hyderabad. Cereals (Market rice, Bajra, Ragi, Korra, Wheat, Jowar, Low GI Rice, Barley, Maize). Pulses (Red Gram Dhal, Bengal Gram Dhal, Bengal Gram whole, Green Gram Whole, Green Gram dhal, Lentil, red kidney bean, Cowpea). Roots and tubers (Colocasia, Potato, yam). Vegetables (Plantain) were subjected to cleaning and made free of dust.

Preparation of samples

All the cereals and pulses were milled in a UDY cyclone mill and made into a fine powder that can be sieved by a 60 mesh sieve. Roots and tubers and vegetables were wiped with a muslin cloth and peeled and the edible portion was sliced into 1-2 cm and immediately surface sterilized by absolute ethanol followed by drying at 40-60°C for 24-36

hours in a hot air oven. Dried samples were ground to a fine powder and stored in air-tight containers for further analysis.

All the chemicals used were of analytical grade from Sigma Aldrich, SRL and Megazyme. Chemical composition was carried out by AOAC (1990) standard protocols and the resistant starch assay was carried out by the Megazyme kit method (K-RSTAR02/17).

Estimation of macronutrients

Macronutrients such as fat, moisture, crude fiber and ash contents were analyzed in the powdered samples by using AOAC (2006) (934.01, 942.05, 962.09 and 920.39, respectively) and crude Protein by the AOAC Kjeldahl method (984.13). The estimation of available carbohydrates was carried out by using the modified Anthrone method (Devindra, 2015).

Estimation of available carbohydrates

Reagents

Sugars

Glucose (>99.5% purity; Sigma Chemical Co., St. Louis, MO, USA) was used in this study.

The standard glucose Stock solution

100 mg glucose in 100 ml of distilled water.

Working standard

10 ml of stock solution was diluted to 100 ml with distilled water (100 µg/ml).

Anthrone reagent

200 mg of anthrone was dissolved in 100 ml of ice-cold sulphuric acid.

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.

Phosphate buffer (0.08 M, pH 6.0)

Dissolve 1.400 g anhydrous dibasic sodium (Na_2HPO_4) and 9.68 g monobasic sodium phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) in 1000 ml distilled water. Check the pH level and adjust if necessary.

NaOH (0.275 N)

Dissolve 11.00 g NaOH in 1000 ml distilled water.

HCL (0.325 M)

Dilute 325 ml 1 M HCL to 1000 ml distilled with water.

Procedure

The day to day foods including cereals, pulses, tubers and vegetables were tested with heat-stable α-amylase, protease and amyloglucosidase to hydrolyze proteins and starch under laboratory conditions. 0.5 g of powdered pizza sample was taken in screw-cap tube followed by adding 5 ml of phosphate buffer then it was allowed to stand for 12 hours

for hydrating the food particle. Then the hydrated samples were treated with alpha-amylase (50 μ l) to degrade the soluble-starch and kept in water bath at 95°C for half an hour. The tubes were then brought to 60°C and pH was adjusted to 7.5 using 0.275 M NaOH before adding Protease (50 μ l) and followed by keeping in water bath at 60°C for half an hour. After removing from bath pH was adjusted to 4.5 using 0.325 M HCl. Then 150 μ l of amyloglucosidase was added and allowed to stand for half an hour at 60°C. The residue formed in the tubes was removed by centrifugation. Further the aliquot was transferred to a volumetric flask (100 ml) and made up the volume using milli-Q water. The glycemic carbohydrate in supernatant was measured using anthrone reagent. Then different volumes of supernatant, 0.2-1 ml was taken in a series of test tubes followed by making the volume to 1 ml using milli-Q water and then 4ml anthrone reagent was added to all the tubes and vortex properly before keeping in boiling water bath for 8 to 10 minutes. After that all the tubes were cooled with running tap water. The concentration of glycemic carbohydrate was measured by plotting the standard glucose curve with the help of optical density value which was obtained from spectrophotometer reading at 630 nm.

Determination of resistant starch

100 mg sample triplicates were weighed and 4 ml of PAA enzyme mixture was added and placed in a shaking water bath for 16hours at 37°C in a horizontal position. Later the samples were treated with 4 ml 99% ethanol and centrifuged at 1500 g for 10 minutes and the supernatant was collected in other tubes. 8ml of 50% ethanol was added and centrifuged for 10 minutes at 1500 g and the supernatant was added to the previously collected tubes. Again 8 ml of 50% ethanol was added and centrifuged for 10 minutes at 1500 g and the supernatant was added to the previously collected tubes. Later the residue was treated with a 2 M KOH ice magnetic stirrer and 8ml of sodium acetate buffer (pH 3.8) and 100 mL amyloglucosidase and treated with GODPOD reagent to estimate the presence of resistant starch in samples. All the supernatants collected were pooled and treated with diluted amyloglucosidase and treated with GODPOD reagent to estimate non-resistant starch. absorbance was measured at 510 nm compared with a reagent blank (0.1 mL of 100 mM sodium acetate buffer (pH 4.5) with GOPOD reagent by 96 well plate reader (Thermo Scientific multiscan go).

Statistical analysis

All experimental analysis was repeated 3 times. The results were presented as a mean from three replications with standard deviation (SD).

RESULTS AND DISCUSSION

The results of the macronutrient content of selected plant foods are given in Table 1. The results of the moisture content of cereal foods tested ranged from 0.23% (Barley) to 7.3%

(Jowar), pulses from 5.05% (Bengal gram whole) to 7% (Cowpea), roots and tubers from 74.7% (Colocasia) to 80.35% (Yam) and plantain 84.67%. The results of the protein among the cereals ranged from 6.99% (Ragi) to 11% (Foxtail), pulses from 16.32% (Bengal gram whole) to 22.92% (Green gram dhal), roots and tubers from 7.74% (Colocasia) to 11.87% (Yam) and plantain showed 6.71%, fat content among the cereals tested was ranged from 2.17% (Market rice) to 6.06% (Maize), pulses from 2.3% (Green gram whole) to 6.53% (Green gram whole), roots and tubers from 0.3% (Yam) to 0.49% (Colocasia) and plantain contains 0.14%, ash content of cereals ranged from 0.44% (Market rice) to 2.16% (Ragi), pulses from 2.58% (Cowpea) to 4.06% (Red gram dhal), roots and tubers from 1.18% (Potato) to 1.38% (Yam) and plantain contains 0.73% and the carbohydrate of cereals ranged from 59.21% (Perl millet) to 80.35% (Market rice), pulses from 19.06% (Red kidney bean) to 47.76% (Green gram dhal), roots and tubers from 11.86% (Potato) to 16.72% (Colocasia) and plantain contains 11.49% respectively. Study results are similar to the proximate composition of the major cereal grains (sorghum, millet, maize and rice) of Iree, Oyo State, Nigeria. The water, oil, ash, protein and carbohydrate contents were in the ranges 9.4-11.0%, 0.3-4.9%, 0.8-2.6%, 6.5-10.9% and 70.7-82.4%, respectively (Adeyeye and Ajewole, 1992). Another study has reported the proximate composition of Oats (*Avena sativa*) and the moisture, crude protein, crude fat, crude fiber, ash and total carbohydrate contents were in the range of 8.5-9.8, 11.9-15.8, 6.7-10.3, 2.1-3.5, 1.2-1.3 and 72.6-74.3 g/100 g DM (Getaneh *et al.*, 2021). The available carbohydrate was found in the range of 75.86% in sorghum to 72.99% in millet, the highest protein in wheat (12.39%), maize 8.58%, ash content ranged from 0.56% for rice to 1.67% for millet, fat and fiber was between 2.50 to 3.94% and 1.09 to 3.19% (Abdulrahman and Omoniyi, 2016). A study reported that the proximate compositions such as carbohydrate, protein, fat, ash and dietary fiber of wheat varieties, sorghum varieties maize varieties (Salamatu *et al.*, 2020). In another study, they reported the macronutrients such as moisture, ash, fat, protein and carbohydrate of cereal grains and black gram soya (Gowri and Bhaminy, 2019). The proximate composition and starch nutritional properties of twenty cooked lentils were studied to identify unique varieties that could be used in value-added foods (Dan *et al.*, 2020). The proximate composition such as Protein, fat, crude fiber, ash and available carbohydrates and energy of both raw taro and yam were studied (Zelalem *et al.*, 2019). Both raw taro and yam had a substantial quantity of the proximate composition and could be promising crops for securing the food supply. The proximate compositions such as moisture, crude protein, ash, crude fiber and carbohydrates of unripe plantain were studied (Adepoju *et al.*, 2012).

The results of the resistant starch, non-resistant starch and total starch content are mentioned in Table 2. From the

table, it is evident that the resistant starch content of cereals analyzed ranged from 0.16% (Foxtail millet) to 5.71% (Jowar), pulses from 1.86% (Bengal gram dhal) to 29.4% (Red gram dhal), roots and tubers from 28.41% (Potato) to 45.7% (Colocasia) and plantain contains 39.88% respectively. The results of the non-resistant starch content of cereals tested ranged from 49.63% (Maize) to 73.18% (Market rice), pulses from 5.05% (Red gram dhal) to 35.98% (Green gram dhal), roots and tubers from 2.43% (Yam) to 19.31% (Potato) and the plantain contains 17.03% respectively and the total starch is the sum of the resistant starch and non-resistant starch. The total starch content of cereals ranged from 50.1% (Maize) to 74.39% (Market rice), pulses from 23.97% (Green gram whole) to 37.22% (Lentil), roots and tubers from 33.62% (Yam) to 48.58% (Colocasia) and the plantain contains 56.91% respectively. The resistant starch (RS) content of twenty-five cereal, potato and legume products and the highest RS concentration reported in the legume group and processed potato products. Among the cereal foods, bread with enclosure of intact rye grains, barley flakes and semolina porridge has an RS level in the higher range (Liljeberg, 2002). The RS content of wheat, Kolam rice, legumes, cooked food product of cereals such as Khichdi, Puri, paratha, Chapatti

and Bhatura, legume preparations such as freshly cooked Pithle, cooked Chole and germinated Moong, Moong Usal and soaked Kabuli chana (Madhuri and Nigudkar, 2014). RS content of raw roots/tubers RS in descending order was tapioca>colocasia>sweet potato>potato>yam (Mahamood *et al.*, 2006). RS content of cereals tested is low. The non-resistant starch and total starch contents were higher in the low amylose rice than in the high amylose rice. The total starch contents of rice varied from 68.57 to 74.76%, whilst the total starch in sweet corn was only 36.23%. The highest amount was observed in the lesser yam (23.25%) followed by cassava root (9.69%). The lowest RS content was found in sweet potatoes. Non-resistant starch of lesser yam and yam bean was lowest, whilst the non-resistant starch in the remaining roots and tubers varied from 49.35% (sweet potato) to 59.61% (taro). The lowest total starch content was found in yam bean, whereas the other roots and tuber were found between 52.54 and 65.70%. They have also reported that the good source of resistant starch is green banana while the non-resistant starch varied from 19.38 to 31.99%. Total starch contents were 62.77, 66.90 and 67.45% in Musa AAA, Musa AAB and Musa ABB (Anuchita Moongngarm, 2013). The RS content within and between each food item category can be influenced by many

Table 1: Chemical composition of commonly consumed plant foods (g/100 g DW)^a.

Food sample	Moisture	Protein	Fat	Ash	Ava. CHO
Cereals					
Market rice	1.57±0.20	7.72±0.12	2.17±0.12	0.44±0.01	80.35±5.57
Barley	0.23±0.03	7.41±0.03	3.06±0.07	0.96±0.00	78.46±3.00
Bajra	2.43±0.12	9.08±0.04	6.01±0.14	1.40±0.01	59.21±1.93
Maize	1.85±0.41	9.20±0.19	6.06±0.24	1.13±0.08	66.56±2.85
Korra	1.56±0.15	11.01±0.01	5.44±0.11	1.13±0.07	59.52±1.59
Wheat	0.61±0.08	9.66±0.02	4.03±0.16	1.27±0.07	64.41±4.02
Ragi	5.39±0.16	6.99±0.10	2.80±0.11	2.16±0.00	60.37±2.82
Low GI rice	0.43±0.23	8.75±0.15	2.28±0.30	0.62±0.05	78.64±8.21
Pulses					
Jowar	7.30±0.20	8.24±0.11	3.81±0.13	1.35±0.01	68.45±4.85
Lentil	6.34±0.29	21.59±0.49	2.68±0.40	3.33±0.02	34.99±5.36
Red kidney bean	5.85±0.15	19.97±0.04	2.39±0.06	3.15±0.01	19.06±2.51
Bengal gram dhal	5.76±0.22	18.51±0.15	6.28±0.07	3.24±0.09	20.70±6.75
Bengalgram whole	5.05±0.03	16.32±0.05	6.53±0.17	2.77±0.02	41.62±0.77
Green gram whole	6.52±0.22	22.29±0.19	2.30±0.41	3.79±0.02	35.44±4.89
Cowpea	7.00±0.14	20.68±0.01	2.94±0.08	2.58±0.01	46.51±2.94
Green gram dhal	6.06±0.29	22.92±0.18	2.57±0.20	3.73±0.02	47.76±0.95
Roots and tubers					
Red gram dhal	5.11±0.73	21.72±0.01	3.55±0.29	4.06±0.15	39.99±4.48
Yam	80.35±0.0	11.87±0.14	0.30±0.05	1.38±0.02	12.81±1.17
Colocasia	74.70±0.0	7.74±0.11	0.49±0.01	1.27±0.00	16.72±0.98
Potato	78.14±0.0	7.75±0.11	0.36±0.07	1.18±0.02	11.86±0.32
Vegetable					
Plantain	84.67±0.0	6.71±0.06	0.14±0.05	0.73±0.00	11.49±1.04

^aEach value is the average of triplicate determinations. ±, One SD.

Table 2: Resistant starch content in cereals, pulses, roots and tubers and vegetables (g/100DW)^a.

Food sample	RS	NRS	TS
Cereals			
Market rice	1.211±0.13	73.18±3.275	74.39±3.391
Bajra	1.449±0.333	57.84±0.64	59.29±0.805
Ragi	1.498±0.073	55.36±0.977	56.85±1.037
Korra	0.167±0.054	65.55±2.373	65.72±2.411
Wheat	3.005±0.512	49.87±1.899	52.88±1.402
Jowar	5.717±0.082	49.82±1.716	55.54±1.779
LowGI rice	3.539±0.295	66.61±5.626	70.15±5.462
Barley	1.067±0.01	62.9±0.207	63.97±0.207
Maize	3.464±0.338	46.63±0.686	50.1±0.786
Pulses			
Red gram dhal	29.4±0.967	5.05±0.086	34.43±0.949
Bengal gram dhal	1.859±0.072	26.18±0.652	28.04±0.706
Bengal gram whole	4.638±0.248	24.51±0.914	29.15±0.974
Green gram whole	4.371±0.229	19.59±3.803	23.97±3.907
Green gram dhal	3.49±0.156	35.98±2.455	39.47±2.472
Lentil	4.65±0.401	32.57±0.613	37.22±0.704
red kidney bean	27.85±0.745	1.173±0.215	29.03±0.878
Cowpea	2.671±0.137	29.91±1.398	32.58±1.277
Roots and tubers			
Colocasia	45.7±4.227	2.883±0.237	48.58±4.424
Potato	28.41±2.358	19.31±0.249	47.73±2.588
Yam	31.19±1.379	2.431±0.376	33.62±1.08
Vegetables			
Plantain	39.88±2.764	17.03±0.702	56.91±2.906

^a Each value is the average of triplicate determinations. (±, One SD).

factors such as breeding techniques that can increase the amylose to amylopectin ratio of the starch granule to allow for RS formation during the cooling process (Diane *et al.*, 2013).

CONCLUSION

In conclusion, the carbohydrate was the major component of all cereals tested followed by pulses, roots, tubers and vegetables. The protein content showed higher in pulses followed by cereals very low percent of protein showed in roots, tubers and vegetables. The resistant starch content of all commonly consumed plant food such as cereals, pulses, roots and tubers and plantain. Pulses contain higher resistant starch content compared to cereals. The study implied that resistant starch-rich plant food could be a source of RS and could have commercial possibilities of their own or as a base with other foods. However, much investigation is still required to fully understand the application of these raw materials as natural RS sources, particularly the stability of RS during processing to be a food product. Moreover, the basic knowledge gained from this study would be useful information to design and develop new food-based products. The data about RS content in food plants could be applied to estimate the intake of RS in Thai and other consumer diets.

Conflict of interest

All authors declared that there is no conflict of interest.

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