## Original Research Article

# Preliminary study of ratio of amylose and amylopectin as indicators of glycemic index and in vitro enzymatic hydrolysis of rice and wheat starches

## Gowri Murugadass, Kavita Dipnaik*

Department of Biochemistry, Lokmanya Tilak Municipal Medical College, Sion, Mumbai-400022, Maharashtra, India

**\*Correspondence:**
Dr. Kavita Dipnaik,
E-mail: kavita.dipnaik@yahoo.com

**ABSTRACT**

**Background:** Wheat and rice form the staple food of large majority of the population throughout the world. Starch is a carbohydrate which is a homopolymer consisting of a large number of D-glucose units joined by α- glycosidic bonds. Starch can be separated into two fractions-amylose and amylopectin. Amylose, Amylopectin ratio is an indicator of Glycemic Index. Starches with lower amylose content will have higher glycemic indexes. Invitro digestion of starch provides efficient means of analysing carbohydrate digestibility and hence glycemic properties of foods. Invitro digestion indicates how a given food item is likely to behave in vivo, in terms of rate and extent of sugar release from starch, by stimulating physiological processes occurring in the mouth, stomach and small intestine. The rate of starch hydrolysis which gives rise to sustained release of reducing sugars would be preferred by the diabetics.
**Methods:** In our study, starches of wheat and rice were first isolated. Amylose and Amylopectin ratio was determined to predict the glycemic index of both. Wheat and rice having the same amylose and amylopectin ratio were selected for our study. These isolated starches were subjected to invitro enzymatic hydrolysis by salivary and pancreatic amylases. Reducing sugars released after hydrolysis and incubation of 0, 5, 10, 15, 20 and 30 minutes were estimated by Folin-Wu method. Statistical analysis was carried out in the form of unpaired student's t-test to find significant difference between means of reducing sugars release by wheat and rice during enzymatic hydrolysis.
**Results:** Having compared the starches of wheat and rice having the same amylose-amylopectin ratio, it was seen that the rice had low digestibility than the wheat starch. Rice starch releases less amount reducing sugars gradually while the wheat starch releases more reducing sugars rapidly in a short period of time. This can be due to amylopectin A which might be present in larger quantities in wheat starch than in rice starch which assists in rapid digestion of wheat starch.
**Conclusions:** Our findings revealed that rice starch released less reducing sugars gradually over a period of time while wheat starch released more reducing sugars rapidly. So, rice is the best option for diabetics for consumption because of its comparatively low release of reducing sugars as compared to wheat.

**Keywords:** Amylase, Amylopectin, Amylose, Glycemic index, Starch

## INTRODUCTION

Starch is a carbohydrate which is a homopolymer consisting of a large number of D-glucose units joined by α- glycosidic bonds. It is the most common carbohydrate in human diets and is contained in large amounts in such staple foods as potatoes, wheat, maize, rice, and cassava. The white, granular, organic chemical is a soft, white, tasteless powder that is insoluble in cold water, alcohol, or other solvents. The basic chemical formula of the

starch molecule is $(C_6H_{10}O_5)n$. Starch can be separated into two fractions–amylose and amylopectin.[1]

Amylose is a helical polymer made of α-D-glucose units, bound to each other through α(1→4) glycosidic bonds. The carbon atoms on glucose are numbered, starting at the aldehyde (C=O) carbon, so, in amylose, the 1-carbon on one glucose molecule is linked to the 4-carbon on the next glucose molecule byα(1→4) bonds.[1]

Amylopectin is a highly branched molecule, with (1→4)-linked α-D-glucosyl units in chains joined by (1→6) linkages. It consists of three types of branch chains. A-chains are those linked to other chains (B- or C-) by their reducing ends through α -D-(1→6) linkages, but they are not branched themselves. B-chains are those linked to another B-chain or a C-chain, but B-chains are branched by A-chains or other B-chains at O-6 of a glucosyl unit. Each amylopectin molecule has only one C-chain, which carries the sole reducing end of the molecule.[1]

The ability of a meal preparation to raise the blood glucose is called as glycemic index. Not all complex carbohydrates are digested at the same rate within the intestine. Hence, their hyperglycemic effect varies from immediate to a rather slower one. The glycemic index is high for bread, potatoes and table sugar and low for legumes whole grain cereals. Simple carbohydrates such as glucose, sucrose have a high glycemic index compared to complex carbohydrates such as starch. This may be because the digestion and absorption of complex carbohydrates is slow and they slowly contribute to an increase in blood sugar. When carbohydrates occur in combined form with fats, fibre and protein, the glycemic index is low.[2,3]

Amylases catalyse the hydrolysis of starch into maltose. In the digestive systems of humans and many other mammals, an alpha-amylase called ptyalin is produced by the salivary glands, whereas pancreatic amylase is secreted by the pancreas into the small intestine.

## METHODS

In this study, wheat and rice having the same amylose and amylopectin content were selected. To check the amylose and amylopectin content, first the starch was extracted using procedures described by Kurusawa et al, and Badenhuizen where the rice and wheat flour were suspended in 0.2% sodium hydroxide solution at 5ºC to remove protein. It was changed repeatedly until it gave negative biuret test. The residue was washed with distilled water and suspended in 0.1N sodium chloride solution under a toluene/xylene layer. It was agitated for 3 hours and allowed to stand overnight to remove all protein without degrading the starch. The upper layer of protein containing toluene complex was removed by decantation and discarded. The starch residue was washed with distilled water. Finally, to get the final product, the water and fat are removed using alcohol.

Ether can be used to get the dried product as it is very volatile and evaporates faster than alcohol. The fine white powdery product is subjected to standard confirmatory test i.e. iodine test to establish that it is starch.[4,5]

Then the estimation of amylose and amylopectin content of starch was carried out by Mc-Cready and Hassid method i.e. 100mg of isolated starch sample was dispersed in 10ml of distilled water. 0.5ml of 1N NaOH solution was added and the solution was warmed in a boiling water bath for about 5 minutes. It was then diluted to 100ml in a volumetric flask. 1ml of this diluted solution was transferred into 50 ml of volumetric flask and neutralised with 1ml of 1N HCl. 1ml of iodine reagent was then added and the solution was diluted to 50ml with distilled water. The blue coloured developed was read in a colorimeter at 660nm.[6]

After that, the starches were subjected to invitro enzymatic hydrolysis using salivary amylase and pancreatic amylase under optimum in vitro conditions (optimum temperature: 37ºC and optimum pH: 6.9). Reducing sugars released after hydrolysis and incubation for 0, 5, 10, 15, 20 and 30 minutes were estimated by Folin-Wu method by standard graph obtained from standardisation process.[7] Standardisation of Folin-Wu method for reducing sugars estimation was carried out using appropriate concentration range of glucose for standard graph. Statistical analysis was carried out in the form of unpaired student's t-test to find significant difference between means of reducing sugars release by wheat and rice during enzymatic hydrolysis.[8]

## RESULTS

Table 1 depicts the concentration of amylopectin and amylose in both wheat and rice are represented in g%. In both wheat and rice, amylopectin and amylose content are exactly the same. The amylopectin content in rice is 80%-85% and that in wheat is 75% according to Guan-Xing Chen et al.[9] The value obtained in this wheat and rice sample is slightly higher than the reference range. The normal range of amylose content in rice is from 3% - 20% according to Abas A et al.[10] And according to Guan-Chen X et al, wheat usually consists of 25% amylose.[9] According to Behall KM et al, compared to amylose, amylopectin led to a greater increase in blood sugar and insulin levels.[11]

**Table 1: Concentration of amylopectin and amylose in wheat and rice starch.**

| Cereal types | Amylopectin in g% | Amylose In g% |
|---|---|---|
| Wheat | 87.3 | 12.7 |
| Rice | 87.3 | 12.7 |

Table 2 shows the amylose and amylopectin ratio in wheat and rice. As, the amylopectin and amylose contents are same in wheat and rice, their ratios are also same in

both of them. Hence, both the starches have similar glycemic index.

**Table 2: Ratio of amylose and amylopectin in wheat and rice starch.**

| Cereal types | Ratio of amylose and amylopectin |
|---|---|
| Wheat | 0.137±0.007 |
| Rice | 0.137±0.001 |

**Table 3: Reducing sugars released in mg% by wheat and rice starch.**

| Time in minutes | Reducing sugars released in mg% by | |
|---|---|---|
| | WHEAT | RICE |
| 0 | 12.5±0 | 4.0±0 |
| 5 | 19.5±0.1 | 5.83±0.1 |
| 10 | 24.25±0 | 7.66±0.1 |
| 15 | 29.25±0 | 9.83±0.07 |
| 20 | 32.5±0.01 | 12.0±0 |
| 30 | 37.5±0.07 | 15.0±0 |

**Table 4: Reducing sugars released in mg% by wheat and rice starch in the following time intervals.**

| Time interval | Reducing sugars released in mg% by | |
|---|---|---|
| | WHEAT | RICE |
| 0-5 | 6.75±0 | 1.83±0.007 |
| 5-10 | 4.5±0.001 | 1.83±0.007 |
| 10-15 | 5.0±0.1 | 2.0±0 |
| 15-20 | 3.25±0.017 | 2.16±0.014 |
| 20-30 | 4.25±0 | 3.0±0 |

Table 3 highlights the reducing sugars released in mg% on enzymatic hydrolysis by salivary and pancreatic amylases by wheat and rice starch in 0, 5, 10, 15, 20, 30 minutes. Wheat starch releases reducing sugars rapidly than the rice starch, which releases the reducing sugars gradually over a period of time.

**Table 5: Statistical analysis of reducing sugars released by wheat and rice starch.**

| Reducing sugars released by wheat starch vs rice starch during time intervals | Calculated 't' value | Table 't' value | Significance* |
|---|---|---|---|
| 0 - 5 | 935.6 | 3.18 | S |
| 5 - 10 | 593.3 | 3.18 | S |
| 10 - 15 | 0 | 3.18 | NS |
| 15 - 20 | 104.8 | 3.18 | S |
| 20 - 30 | 0 | 3.18 | NS |

*Where, S = Significant; NS = Non-significant

Table 4 highlights the reducing sugars released in mg% on enzymatic hydrolysis by wheat and rice starch during the time intervals 0-5, 5-10, 10-15, 15-20 and 20-30 minutes. During the time interval wheat released high amounts of reducing sugars while rice released low amounts. As time progressed the reducing sugars released by wheat decreased while that of rice increased. So, wheat releases more sugar into the blood more rapidly while rice releases sugars gradually.

Table 5 shows the shows the statistical analysis (t-test) of reducing sugars released by wheat and rice starch.

## DISCUSSION

The wheat and rice starches selected had the same amylose-amylopectin ratio, hence their glycemic index were also same. The extracted starches were subjected to invitro enzymatic hydrolysis by salivary amylase and pancreatic amylase and the reducing sugars released in mg% during the time intervals 0-5, 5-10, 10-15, 15-20 and 20-30 minutes were estimated. It was seen that the reducing sugars released by wheat starch was highest for the time interval 0-5 minutes but subsequently falls as time progresses. But the opposite is seen in rice starch, it releases reducing sugars lowest during the time interval 0-5 minutes but subsequently increases as time progresses. This establishes the fact that wheat is digested faster than the rice, this can be due to a specific type of amylopectin i.e. amylopectin A which might be present in large amounts in wheat than in rice which helps in rapid digestion of wheat starch which in turn increases the blood sugar level rapidly as told by William Davis in his book "Wheat belly".[12]

Hence, wheat starch has higher rate of digestibility of starch as compared to rice.

Limitation: Different varieties of wheat and rice samples having different amylase-amylopectin ratio were not studied for their digestibility behaviour. Also, the digestibility behaviours of different cultivars of wheat and rice have not been studied

## CONCLUSION

Amylose and amylopectin ratio can predict the glycemic index of wheat and rice. Having compared the starches of wheat and rice having the same amylose-amylopectin ratio, it was seen that the rice had low digestibility than the wheat starch. Rice starch releases less amount reducing sugars gradually while the wheat starch releases more reducing sugars rapidly in a short period of time. This can be due to amylopectin A which might be present in larger quantities in wheat starch than in rice starch which assists in rapid digestion of wheat starch. Rice is the best option for diabetics for consumption because of its comparatively low digestibility than wheat, as sustained slow release of reducing sugars is desirable in diabetics.

## REFERENCES

1. James B, Roy W. Starch: Chemistry and technology. Food Science and Technology International Series. Edited by Academic Press-Elsevier. 2009.
2. Chawla R, El-Metwally TH, Suchanda S. Textbook of Medical Biochemistry. 2nd ed. Wolters Kluwer Publications;2017.
3. Rafi MD. Textbook for Biochemistry for Medical students. 2nd ed. Universities Press;2014.
4. Kurusawa H, Kanauti Y, Yamamoto I, Hayakawa TI. Agricultural Biological Chemistry. Taylor and Francis online. 1969;33:798.
5. Badenhuizen NP. Methods in Carbohydrate Chemistry. by RL Whistler, Academic Press Inc., New York, NY. 1964:14.
6. McCready RM, Hassid WZ. The separation and quantitative estimation of amylose and amylopectin in potato starch. J Am Chem Soci. 1943;65(6):1154-7.
7. Folin O, Berglund H. A colorimetric method for the determination of sugars in normal human urine. J Bio Chem. 1922 Mar 1;51(1):209-11.
8. Khanal AB. Mahajan's methods in biostatistics for medical students and research workers. 8th Edition, Jaypee Brothers Medical Publishers (P) Ltd;2016.
9. G Chen GX, Zhou JW, Liu YL, Lu XB, Han CX, Zhang WY, et al. Biosynthesis and regulation of wheat amylose and amylopectin from proteomic and phosphoproteomic characterization of granule-binding proteins. Scientific reports. 2016;6:33111.
10. Wani AA, Singh P, Shah MA, Schweiggert-Weisz U, Gul K, Wani IA. Rice starch diversity: Effects on structural, morphological, thermal, and physicochemical properties-a review. Comprehensive Rev Food Sci Food Safety. 2012 Sep;11(5):417-36.
11. Behall KM, Scholfield DJ, Yuhaniak I, Canary J. Studies on Diets containing high amylose vs amylopectin starch: effects on metabolic variables in human subjects. Am J Clin Nutr. 1989;49(2):337-44.
12. Davis W. Wheat belly: lose the wheat, lose the weight, and find your path back to health. Rodale Books; 2014 Jun 3.