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Effect of domestic processing methods on the starch, non-starch polysaccharides and in vitro starch and protein digestibility of three varieties of rice with varying levels of amylose

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Abstract

The effect of processing on the *in vitro* protein and starch digestibility of three varieties of rice (*Doongara, Inga* and *Japonica*) with different levels of amylose was studied. The effect of heating processes on the amount of protein, amylose, total starch, and non-starch polysaccharide (NSP) and resistant starch (RS) contents was also analysed. Results indicated a highly significant increase in the protein and starch digestibilities of rice. Pressure-cooking rendered starch and protein more digestible. The levels of amylose affected the digestibility of starch, but not protein digestibility per se. Boiling and pressure-cooking caused only small changes in the total RS and NSP contents in all the three varieties but some redistribution from insoluble to soluble components was observed.

Keywords: Starch; Resistant starch; Protein digestibility; Starch digestibility; Amylose; Non-starch polysaccharides

1. Introduction

Starch is a major component of the diets for many populations. It is composed mainly of the macromolecules, amylose and amylopectin. Starchy foods are seldom eaten raw and must undergo a heat treatment in order to become palatable and highly bioavailable. When starchy foods are heated in excess water, the structure of the starch granules is altered by the loss of crystallisation of amylopectin, followed by swelling, hydration and solubilisation. This process is called gelatinisation. The hydration of starch followed by thermal processing results in the production of retrograded starch in which the starch molecule chain becomes realigned and linked either to themselves or to other food components such as protein (British Nutrition Foundation, 1990).

Responses of starch to heating in different moisture conditions vary with the type of starch. Starch high in amylose swells more slowly than the starch rich in amylopectin, and exhibits a loss of order within the granule, followed by its destruction (Colonna & Mercier, 1985). Further, heating leads to disruption of starch complexes and the molecules adopt a more random orientation. In food systems, a certain order of the starch molecules is likely to be always present and this order is very important. Apart from imparting texture to food, it also affects starch availability when consumed. The effect of processing of starch in the food can vary considerably, affecting the digestion of the starch and thus its nutritive value.

Heat processing of food also influences protein digestibility in different ways. Firstly, by modifying the tertiary and secondary structure (''denaturation'') of the protein, thus increasing protein digestibility. Secondly, amino acid side chains may be altered, thus delaying the action of certain digestive enzymes, forming cross-links within or between molecules that lessen the digestibility of the whole protein molecule.

Rice, is an important source of starch and protein in many parts of the world. Parboiling, milling and extrusion are the common methods for industrial processing of rice, while boiling or steaming and pressure cooking are the ordinary home processing methods. There are several studies on the effect of food processing on the starch, RS, NSP content of rice (Eggum, Juliano, Perez & Acedo, 1993; Marsono & Topping, 1993; Parchure & Kulkarmi, 1999). However, no studies have been done

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on the effect of food processing on the starch and protein digestibility of rice varieties with varying levels of amylose. Hence the present study was undertaken to investigate the changes in starch and NSP due to processing and the in vitro digestibility of protein and starch of three varieties of rice of high, intermediate and low levels of amylose.

2. Materials and methods

2.1. Samples

Certified rice (*Oryza sativa*) cultivars, *Doongara*, containing high (31%) amylose; *Inga*, containing medium (20%) amylose and *Japonica*, containing low (11%) amylose were obtained from Rice Growers Co-operative Ltd. Leeton, New South Wales, Australia.

2.2. Processing of rice samples

Raw rice was boiled in tap water for 20 min (rice: water, 1:2, and w/v). Pressure-cooking of rice was done using a domestic pressure cooker at 15 psi for 10 min (rice:water, 1:2, and w/v).

2.3. Chemical analysis

2.3.1. Total and resistant starch

Determination of the different starch fractions of rice was performed by the method of Englyst, Kingman and Cummings (1992). The analysis was done under controlled enzymatic hydrolysis followed by colorimetric measurement of the glucose released. Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured following incubation with pancreatic amylase and amyloglucosidase at 37°C. The RDS is the glucose released after 20 min and SDS, the glucose released after a further 100-min incubation. Resistant starch (RS) is the starch that is not hydrolysed after 120-min incubation. Total starch (TS) was determined as the glucose released by enzymatic hydrolysis after gelatinization in boiling water and hydrolysis with potassium hydroxide to disperse the retrograded starch. Samples were analysed in triplicate and results expressed as grams of starch/100 g of dry weight of food.

2.3.2. Determination of amylose

Amylose was determined using the method developed by Martinez, (Martinez & Prodolliet, 1996). The sample was dissolved in dimethyl sulphoxide and precipitated with ethanol. The extracted sample was redissolved in ureadimethylsulphoxide and the resulting solution was defatted with ethanol to remove all lipids present in the sample. An aliquot of the lipid free solution was reacted with iodine and the absorbance of the blue-coloured amylose–iodine complex determined the iodine binding capacity of starch (Blue value). The correction factors proposed by Morrison and Laignelet (1983) was used to convert the blue value and amylose was calculated using the suggested regression equation.

$$\% \text{ amylose} = (28.414 \times \text{Blue value}) - 6.218$$

2.3.3. Insoluble and soluble NSP determination

Insoluble and soluble NSP was analysed by a modified enzymatic method suggested by Englyst and Cummings (1987) and Theander and Westerlund (1986). Samples were gelatinised with dimethyl sulphoxide (DMSO) with addition of acetate buffer and incubated with α-amylase at 95°C for 30 min then followed by incubation with pullulanase and amyloglucosidase at 40°C overnight. The insoluble NSP precipitate was hydrolysed with sulphuric acid and the constituent monosaccharides were estimated by gas–liquid chromatography as alditol acetates. The soluble NSP (in the supernatant) was precipitated with 80% ethanol and hydrolysed with sulphuric acid. Constituents of monosaccharides were determined as for the insoluble NSP.

2.3.4. Nitrogen analysis

Nitrogen was analysed using a Leco Protein Analyser (Leco FP – 428, Leco Corporation, St. Joseph, MI, and USA). A factor of 5.95 was used to compute the protein value for rice.

2.3.5. In vitro starch digestibility

In vitro starch digestibility was assessed by the method of Singh et al. (1982). The reducing sugars were estimated with dinitrosalicylic acid. The sample was dispersed in phosphate buffer and incubated with pancreatic amylase for 2 h. After the incubation period, dinitrosalicylic acid was added. Maltose was used as a standard and in vitro starch digestibility was expressed in terms of mg maltose released per gram of sample.

2.3.6. In vitro protein digestibility

In vitro protein digestibility using a multienzyme technique by Hsu, Vavak, Satterlee and Miller (1977) was followed. The multienzyme system consisted of trypsin, chymotrypsin and peptidase. In this procedure, digestibility is estimated by determining the drop in pH caused by enzymatic digestion. The drop in pH after 10 min suspension of the sample in the enzyme system was taken as % protein digestibility.

2.4. Statistical analysis

Results obtained were analysed by one way analysis of variance (ANOVA), followed by LSD to test for differences, using SPSS/PC+ program, version 7.

3. Results and discussion

3.1. Total starch content

No significant differences in starch content (Table 1) were observed between raw and boiled *Doongara* rice while raw and pressure-cooked rice differed significantly. Significant differences ($p < 0.05$) were found between raw, boiled and pressure cooked, *Inga* and *Japonica* varieties. These differences are probably explained by the gelatinisation effect brought on by the effect of heat on starch in excess water. This rendered the starch more susceptible to α-amylase, because of the swelling of the amorphous phase of the granule, contributing to the disruption of the crystalline region.

3.2. Effect of processing on the amount of resistant starch

Results showed (Table 1) that raw rice had the highest amount of resistant starch. The high amount of resistant starch in raw rice can be attributed to the nature of the starch, which is of the B-type crystal structure, which is highly resistant to α-amylase (Hibi, Kiramura & Kuge, 1990). The processes of boiling and pressure-cooking produced an appreciable amount of resistant starch in *Doongara* and *Inga* rice. Boiling compared to pressure-cooking resulted in increased resistant starch in *Japonica* though not significant. The amylose content, amount of water and the processing temperature used during cooking influence the formation of resistant starch. Up to 2.8% of resistant starch was found in pressure-cooked *Doongara* rice which contained high amylose (31% amylose) in the raw state and 1.6% of resistant starch in boiled and pressure cooked *Inga* which is a medium amylose (20% amylose) rice. It was observed that rice with a high amylose content in the raw state formed more resistant starch on processing compared to the low and intermediate amylose rices due to the presence of higher amounts of the linear component of starch. When this starch is gelatinised or pasted in water and allowed to cool, the amylose molecules align themselves or associate with each other and form a rigid gel (retrogradation). Resistant starch is produced as the insoluble crystallite formed by the process of controlled retrogradation (Eggum et al., 1993).

Table 1
Total starch and resistant starch contents of rice (g/100g dry weight)[a]

Rice	*Amylose*	TS[b]	RDS[c]	SDS[d]	RS[e]
Doongara					
Raw	31.1a	82.1b	12.7a	56.5b	12.9c
Boiled	30.8a	81.8b	40.0c	39.6a	2.2a
Pressure cooked	32.0a	80.0a	37.2b	40.0a	2.8b
Inga					
Raw	20.2a	83.6c	21.7a	52.7b	9.2b
Boiled	20.0a	81.0b	47.6c	31.8a	1.6a
Pressure cooked	20.7a	78.2a	45.3b	31.3b	1.6a
Japonica					
Raw	11.5b	83.2c	60.1a	14.6a	8.6b
Boiled	10.4a	81.6b	63.3b	17.3b	1.0a
Pressure cooked	11.5b	79.7a	61.4a	17.4b	0.8a

[a] Values are means of triplicate analyses. Mean values within the columns with different letters are significantly different at $p < 0.05$.
[b] TS – Total starch.
[c] SDS – Slowly digestible starch.
[d] RDS – Rapidly digestible starch.
[e] RS – Resistant starch.

3.3. Effect of processing on the amylose content of rice

The process of boiling consistently decreased the amount of amylose in all rice varieties. The decrease was significant in Japonica. It may be during cooking that amylose can be leached out from the intact starch granule, leading to a change in the proportion of amylose and amylopectin (Schweizer, Reimann, Solms, Eliasson & Asp, 1983). Pressure-cooking was observed to increase the amylose contents of *Doongara* and *Inga*.

3.4. Effect of processing on the non-starch polysaccharide (NSP) content of rice

Table 2 shows the amount of NSP in the three varieties of rice. Boiling and pressure-cooking did not have a marked increase on the amount of total NSP in *Doongara* and *Inga* excepting in *Japonica*. However, changes were seen in the soluble and insoluble fractions. There was a significant increase in the soluble NSP contents from the raw in all the three varieties of rice on boiling and pressure-cooking (*Doongara*, raw: 1.2 g/100 g, boiled: 1.6 g/100 g, pressure-cooked: 1.6 g/100 g;

Table 2
Non-starch polysaccharide (NSP) content of rice (g/100 g dry wt basis)[a]

Rice	Soluble NSP	Insoluble NSP	Total NSP
Doongara			
Raw	1.2a	0.6b	1.8a
Boiled	1.6b	0.3a	1.9a
Pressure-cooked	1.6b	0.4a	2.0a
Inga			
Raw	1.0a	0.6b	1.6a
Boiled	1.3b	0.3a	1.6a
Pressure-cooked	1.6c	0.2a	1.8a
Japonica			
Raw	0.5a	0.5a	1.0a
Boiled	1.4b	0.4a	1.8b
Pressure-cooked	1.3b	0.5a	1.9b

[a] Values are means of triplicate analyses. Mean values within the columns for each rice variety with different superscripts are significantly different at $p < 0.05$.

Inga, raw: 1.0 g/100 g, boiled: 1.3 g/100 g, pressure-cooked: 1.6 g/100 g and *Japonica*, raw: 0.5 g/100 g, boiled: 1.4 g/100 g and pressure-cooked: 1.3 g/100 g). A corresponding decrease in the insoluble fraction was noticed excepting in the case of *Japonica*. While in *Japonica* the insoluble NSP was not affected by processing, it was the soluble NSP that was significantly increased during boiling and pressure-cooking. Excepting in *Japonica*, it is quite clear from our study that boiling and pressure-cooking caused only small changes in the total NSP content, but some redistribution from insoluble to soluble components was observed.

3.5. Effect of processing on the protein content of rice

Protein contents in *Doongara* (raw: 8.6 g/100 g) and *Inga* (raw: 7.6 g/100 g) did not show a significant change when cooked (boiled *Doongara*, 8.2 g/100 g; pressure-cooked, 8.3 g/100 g; boiled *Inga*, 7.5 g/100 g; pressure-cooked, 7.5 g/100 g) excepting in *Japonica* (raw: 7.4 g/100 g) in which a significant decrease was observed on boiling (6.8 g/100 g) and pressure-cooking (6.7 g/100 g).

3.6. Effect of processing on the in vitro starch digestibility

Results of *in vitro* starch digestibility (Fig. 1) showed that *Doongara* variety (raw, 25.8%) was rendered more digestible when subjected to boiling (54.3%) and pressure-cooking (65.2%); *Inga* (raw, 28.2%) was more digestible when boiled (70.2%) and pressure-cooked (67%) and *Japonica* (raw, 35.6%) was more digestible when boiled (74.6%) and pressure-cooked (75.1%). Boiling and pressure-cooking in general, caused significant increases ($p < 0.05$) in starch digestibility in all the three varieties when compared to raw. Between the varieties, *Doongara* had lower starch digestibility when processed compared to *Inga* and *Japonica* as a result of its high amylose content.

The results showed positive correlation between the amount of resistant starch and amylose content, which

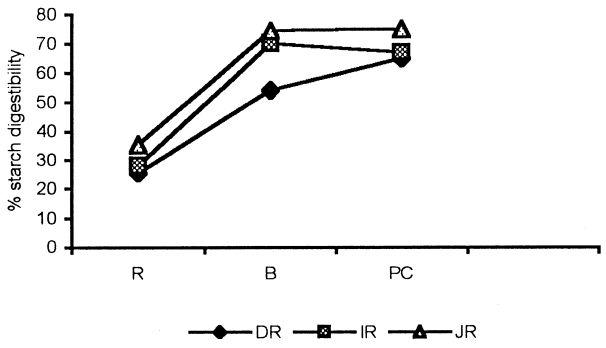

Fig. 1. Effect of processing on the in vitro starch digestibility of three varieties of rice.

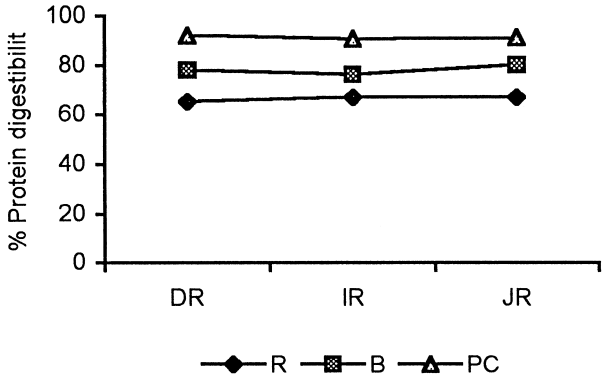

Fig. 2. Effect of processing on the in vitro protein digestibility of three varieties of rice.

may be attributed to the content and chain length of the amylose component of the starch that causes the low digestibility (Rao, 1976). This observation was also made by Berry (1986) and Sievert and Pomeranz (1989).

The starch digestibility in rice is affected overall by the degree of gelatinisation during cooking, the granule particle size, the amylose/amylopectin ratio, starch protein interaction, amylose/lipid complexes and the level of resistant starch. In our study the marked improvement of starch digestibilities in processed rice may be attributed to the gelatinization of starch granules, characterised by irreversible swelling of the granules, increase in viscosity as the order and crystallinity of the starch molecules are broken down by heat allowing more water penetration and hydration of the granules (Juliano, 1984). The significant increase in RDS and corresponding decrease in the amount of SDS rendered the samples more digestible.

3.7. Effect of processing on the in vitro protein digestibility

The overall protein digestibility of the three varieties of rice was significantly improved through processing. Boiling improved the protein digestibility, with a range of 76.5–80.5 and 90.9–92.2% for pressure-cooking (Fig. 2). The increase in protein digestibility of rice by boiling and pressure-cooking may be attributed to the inactivation of proteinase inhibitors and the opening up of the protein structure through denaturation. Cooking could have destroyed the anti-nutritional factors present in rice and rendered rice protein more digestible. This study revealed an in vitro protein digestibility of 76.5–80.5% on boiling, which is close to the reported in vivo value of 85% in humans (Eggum, 1973).

4. Conclusion

The results of the study clearly indicated that there was a significant decrease in the total starch contents on

pressure-cooking of all the three varieties. Both boiling and pressure-cooking significantly increased the levels of RDS in all three varieties. A corresponding significant decrease in SDS and RS was noticeable. The variety *Doongara* showed higher levels of RS compared to the other two varieties explaining that the level of RS is influenced by a number of factors such as: amylose content, amount of water used for cooking and the processing temperatures. The increase in RDS on processing improved the starch digestibility significantly. The boiling process seemed to decrease the amylose contents in all the three varieties and significantly in the low-amylose *Japonica* variety. Pressure-cooking increased the amylose content of *Doongara* and *Inga*. This could be attributed to the process of retrogradation. While there were no significant changes in the total NSP levels in all three varieties, a re-distribution in the soluble and insoluble fractions were observed. Protein digestibility improved on processing in all three varieties of rice. The presence of RS and NSP did not have an effect on the digestibility of protein and starch. However the level of amylose affected starch digestibility.

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