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ORIGINAL ARTICLE

Processing and storage of Indian cereal and cereal products alters its resistant starch content

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Abstract Resistant Starch (RS) is prebiotic in nature and is defined as the sum of starch and products of starch degradation not absorbed in small intestine of healthy individuals but later are fermented by natural microflora of the colon to produce short chain fatty acids. RS acts as a nutraceutical and its consumption leads to many health benefits. The aim of the study is to analyze the RS content in raw and processed cereals and cereal products and determine various processing and storage effects on the RS content. RS content in raw cereals ranged from 0.53 g% (pearl millet)—2.09 g% (rice). Of all the processing techniques applied in the study, roasting, baking and boiling increased the RS content followed by shallow frying. Steaming and frying showed a decrease in RS content. The puffed, flaked and extruded cereal products obtained from market when analyzed also showed very less retention of RS content. Storage of different cereal products at 4°C up to 12 and 24 h significantly increased RS content. Amylose showed a higher correlation with RS in maize produces than in other cereal products.

Keywords Resistant starch · Amylose · Cereals · Processed cereals · Processing · Storage

Introduction

Cereals occupy an important place in human nutrition globally. They are rich and inexpensive source of carbohydrate (55–75%) and protein (7–12%) and contribute substantially to the energy intakes. Cereals play an important role in the diets of individuals suffering from metabolic disorders. Grains are major sources of Resistant Starch (RS) and dietary fibers which could explain in part their beneficial role in humans. RS is defined as the sum of starch and products of starch degradation not digested and absorbed in small intestine of healthy individuals but later are fermented by natural microflora of the colon to produce shortchain fatty acids (Berry 1986). RS has been categorized as RS_1, RS_2, RS_3 and RS_4. RS_1 represent tightly bound starch molecules wrapped in fiber shell that is physically inaccessible to digestive enzymes (Bird et al. 2000; Sajilata et al. 2006) and is found in partly milled grains and seeds. RS_2 is ungelatinized starch granule, which is unavailable to amylotic enzymes due to its compact and unhydrated structure. RS_3 is the retrograded or recrystallized starch and is found in most of the heat processed and cooled foods (Muir and O'Dea 1992; Sajilata et al. 2006). RS_4 is the chemically modified form including the esterified and cross bonded starch that cannot be broken down, as the modification process renders the structure inaccessible to digestion by α amylase (Sajilata et al. 2006). RS has assumed importance in our daily diets due to its various beneficial health properties mostly mediated by short chain fatty acids produced during its fermentation in the large intestine (Premavalli et al. 2006, Sharma et al. 2008). RS may be either present naturally in foods or may be generated during various processing, cooking or storage conditions. Formation of RS during processing of starchy foods is affected by various factors such as water content, pH, heating temperature and time, number of heating and cooling cycles, freezing, drying and presence of added additives such as oil, spices, and salts. RS formation also depends on amylose/amylopectin ratio, starch protein interaction, amylose lipid complexes and rate of starch

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retrogradation (Englyst et al. 1992; Bravo et al. 1998; Mahadevamma and Tharanthan 2004). Few studies have been carried out to determine the RS content of raw and processed roots and tubers, cereals and legumes (Kavita et al. 1998; Mahadevamma and Tharanthan 2004; Katyal et al. 2005; Mahmood et al. 2006; Yadav et al. 2010). However limited literature is available on the RS content of popularly consumed cereal based food products of Gujarat, India. Therefore the present study aimed at analyzing the RS content of raw cereals and their processing and storage changes.

Materials and methods

Sample collection Four cereals—wheat (*triticum aestivum*) (GW1 variety), rice (*orzya sativa*) (GR11 variety), maize (zea mays) (Narmada Moti variety) and pearl millet (*pennisetum americanum*) (GHB526 variety) were collected from 4 different Research stations located in Arnej, Anand, Navagam and Panchmahal districts of Gujarat state, India. All the grains procured were 6–10 months old and were collected in clean sample collectors. The flours from wheat, rice, maize and pearl millet were prepared by using Navdeep flour mill (80 meshes).

Methods of processing A total of 18 products were processed with different domestic treatments commonly applied in Indian households. *(a) Boiling*: Broken wheat was prepared by milling wheat grains coarsely in Dalia milling machine at the Anand Agriculture Research station with the grain size 1–1.5 mm. *Daliya* (sweet porridge) was prepared by adding 10 g sugar and 150 ml milk in 20 g of broken wheat and boiled for 10 min. Rice was boiled with filtered tap water (1:2 w/v) for 15 min. *(b) Steaming*: *Khichhu* (steamed rice flour) is a traditional recipe cooked in filter tap water (1:2 w/v) with added salt, green chili and cumin seeds for 15 min. *Muthiya* (steamed wheat flour) is a traditional Gujarati snack recipe. Coarse wheat flour was taken with filtered tap water (1:2 w/v) and salt and spices were added for required taste. The mixture was cooked for 20 min. *(c) Roasting:* Unleavened bread was prepared from wheat flour (*Chapati*), Maize flour (*Rotla*) and pearl millet flour (*Rotla*) at 150–200°C for 1–2 min. *(d) Shallow frying: Paratha* is shallow fried unleavened bread prepared from wheat flour and *Thepla* is shallow fried unleavened bread from pearl millet flour. *Paratha* and *Thepla* were prepared by shallow frying in 5 ml of sunflower oil each at 150–200°C for 2 min. *(e) Frying: Puri* was prepared by rolling the small sized wheat dough (100 g wheat flour with 20 ml water and 10 ml of oil) followed by deep frying for 20 s until puffed. *Dhebra* was prepared in the similar manner as *puri* except that the 100 g pearl millet dough is added with 10 g curd, 5 g jaggery and spices and salt for taste. *Rice papadi* is a dry crunchy side dish of Indian meal and is prepared from rice flour. *Papadi* was prepared by adding filtered tap water (50 ml) to rice flour (100 g) containing salt, green chili and cumin seeds. The mixture was steamed and was smeared by cotton seed oil till it become soft and non sticky. *Rice Papadis* were rolled and dried in sun for 12–16 h and then stored. Before consumption the dry *Papadis* were deep fat fried for 30 s with excess oil drained off. *(f) Popping:* Maize was popped for 1 min in a heavy bottom covered vessel.

(g) Puffing and Flaking: Commercially available rice flakes and puffed rice were collected from local market.

(h) Extrusion: Commercially available noodles was collected from market. It was further boiled and analyzed for its RS and amylose content.

Sample preparation of processed foods All the products were dried in a hot air oven at temperature ranging between 80–100°C until two constant readings were obtained (AOAC 1990). The samples were then powdered and stored in air tight containers until analysis. The freshly processed samples were immediately dehydrated and analyzed for its RS content and the same samples stored at 4°C for 12 and 24 h were analyzed for RS content after dehydrating.

Analytical procedures All the 4 replicate samples were analyzed in duplicates for the following parameters.

Moisture determination Moisture content of the cereals was determined using IR moisture analyzer (AOAC 1990) in which 2 g of sample was kept for 2–3 min at 130°C.

Amylose determination Amylose content was determined by the method of Juliano et al. (1981). Amylose was extracted and solubilized by treating the samples with glacial acetic acid and sodium hydroxide. The mixture was further diluted and treated with Iodine solution and the colored complexes formed were measured at 620 nm in Spectronic 20D+ and compared with amylose standard.

Resistant starch (RS) determination Finely powdered cereals and cereal products were centrifuged with 80% alcohol and treated with 1 ml pepsin (Sigma Aldrich Co, Bangalore) to remove protein and 3 ml α amylase (30U α amylase in 1 ml tris maleate buffer) to remove digestible starch. α amylase is taken from the source A 3176 Type IV B from porcine pancreas, Sigma Aldrich Co. Bangalore). After centrifugation residues were dispersed in 2 M KOH and amyloglucosidase [(6U/ml) Fluka 10115 from *Aspergilus Niger*, Sigma Aldrich Co. Bangalore]. The liberated glucose was quantified by glucose oxidase assay method. RS was calculated as glucose x 0.9 (Champ et al. 1995).

Statistical analysis Statistical analysis was performed using Statistical Package for Social Sciences (SPSS 15.0v) software. Results were expressed as mean values ± standard deviations of cereals and cereal products. Student t test was performed to determine the effect of processing between raw and processed products. ANOVA was performed to determine the storage effect on amylose and RS content of cereals and cereal products. Pearson Correlation was performed to examine the correlation between amylose and RS.

Results and discussion

Effect of processing on RS and amylose content of various cereal products The RS and amylose content of raw and processed cereals analyzed are shown in Table 1. The RS content of raw cereals ranged from 0.53 g% in pearl millet to 2.03 g% in rice. Different processing methods used in the study showed a significant difference between the RS content of raw and processed cereals.

Roasting showed a significant increase in RS content by 1.3, 1.5 and1.7 fold in maize *rotla, chapatti* and pearl millet *rotla* respectively (Table 1). Dry heating has a detrimental influence on the starch digestibility which might be due to the trans glycosidation reactions. These chemical alterations of starch takes place under conditions like roasting and shallow frying leading to formation of atypical glycosidic bonds and the concomitant reduction in amyloytic susceptibility resulting in formation of RS (Siljestorm et al. 1989). The study also showed 1 fold increase in amylose content upon roasting which may also be one of the factors for increased RS content in cereals on roasting. The RS content of unleavened bread, *chapatti* estimated in the present study was 2.90 g%. It was slightly higher than that reported by Kavita et al. (1998) and Platel and Shurpalekar (1994) which showed 0.50 g% and 1.90 g% respectively. Both the studies were carried out in Mysore, India and difference in the RS content may be due to the varietal differences and the climatic conditions (Vaidya and Sheth 2010).

As shown in Table 1, boiling resulted in a non significant increase in both *daliya* (broken wheat porridge) and rice by 1 fold (2.06 g%). Tovar and Melito (1996) and Platel and Shurpalekar (1994) also showed the similar trend in RS content of boiled rice. Upon steaming RS content of rice flour (*khichhu*) decreased by 1 fold (1.97 g%) whereas steamed wheat flour, *muthiya* (1.88 g%) retained its RS content as the raw wheat (1.86 g%). During cooking starch granules are gelatinized and partly solubilized becoming available to digestive enzymes. Because these samples were analyzed immediately after cooking, it should be assumed that no retrograded starch was formed. However RS content is also affected by starch-protein interaction resulting a fraction of starch not digested by amyloytic enzymes in these processed cereals. Wheat having high protein content than rice, the starch-protein interactions takes place in wheat. The RS content retention was high in wheat whereas it decreased in rice upon steaming.

Baking bread increased the RS content in wheat by 1.7 fold (2.90 g %). It is similar to that of roasting *chapatti*. Liljeberg et al. (1996) showed that the lactic acid during dough preparation and subsequent baking promotes starch retrogradation in bread. The formation of RS takes place within hours after gelatinization of starch. It involves recrystalization of the amylose fraction where as staling involves retrogradation of amylopectin. The slow retrogradation of amylopectin is due to difficulties with which bulky molecules crystallize. On other hand, linear amylose molecules associate very easily and therefore retrograde easily. Englyst et al. (1983) also showed high RS content on baking and supported the results of present study.

Shallow frying shows very minimal difference in RS content of wheat flour (2.15 g %) but *thepla* made out of pearl millet showed high RS content (1.27 g %) than the raw pearl millet (0.53 g %). The increased RS content in *thepla* may be due to interferance of other ingredients like jaggery, oil and curd lowering the starch digestibility. Frying showed non significant decrease in RS content by 1.3 fold in *puri* and *dhebra*. Frying of sundried *rice papadi* and cornflakes decreased RS content by 6 folds.

The amylose content also similarly decreased on frying in the present study. Mahadevamma and Tharanthan (2004) reported that addition of cooking oil considerably lowered the net RS content. The FFA in the oil may form an inclusion complex with the amylose helix thereby decreasing the content of amylose available for retrogradation and subsequent RS formation. Mahadevamma and Tharanthan (2004) and Platel and Shurpalekar (1994) also showed decrease in RS content on frying.

In the present study, popping showed low RS content (1.69 g%). The rice flake and puffed rice obtained from market also showed low RS content (1.29 g% and 1.18 g% respectively. The products obtained by popping, puffing and flaking are generally completely cooked and serve as ready to eat snack food. During this process starch becomes fully gelatinized, thereby enhancing the digestibility and lowering the amount of RS compared with other thermally processed products (Mahadevamma and Tharanthan 2004).

Extrusion cooking is becoming increasingly popular in food processing technology. During this process, the food components undergo total disintegration (Colonna et al. 1984) and starch becomes fully gelatinized, resulting in a possible decrease in molecular entanglement and therefore RS formation is less compared to other processing methods. In the present study noodle from market were

Table 1 Effect of processing on amylose and RS content in cereals and cereal products

Processing methods	Amylose (Raw)	Amylose (Processed)	t test	RS (Raw)	RS (Processed)	t test
Roasting						
Chapatti (wheat flour)	28.5±1.50	28.9±1.29	0.43 NS	1.9±0.05	2.9±0.74	2.80*
Rotla (pearl millet flour)	20.2±0.29	21.4±0.95	2.14 NS	0.53±0.03	0.91±0.25	3.04*
Rotla (maize flour)	26.2±1.73	32.3±0.47	6.49**	1.7±0.06	2.3±0.36	3.13*
Boiling						
Daliya (broken wheat)	28.5±1.50	26.2±0.78	8.25**	1.9±0.05	2.1±0.30	1.31 NS
Rice	29.3±0.94	30.9±0.72	2.73*	2.0±0.10	2.1±0.58	1.81NS
Steaming						
Khichhu (rice flour)	29.3±0.94	32.4±0.59	4.25**	2.0±0.10	1.9±0.12	0.84 NS
Muthiya (wheat flour)	28.5±1.50	26.6±0.61	2.28 NS	1.9±0.05	1.8±0.13	0.21 NS
Baking						
Bread (wheat flour)	28.5±1.50	31.3±0.95	2.98*	1.9±0.05	2.9±0.31	6.53***
Shallow frying						
Paratha (wheat flour)	28.5±1.50	28.5±0.56	0.04 NS	1.9±0.05	2.2±0.44	1.29 NS
Thepla (pearl millet flour)	20.2±0.29	23.8±0.50	11.6***	0.53±0.03	1.3±0.32	4.52**
Frying						
Puri (wheat flour)	28.5±1.50	21.3±0.59	8.56***	1.9±0.05	1.4±0.48	2.12 NS
Dhebra (pearl millet flour)	20.2±0.29	14.2±0.53	19.8***	0.53±0.03	0.65±0.19	2.23 NS
Rice papadi (rice flour)	29.3±0.94	17.9±0.64	19.2***	2.0±0.10	0.03±0.04	36.9***
Corn flakes (maize)	26.2±1.73	26.7±0.90	0.52 NS	1.7±0.06	0.28±0.18	15.1***
Popping						
Popcorn (maize)	26.2±1.73	29.1±0.83	2.88*	1.7±0.06	1.6±0.33	0.22 NS
Puffing						
Murmura (puffed rice)	–	34.2±0.57	–	–	1.3±0.48	–
Flaking						
Poha (rice flakes)	–	34.2±0.91	–	–	1.2±0.31	–
Extrusion & Boiling						
Noodles (wheat flour)	–	12.4±0.52	–	–	1.7±0.74	–

[The results for processed foods are expressed on dry weight gram % basis. Each value is expressed as mean±SD of 4 replicate done in duplicates; Different alphabets as prefixes shows significant difference;* $P<0.05$; ** $P<0.01$***. $P<0.001$]

analyzed for its amylose and RS content and it showed comparatively lower RS content in wheat (1.72 g %) than the raw. Camire and Flint (1991) reported low RS content during extrusion cooking where as 2 fold increase in RS content was observed in extrusion cooked bengal gram by Mahadevamma and Tharanthan (2004).

Effect of storage on RS content of cereal and cereal products The effect of storage on RS content of cereals and cereal products are shown in Table 2. During cooking starch granules are gelatinized and partly solubilized becoming available to digestive enzymes and no retrogradation takes place. The formation of RS 3 which is retrograded amylose takes place within hours after heating, during cooling period. It involves recrystalization of the amylose fraction (linear-(1,4)-α-D-glucose) and retrogradation of amylopectin (branched (1,4),(1,6)- α-D-glucose). The linear amylose molecules associate very easily and therefore retrograde easily than amylopectin (Sajilata et al. 2006). In our experiment, the samples that showed less RS content when analyzed immediately after cooking had a relative increase in RS formation on storing at 4°C during 12 and 24 h. The wheat products (*chapatti* and bread) showed a significant increase of 1 fold in RS content when stored at 4°C for 12 h (3.5 g % and 3.03 g % respectively) while a further increase of 1 fold in RS content was observed when analyzed after 24 h (3.7 g % and 3.50 g % respectively). The similar results were observed by Bravo et al. 1998. *Muthiya* (steamed wheat flour) and *khichhu* (steamed rice flour) which had less RS content when estimated immediately after cooking significantly increased after storage at 4°C for 12 h and 24 h (Table 2). The lower RS content in freshly cooked samples reflects better amylose and amylopectin availabilities. Upon cooling and

Table 2 Effect of storage on RS content in cereals and cereal products

CEREALS	0 h (g %)	12 h (g %)	24 h (g %)	F test
WHEAT				
Chappati	2.9±0.74^{a}	3.5±0.43^{ab}	3.7±0.39^{b}	5.37*
Paratha	2.2±0.44	2.5±0.40	2.6±0.37	1.37^{NS}
Puri	1.4±0.38	1.5±0.39	1.6±0.51	0.23^{NS}
Muthiya	1.9±0.13^{a}	3.6±0.64^{b}	3.7±0.19^{b}	10.63**
Daliya	2.1±0.30^{a}	2.1±0.4^{b}	2.9±0.12^{b}	17.12**
Noodles	1.7±0.74	1.9±0.21	2.1±0.20	0.82^{NS}
Bread	2.9±0.31^{a}	3.0±0.16^{ab}	3.5±0.23^{b}	11.88**
RICE				
Rice	2.1±0.58^{a}	3.9±0.31^{ab}	4.1±0.28^{b}	15.91**
Khichhu	1.9±0.12^{a}	3.9±0.25^{b}	3.8±0.17^{c}	67.27***
Papadi	0.03±0.04	0.06±0.02	0.08±0.03	2.01^{NS}
MAIZE				
Rotla	2.3±0.36^{a}	2.9±0.23^{ab}	3.1±0.17^{b}	9.83**
Popcorn	1.7±0.33	1.7±0.20	1.9±0.52	0.88^{NS}
Corn flakes	0.28±0.18^{a}	0.69±0.61^{ab}	1.2±0.47^{b}	7.15**
PEARL MILLET				
Rotla	0.9±0.25^{a}	1.3±0.07^{b}	1.5±0.09^{c}	12.97**
Thepla	1.3±0.32	1.5±0.60	1.7±0.42	1.18^{NS}
Dhebra	0.67±0.26	1.04±0.51	1.2±0.20	2.65^{NS}

[The results for processed foods are expressed on dry weight gram % basis. Each value is expressed as mean±SD of 4 replicate done in duplicates; Different alphabets as prefixes shows significant difference;* P<0.05; ** P<0.01***. P<0.001]

storing these amylose and amylopectin chains undergo retrogradation resulting in high RS contents (Kavita et al. 1998). The shallow fried and fried wheat products (*paratha and puri*) also showed an increase in RS content on storage for 12 and 24 h but had no significant difference. Similarly *rice papadi* (sundried and later fried rice product) and *thepla* and *dhebra* (shallow fried and fried pearl millet products) also had no significant difference in RS content on storage (Table 2). This may be due to lack of availability of starch for retrogradation as most of them form starch lipid complexes during processing. Again there showed a slight increase of RS content upon storage of popcorn but it

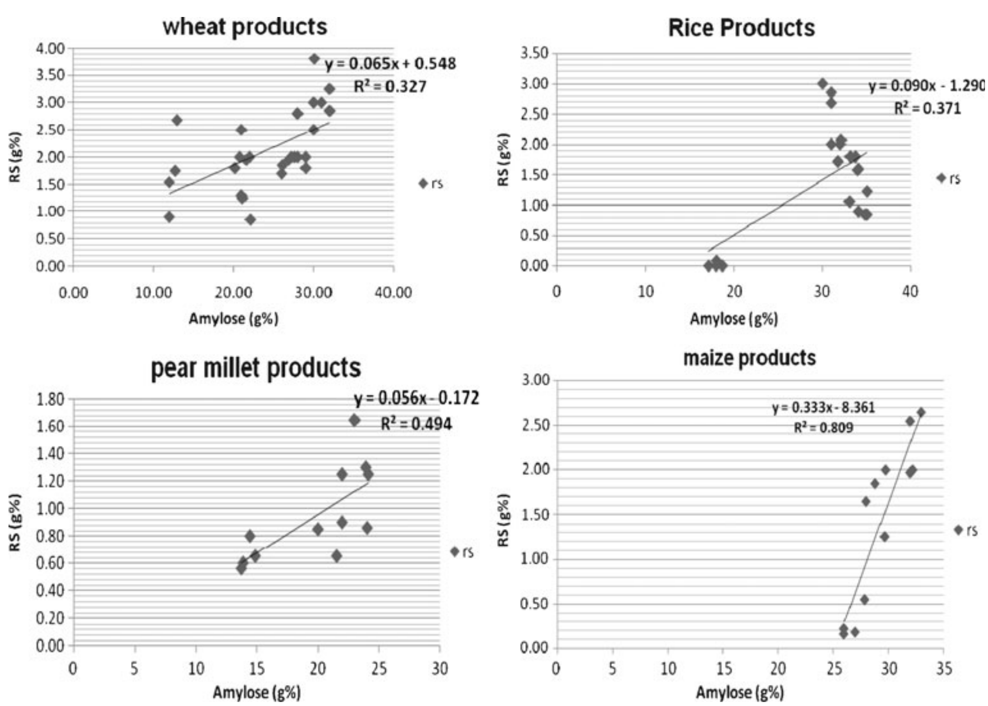

Fig. 1 Relation of amylose and resistant starch in different processed cereal products

did not differ significantly. Englyst and Hudson (1996) revealed that starchy products when undergo moist heating process and cooled undergo more retrogradation than dry heating.

The nature of starch in terms of amylose and amylopectin is an important factor for the starch digestibility and in turn affect the formation of RS. The starch consists of 2 main structural components, amylose and amylopectin. Amylopectin has a much larger surface area per molecule than amylose. Furthermore, the glucose chains of amylose starch are more bound to eachother by H^+ bonds making them less available for amylitic attack than amylopectin, which has many branched chains of glucose. The difference in the rate of digestibility between amylose amylopectin starches may be due to larger surface area of amylopectin (Thorne et al. 1983). We also tried to observe the effect of amylose content on the RS of cereal products. A strong association of amylose and RS content is observed in maize products but very weak positive association is seen in wheat, rice and pearl millet products (Fig. 1).

Conclusion

Depending on the cereal variety and type of processing applied, the RS content of cereals varied largely. Of all the processing techniques applied to study the alterations in RS content of various cereal products, roasting, baking and boiling not only retained the maximum RS but showed an increase in its content followed by shallow frying. Steaming and frying showed a decrease in RS content. The puffed, flaked and extruded cereal products obtained from market when analyzed also showed very less retention of RS content. Storage of different cereal products at 4°C upto 12 and 24 h significantly increased RS content. Amylose showed a higher relation with RS in maize produces than in other cereal products. Therefore depending on the use of foods, an appropriate processing method could be recommended. Considering malnutrition, processing methods leading to highest starch digestibility and low RS formation should be used. In turn, diabetic patients should consume cereals with high RS content eliciting a low postprandial glycemic response with the appropriate processing methods.

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