

Effect of storage on resistant starch and amylose content of cereal–pulse based ready-to-eat commercial products

Mohana Kumari^a, Asna Urooj^{a,*}, N. Narayan Prasad^b

^a Department of Studies in Food Science and Nutrition, University of Mysore, Mysore 570006, India

^b Department of Biochemistry and Nutrition, Defence Food Research Laboratory, Siddhartha Nagar, Mysore 570011, India

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Abstract

A wide range of ready-to-eat (RTE) foods, with varied shelf life are commercially available to meet the increasing demand for convenience foods, both by the Armed Forces and the public at large. The study evaluated the effect of storage on the resistant starch (RS) and amylose content of selected ready-to-eat (RTE) cereal–pulse based processed foods viz., *pongal*, *khara bhath*, *dal fry*, *bisibele bhath*, *rajmah* and *kesari bhath*, developed by Defence Food Research Laboratory, Mysore. RS was quantified directly in the residues obtained after removing digested starch in simulated physiological conditions. Nutrient composition and carbohydrate profile of the foods were also analyzed. The carbohydrate profile indicated low amounts of sugars, except in case of *kesari bhath*. The total starch content ranged from 14.5 to 24 g% while amylose ranged from 1.2 to 7.2 g%, respectively. The total and resistant starch in the RTE foods varied depending on the ingredients used and type of processing. Foods containing higher amylose content were found to have maximum increases in RS content after storage. Storage at ambient conditions resulted in significant increases ($p < 0.05$) in RS and TS content of RTE foods. The findings reveal that the RTE foods studied hitherto contained appreciable quantities of RS, which further increased on storage. © 2006 Elsevier Ltd. All rights reserved.

Keywords: Processed foods; Resistant starch; Amylose; Storage

1. Introduction

Starch in food may resist digestion in the small intestine based on the type of contained starch and processing conditions employed (Berry, 1986; Englyst & Cummings, 1987). The starch molecule undergoes several physical modifications during processing. The gelatinized starch particularly the amylose fraction, may revert to a form which could be highly resistant to hydrolysis with α -amylase (Annison & Topping, 1994) and is called Resistant Starch (RS). Based on the diverse physiological property and in relation to the *in vitro* digestibility characteristics, starch has been classified nutritionally as rapidly digestible, slowly digestible and resistant starch (Englyst, Kingman, &

Cummings, 1992). Resistant starch (RS) has attracted greater interest amongst both the nutritionists and food industry, due to its contribution to the non-digestible carbohydrate component of the diet and the physiological implications viz., colonic fermentation, post-prandial glycemia, faecal bulking, intestinal transit time and energy value of foods (Abia, Buchanan, Saura-Calixto, & Eastwood, 1993; Annison & Topping, 1994; Danone, 2001; Gee, Johnson, & Lind, 1992; Livesey, 1990).

RS may be found in both unprocessed foods such as raw potatoes and bananas and in processed foods. Processed foods invariably undergo storage at moderate or low temperatures before consumption. Storage of foods is also a contributing factor to the changes in the available starch content of the product. The quantity of RS formed, during processing/storage depend on the severity of the processing conditions like temperature, pH, moisture, number of heating/cooking cycles adopted, condition of storage, etc. In

* Corresponding author. Tel.: +91 9448489334; fax: +91 821 2419301/2419363.

E-mail address: asnaurooj@foodsci.uni-mysore.ac.in (A. Urooj).

addition, many of the processing treatments such as freezing autoclaving, etc., are also known to have significant impact on the fermentation of RS (Goni, Garcia-Diz, Manas, & Calixto, 1996; Siljestrom, Eliason, & Bjorck, 1989 & Sievert & Pomeranz, 1989).

In view of the increasing interest in the physiological and functional characteristics of RS and its relation to the processing conditions adopted in the development of ready-to-eat (RTE) or easy-to-reconstitute (ETR) foods, the present study was undertaken to evaluate some of the commercially available processed foods for their RS and amylose content. Since storage of foods is known to have an impact on the RS content, the products were also analysed after storage at ambient conditions. In addition, the nutrient composition of the products, have also been evaluated.

2. Materials and methods

The processed foods, selected for the study, were provided by M/s MTR Products, Bangalore, India, the technology of which has been developed by Defence Food Research Laboratory, Mysore (India). The ingredients used and the process of development are given in Table 1. The samples for analysis were drawn from two batches of each of the prepared products. One batch of the products were stored in an insect proof chamber at ambient conditions. The samples were analyzed for RS, amylose and total starch before and after storage (6 months).

The products on cooking/preparation, generally 65–70% cooked except in the case of kesari bhath, are packed/heat sealed in quantities of 250 g each in retort pouches of suitable size. These pouches are then placed in aluminium moulds specially designed for the purpose and arranged vertically in suitable wire mesh baskets. The baskets are loaded into an autoclave. The steam pressure when slowly raised reaches a level of 10 lbs compressed air is let in to raise the pressure to a total of

20 lbs. The duration of exposure (30–30 min) and the temperature thereof, to this high pressure varies from product to product and is dependant on the F_0 value for the product, which generally ranges between 6.0 and 6.5. Subsequently the steam is cut off and the pressure of 20 lbs is maintained with air alone followed by cooling by letting in water inside the autoclave for 6–10 min. The water flow is stopped while slowly releasing the air pressure inside the autoclave. The pouches containing the product are then dried at 70 °C to remove the superficial water and are ready for storing/transportation.

The contents of the individual sachets were thoroughly homogenized using a domestic/kitchen mixer. One portion of the homogenized material was dried in an oven at 50 °C powdered and passed through a 60 mesh (British standard screen) for analysis of protein fat, sugars and dietary fiber. The other portion was stored in a deep freezer (−18 °C) until analyzed for, total starch (TS), RS and amylose, which was completed within a week from the date of homogenization, avoiding repeated thawing and freezing.

2.1. Analytical methods

2.1.1. Proximate composition

All the products were analyzed for moisture, protein ($N \times 6.25$), crude fat, total ash by the AOAC methods (AOAC, 1984). The total nitrogen in dried food samples was determined by micro-kjeldahl procedures. A factor of 6.25 was used to convert 'N' (nitrogen) value into protein. Crude fat was estimated by solvent extraction in a Soxhlet apparatus for 14–16 h with petroleum ether. Ash was determined by drying sufficient homogenized food sample to give dry weight of 2 g overnight in an oven (100 °C), charring on a hot plate for 1 h and then ashing in a muffle furnace at 550 °C for 4–5 h. The weight was recorded after cooling. The difference in weight gives an estimation of ash content.

Table 1
List of various RTE products, their major ingredients and methods of processing

Item	Ingredients used	Processing details
1. Pongal	Rice, green gram dhal (<i>Phaseolus aureus</i> roxb), hydrogenated vegetable oil, lime concentrate, cashewnut, green chilli, pepper, ginger, cumin, desiccated coconut, coriander and curry leaves	Ghosh et al., 1980; Krishnappa et al., 1982; Sabapathy et al., 2001; Sabapathy and Bawa, 2003
2. Khara bhath	Semolina, carrot, ghee, coconut, lime concentrate, cashewnut, Bengal (<i>Cicer arietinum</i>) and black gram dhal (<i>Phaseolus mungo</i> roxb), sugar, green chilli, ginger, mustard coriander leaves, curry leaves, turmeric powder and salt	
3. Dal fry	Green gram dhal, tomato, onion, ghee, red gram dhal, salt, ginger, garlic, green chilli, black gram dhal, coriander leaves and lemon concentrate	
4. Bisibele bhath	Rice, redgram dhal (<i>Cajanus cajan</i>), carrot, beans, hydrogenated oil, coconut, cashewnut, red chilli (dry), jaggery, mustard, curry leaves, salt and spices	
5. Rajma masala	Rajma (<i>Phaseolus vulgaris</i>), tomato, onion, green gram, hydrogenated oil, green chilli, coriander powder, ginger, coriander leaves, garlic, chilli powder and spices	
6. Kesari bhath (sweet semolina pudding)	Semolina, vermicelli, sugar, hydrogenated oil, milk, cashewnut, raisins, cardamom and saffron	

2.1.2. Free sugars

Reducing and total sugar contents were measured by the micro method of Shaffer Somogyi (AOAC, 1984) which is based on the iodometric titration of cuprous oxide with sodium thiosulphate, using starch as indicator. The sugar values were calculated according to table values.

2.1.3. Dietary fiber

The total dietary fiber (TDF) was measured as the sum of water-soluble and water-insoluble fractions, based on digestion of food sample (1 g) with enzymes, as described by Asp, Johnson, Hollmer, and Siljestrom (1983). The enzymatic hydrolysis of starch and protein was carried out in three steps: gelatinization in the presence of a heat-stable α -amylase (100 mg, 100 °C, 15 min, pH 6.0), incubation with pepsin (100 mg, 40 °C, 60 min, pH 1.5) and incubation with pancreatin (100 mg, 40 °C, 60 min, pH 6.8). Insoluble dietary fiber (IDF) was recovered by filtration with celite as the filter aid. Soluble dietary fiber (SDF) was precipitated from the filtrate with four volumes of 95% ethanol and recovered by filtration. The fiber values were corrected for indigestible protein (kjeldahl N \times 6.25) and ash (ignition at 525 °C, 5 h).

2.1.4. Starch

The total starch (TS) content was analyzed using controlled enzymic hydrolysis with invertase (E.C 3.2.1.26, to hydrolyse sucrose), heat stable α -amylase and amyloglucosidase (E.C 3.2.1.3) (Englyst et al., 1992). A schematic representation of the method is given in Fig. 1. Foods were analyzed with minimal pretreatment on as is basis. A value

for free glucose (FG) was obtained as glucose released from the food after 30 min. A second measurement total glucose (TG) was obtained by gelatinization of the starch in boiling water and treatment with KOH (7 M) at 0 °C to disperse any retrograded amylose, followed by complete enzymic hydrolysis with amyloglucosidase. Values for TS are obtained by correcting TG for free glucose obtained by separate analysis. Total starch values are calculated as glucose \times 0.9 [TS = (TG – FG) \times 0.9].

2.1.5. Resistant starch

The RS content was quantified directly in fresh food samples according to the method of (Goni et al., 1996). To portions of food samples (equivalent to 100 mg of dry matter) KCl–HCl buffer (10 ml, pH 1.5) was added, and homogenized. Pepsin solution (0.2 ml) (1 g pepsin/10 ml KCl–HCl) was added, mixed thoroughly and shaken continuously for 60 min at 40 °C. Cooled to room temperature, 9 ml Trismaleate buffer (0.1 M, pH 6.9) and 1 ml of α -amylase solution (40 mg α -amylase/ml Tris maleate buffer) was added, incubated at 37 °C for 16 h with constant shaking. Samples were centrifuged (15 min, 3000 \times g) and supernatants discarded, re-suspended in 10 ml distilled water and the centrifugation repeated. The residues were moistened with distilled water (3 ml), 3 ml KOH (4 M) was added, mixed well and kept at room temperature with constant shaking for 30 min. Then, 5.5 ml of HCl (2 M), 3 ml of sodium acetate buffer (0.4 M, pH 4.75) and amyloglucosidase (80 μ l, E.C.3.2.1.3) were added, mixed thoroughly and incubated at 60 °C for 45 min with constant shaking. Samples were centrifuged (15 min, 3000 \times g) and

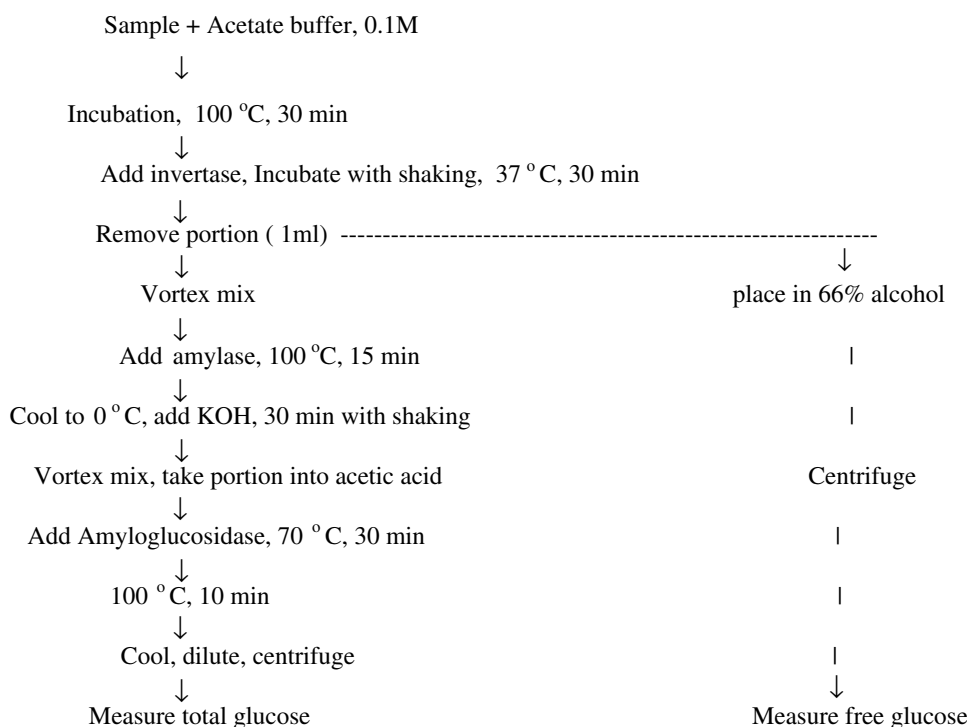


Fig. 1. Analytical procedure for estimation of total starch.

the supernatants collected. The residues were resuspended in 10 ml distilled water, centrifugation repeated. The supernatants were combined with the water washes and adjusted to a final volume of 25–1000 ml with distilled water, depending on the RS content. Total glucose was analyzed using a GOD-POD reagent (Dr. Reddy's Laboratories, Hyderabad, India). A glucose standard solution (10–60 µg/ml) was used. To 1 ml of the reagent solution 20 µl of supernatant was added, mixed and placed in a water-bath for 10 min at 37 °C. Absorbances at 510 nm were measured against blank reagent. The resistant starch was calculated as glucose (mg) × 0.9. The RS content was estimated in both fresh and stored samples (6 months).

2.1.6. Amylose

All the samples – both fresh and stored were analyzed for the amylose content by the colorimetric method based on the reaction between amylose and iodine. To the defatted sample (100 mg), ethanol (1 ml, 95%) and NaOH (1 N, 9.2 ml) were added and left overnight and made to volume (100 ml). To an aliquot (5 ml), acetic acid (1 N, 1 ml) and iodine solution (2 ml, 0.2% I₂ in 2% KI) were added and the volume made up to 100 ml with distilled water and mixed, after 20 min the absorbance was measured at 620 nm using as blank 5 ml 0.09 N NaOH, to which acetic acid (1 ml) and iodine solution (2 ml) were added in 100 ml total volume (Juliano, Perez, & Blakeney, 1981). The above analysis were carried out in 2 replicates.

2.1.7. Statistical analysis

The data obtained with respect to amylose, TS and RS (fresh vs stored) were analysed by the students 't' tests.

3. Results and discussion

The proximate composition of the different foods, are given in Table 2. All RTE foods were found to contain high moisture ranging between 73.2% and 84.2% except *kesari bhath*, which had only 42.8%. On moisture-free basis, all the products except *kesari bhath* were found to be good sources of protein due to the presence of pulses/whole grams as a component. The fat content ranged from 13.3% to 38.4% with 3 foods having >30% (*rajmah masala*, *pongal* and *khara bhath*). All the foods provided nearly similar amounts of energy per serving except *dhal fry* which appeared to be calorie dense, providing 3428 kJ/serving.

All the foods contained low amounts of free sugars (Table 3) ranging from 0.27% to 2.2% and 4.2% to 7%, respectively, with the exception of *kesari bhath*, which could be attributed to the use of higher quantities of cane sugar in its preparation. The total starch content ranged from 14.5 to 24 g%. The IDF and TDF content of *rajmah* and *bisi bele bhath* were higher than the values for other foods, which is due to the presence of whole grams/split gram. All the foods had a mean SDF content of 2.76 g, with the exception of *rajmah* (6.5 g). It is observed that, the carbohydrate profile of the foods studied varied depending on the ingredients used and the type of processing employed viz., frying, boiling, autoclaving.

The changes observed in the amylose, resistant starch (RS) and total starch (TS) content of various foods before and after storage (6 months) are given in Table 4. Among the foods, *khara bhath* and *bisi bele bhath* had highest amylose content. Storage of RTE foods under ambient conditions, resulted in a significant decrease ($p < 0.05$) in the amylose content in 3 foods viz., *pongal*, *khara bhath* and *bisi bele bhath*. The presence of amylose in the starchy foods is also known to influence the formation of RS in processed foods. All the foods contained amylose in the range from 1.2 to 7.2 g%.

All the foods contained appreciable amounts of RS ranging from 2.2 to 3.1 g/100 g (fresh weight basis). Storage resulted in changes in RS content of RTE foods as well, the increases were significant ($p < 0.05$) in 4 foods. All the foods studied had appreciable amounts of RS, which indicates that processing treatment, favors formation of RS in these foods. Similar results have been reported in an earlier study (Namratha, Asna, & Prasad, 2002). Several reports in the literature indicate the presence of RS in processed foods such as bakery foods (Kale, Kotecha, & Chavan, 2002) and cereal based foods (Englyst & Hudson, 1996; Muir & O'Dea, 1992; Namratha et al., 2002; and Tharanathan & Tharanathan, 2001).

Small increases in the TS content of all the foods was observed after storage. The mean increases ranged from 0.6% to 1.6% on fresh weight basis. These differences were significant ($p < 0.05$) for 5 foods, except *kesari bhath*. Perhaps the physical and chemical modifications in the starch structure during storage contributes to increased availability of starch to enzyme action.

Foods containing higher amylose content were found to have maximum increases in RS content after storage which

Table 2
Proximate composition of RTE foods (g/100 g dry basis)

Product	Moisture	Crude protein	Fat	Carbohydrate ^a	Total ash	Crude fiber	Cals (kJ) per serving of 300 g
Pongal	4.2	22.2	32.3	33.1	8.2	1.3	2574
Khara bhath	4.4	28.0	31.7	29.9	6.0	0.8	1791
Dal fry	5.8	20.3	27.5	39.6	6.8	2.7	3428
Bisebele bhath	4.3	16.0	18.2	52.9	8.6	3.7	987
Rajmah masala	5.2	24.2	38.4	21.1	11.1	8.9	1222
Kesari bhath	4.8	6.3	13.3	75.1	0.5	0.2	3332

^a The values obtained are by difference values are mean of 2 replicate analysis.

Table 3
Carbohydrate profile of RTE foods (before storage: g/100 g on moisture-free basis)

Product	Sugars		Total starch	Dietary fiber			Total carbohydrates ^a
	Total	Reducing		Soluble	Insoluble	Total	
Pongal	4.2	0.33	24.0	2.11	6.12	8.23	36.43
Khara bhath	4.7	0.27	21.0	2.64	6.70	9.30	35.00
Dal fry	7.0	0.33	17.0	3.70	6.90	10.60	34.60
Bisebele bhath	4.2	1.50	17.5	3.36	9.97	13.33	35.03
Rajmah masala	6.8	2.20	14.5	6.46	12.40	18.86	40.16
Kesari bhath	23.2	12.60	22.0	6.00	8.80	14.80	60.00

^a Values are sum of total sugars + Total starch + Total dietary fiber values are mean of 2 replicate analysis.

Table 4
Changes in amylose, resistant starch and total starch content^d of RTE Foods on storage

Product	Amylose		Resistant starch		Total starch	
	Before	After	Before	After	Before	After
Pongal	0.57 ± 0.03 (3.6)	0.38 ± 0.01 ^c (2.4)	2.8 ± 0.40 (17.7)	3.3 ± 0.25 ^b (21.5)	3.7 ± 0.50 (24.0)	4.5 ± 0.26 ^b (28.9)
Khara bhath	1.94 ± .06 (7.2)	1.64 ± 0.09 ^b (6.0)	2.43 ± 0.15 (8.6)	3.0 ± 0.53 ^d (11.2)	5.6 ± 0.56 (21.0)	7.0 ± 0.17 ^b (25.8)
Dal fry	0.81 ± 0.20 (3.6)	0.62 ± 0.10 ^d (2.8)	2.2 ± 0.17 (9.9)	2.7 ± 0.23 ^b (12.2)	3.8 ± 0.36 (17.0)	5.4 ± 0.44 ^c (24.5)
Bise bele bhath	1.18 ± 0.16 (6.3)	0.69 ± 0.12 ^b (3.5)	2.2 ± 0.10 (11.8)	3.37 ± 0.15 ^c (16.0)	3.3 ± 0.26 (17.5)	4.3 ± 0.36 ^b (23.0)
Rajmah Masala	0.68 ± 0.07 (3.6)	0.68 ± 0.05 ^d (3.6)	3.1 ± 0.30 (16.3)	3.3 ± 0.30 ^d (17.4)	4.3 ± 0.26 (14.5)	5.8 ± 0.36 ^c (20.0)
Kesari bhath	0.69 ± 0.04 (1.2)	0.69 ± 0.07 ^d (1.2)	2.7 ± 0.10 (4.7)	3.1 ± 0.20 ^b (5.4)	12.5 ± 0.46 (22.0)	13.2 ± 0.31 ^d (23.0)

Value in parenthesis indicate on moisture-free-basis.

^a g/100 g fresh basis.

^b $p < 0.05$.

^c $p < 0.01$.

^d NS – Not significant.

is attributed to the retrogradation of amylose. Storage of RTE foods had a varied effect on both the amylose and the RS content of the RTE foods. There was a decrease in the amylose content in four of the RTE foods viz., *pongal*, *khara bhath*, *dal fry* and *bise bele bhath* and a concomitant increase in the RS content. In *rajmah masala* and *kesari bhath*, no significant changes were observed in amylose and RS content after storage. Thus, it appears that processing treatments used, storage period and amylose content of foods are the important factors influencing the RS in selected RTE foods. The RS content of the various foods rank as follows:

Before storage: Rajmah > Pongal > Kesari bhath > Khara bhath > Dhal fry ≥ Bisibele bhath.

After storage: Bisibele bhath > Pongal > Rajmah > Kesari bhath > Khara bhath ≥ Dhal fry.

The above findings indicate that RTE foods contain appreciable amounts of RS and that storage (6 months) further increases the RS content. A change in life style has created a demand for pre-packed/processed RTE/ETR foods, which are not only convenient but also have a longer shelf life.

4. Discussion

It is well known that foods are subjected to various processing treatments before consumption. During processing, the starch molecule undergoes several physical modifica-

tions depending on the type of contained starch and severity of the conditions employed leading to the formation of resistant starch (Annisson & Topping, 1994; Goldblith, 1971). In processed foods, various factors like degree of milling, heating, freezing, drying, acid/alkaline conditions, moisture level during cooking or baking, the presence of fat and the initial amylose to amylopectin ratio are known to influence the formation of RS and IDF (Goni et al., 1996; Muir & O'Dea, 1992 and Ring, Gee, Whittam, & Johnson, 1988).

5. Conclusion

The present study reports the effects of storage on starch components viz., amylose, resistant and total starch content in some of the popular RTE foods procured from the local market.

Since the presence of RS in processed RTE foods, has different physiological implications, it is suggested to be beneficial in the management of obesity and diabetes. RS undergoes fermentation in the large bowel, producing short chain fatty acids, which are implicated to exert a protective effect against development of colon cancer (Annisson & Topping, 1994; Goni et al., 1996; Haralah-ampu, 2000). On the contrary, consumption of RTE foods containing RS may be undesirable for children, adolescents, sports persons as it decreases the available energy. In view of this, studies are needed to evaluate the physiological effect of long-term consumption of RTE foods.

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