ORIGINAL ARTICLE

Measurement of resistant starch in cooked cereal-based foods

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Abstract

Resistant starch, which has been defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals, is considered beneficial for health due to its effects on the human bowel and on carbohydrates and lipids metabolism. Cereals are daily consumed by the Italian population as bread, pasta, breakfast cereals, gruels, etc. Cereal-based foods (50 samples) belonging to the above categories were analysed, both raw and after cooking, by the *in vitro* method of McCleary and Monaghan (standard AOAC 2002.02 and AACC 32-40 methods) in order to assess their resistant starch content. Cooked potatoes and banana (raw and cooked) were used as a comparison. The cooked foods and also the bread samples were cooled at the temperature compatible with their consumption, rapidly frozen and freeze dried in order to standardize the analytical protocol. Freeze drying of cooked foods was important in achieving repeatability of measurements. Within each category of food, different amounts of resistant starch were found depending mainly on the nature of the cereals used as raw material and on other added flours (i.e. legume flours). Within all cereal-based samples values in the range 0.1–3.4% d.m. were found.

Introduction

Starch, which is the major dietary source of carbohydrates, is the most abundant storage polysaccharide in plants (Ellis *et al.*, 1998). The relatively recent recognition of incomplete digestion and absorption of starch in the small intestine as a normal phenomenon, has raised interest in non-digestible starch fractions (Cummings & Englyst, 1991; Englyst & Geoffrey, 1996). These are called 'resistant starches (RS)' and extensive studies have shown them to have physiological functions similar to those of dietary fibre (Asp, 1994; Eerlingen & Delcour, 1995). RS have been shown to produce a large amount of butyrate all along the colon that has been observed to have a range of effects on cell metabolism, differentiation, progression and growth of colon tumours (Champ *et al.*, 2003).

The diversity of the modern food industry and the enormous variety of food products it manufactures requires starches that can tolerate a wide range of processing techniques and preparation conditions (Visser *et al.*, 1997). These demands are met by modifying native starches with chemical, physical and enzymatic methods (Betancur & Chel, 1997), which may lead to the formation of indigestible residues.

The term 'RS' was first coined by Englyst *et al.* (1982) to describe a small fraction of starch that was resistant to hydrolysis by exhaustive α-amylase and pullulanase treatment *in vitro*. However, RS is now defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals (Asp & Bjorck, 1992). RS is naturally occurring, but it can also be produced by the modification of starch during the processing of foods.

RS, based on their nutritional characteristics, as summarized in a review by Sajilata *et al.* (2006), can be subdivided in four fractions: RS_1 is the physically inaccessible form such as partly milled grains and seeds; RS_2 represents starch that is in a certain granular form and resistant to enzyme digestion; RS_3 represents the most RS fraction and it is mainly retrograded starch formed during cooling of gelatinized starch; RS_4 is the RS where novel chemical bonds other than

α-(1–4) or α-(1–6) are formed. Modified starches are included in this category.

Different RS fractions can coexist in the same food. RS_3 and RS_4 are not digested by mammalian intestinal enzymes and are partly fermented in the colon (Englyst *et al.*, 1992; Cummings *et al.*, 1996). RS is estimated to be approximately 10% (2–20%) of the amount of starch consumed in the Western diet (Stephen *et al.*, 1983).

There are currently a number of methods available for the measurement of RS (Champ *et al.*, 2003). Digestion and absorption in the human gastrointestinal tract is a dynamic process in which many enzymes, including proteases and amylases, work in concert to disrupt the three dimensional and then the intermolecular structure in foods. Macromolecules are hydrolysed as they become exposed and hydrolytic products are rapidly absorbed. The successful analytical method for RS will be one that mimics this process in the human gastrointestinal tract, so that the analytically determined value reflects starch not assimilated in the human intestine. Therefore RS is not constant for different foods made from the same ingredients, as retrograded starch is created through food processing and by cooking and cooling food.

The first and main step of any *in vitro* method to measure the content of RS in foods is the removal of all the digestible starch from the product using thermostable α-amylases (McCleary & Rossiter, 2004). At present, the method of McCleary and Monaghan (2002) is considered the most repeatable and reproducible measurement of RS in plant materials even though some objections to its capacity to measure all RS fractions have been moved (Champ *et al.*, 2003). It is based on the joint action of pancreatic α-amylase and amyloglucosidase (AMG) at pH 6.0, followed by alcohol precipitation. With respect to other methods, it omits the initial heating step at $100\,^\circ C$, so as to more closely mimic physiological conditions.

In the human diet many foods are eaten in a cooked state. Analyses of cooked foods are difficult to perform due to problems encountered in the standardization of the preparation and sampling phases. On the other hand, the measurement of RS in foods has a physiological meaning if it is determined in the actual situation in which the food is consumed and it enters the human digestive system. It is doubtful whether the measurements of RS in raw foods, which have to be further processed at the home level, can be of any use to the consumer.

In consideration of the above and of the fact that several cereal based foods are daily consumed by the Italian population under different forms, cooked samples of pasta of different origins, cooked whole and dehulled grains, cooked maize gruels were analysed together with bread and breakfast cereals by the method of McCleary and Monaghan (2002) (standard AOAC 2002.02 and AACC 32-40) in order to assess their level of RS and results are reported in this paper.

Moreover, in an attempt to standardize the preparation, sampling and handling phases of the RS determination in cooked foods, phases that might have an influence on the repeatability and reproducibility of the analytical result, a new protocol was experimented in our study in which cooked samples soon after cooking and in any case considering the usual conditions under which they are consumed, underwent rapid freezing with liquid nitrogen and freeze drying before RS determination.

Materials and methods

Five samples of dry extruded semolina pasta of different shapes (spaghetti, penne) belonging to different brands together with three samples of dry extruded mixed grain pasta (see Table 1), dry extruded emmer pasta of three different shapes, four dry extruded maize pasta of the same shapes but made with different cultivars, were purchased on the market and analysed after cooking in salted water. Spaghetti of different brands possessed the same diameter. Dehulled einkorn (*Triticum monococcum*), emmer (*Triticum dicoccum*) and barley (*Hordeum vulgare*) dehulled grains, were purchased from the producers in typical growing areas and analysed after cooking in salted water. Maize (*Zea mais*) flours (Polenta) of different granulometry (Bramata, Fioretto) and produced from different cultivars were analysed after cooking in salted water. Samples of brown, white and parboiled rice (*Oryza sativa*) belonging to different cultivars typical of the Italian production were also analysed. All the Italian cultivars belonged to the *Japonica* ssp.

For all samples optimum cooking times were determined by following the indications on the packaging or recommendations by producers. Several samples represented typical products with denominations of origin according to the European legislation.

Samples of bread of different shapes and made from different ingredients (see Table 1) were purchased from artisanal bakeries in the morning and analysed the same day to mimic the actual consumption conditions apart from one sample where an aliquot was left to stale at room temperature for 5 and 30 days in order to assess the influence of storage on RS.

Samples of corn flakes of three different brands were purchased in a supermarket, milled in a laboratory mill and analysed.

Table 1 Resistant starch content in starchy foods (% d.m.)*

Sample	Cooking time (min)	RS (% d.m.)	RS % d.m. (measured after 3 days)
Durum wheat pasta			
Spaghetti N.1	7	1.2	
Spaghetti N.2	8	1.4	
Spaghetti N.3	7	1.2	
Penne rigate N.1	11	1.1	
Penne rigate N.2	11	1.3	
Emmer Pasta			
Penne rigate	10	0.4	
Tagliatelle N.1	8	1.0	
Tagliatelle N.2	10	1.3	
Spaghetti N.1	10	0.4	
Spaghetti N.2	12	0.6	
Mixed grains pasta			
Pennette (spelt and lentils)	10	1.5	
Spaghetti (five cereals)	9	1.4	
Tortiglioni (barley/buckwheat)	11	1.3	
Maize pasta			
Maccheroncini Cv 1	8	0.5	
Maccheroncini Cv 2	10	0.4	
Maccheroncini Cv 3	5	0.4	
Maccheroncini Cv 4	8	0.5	
Maize flour (Polenta)			
Course flour (Bramata) Cv 1	15	0.8	
Fine flour (Fioretto) Cv 1	20	0.6	
Course flour (Bramata) Cv 2	15	0.4	
Fine flour (Fioretto) Cv 2	20	0.5	
Course flour (Bramata) Cv 3	15	1.2	
Fine flour (Fioretto) Cv 3	20	0.5	
Flour Cv 4	15	0.3	
Flour Cv 5	15	0.4	
Flour Cv 6	15	3.4	
Whole grains			
Dehulled Einkorn Cv 1	30	0.8	
Dehulled Emmer Cv 1	30	1.8	2.1
Dehulled Emmer Cv 2	30	1.9	
Dehulled Emmer Cv 3	30	2.7	
Dehulled Emmer Cv 4	30	2.7	2.6
Pearled barley Cv 1	35	2.1	
Rice			
White rice Cv 1	13	0.4	
Parboiled white rice Cv 1	14	0.3	0.3
White rice Cv 2	13	0.6	
White rice Cv 3	15	0.3	
Parboiled white Cv 4	12	0.3	
Parboiled white fast cooking Cv 4	5	0.5	
Brown rice Cv 5	26	0.1	0.1
Brown rice Cv 2	28	0.5	
Bread			
Genzano bread		1.5	
Genzano bread (5 days stale)		1.3	1.4
Genzano bread (30 days stale)		1.5	

Table 1 Continued

Sample	Cooking time (min)	RS (% d.m.)	RS % d.m. (measured after 3 days)
Small Rosetta		0.8	
Small Ciabatta with oil		1.0	
Wholemeal bread		1.2	
Five cereals and soy bread		2.5	
Corn flakes			
Corn flakes n.1		2.2	2.1
Corn flakes n.2		0.2	0.2
Corn flakes n.3		3.1	
Banana			
Raw bananas		9.2	
Cooked bananas	10	1.2	
Potatoes			
Boiled potatoes	40–45	3.9	
Reference standards			
High-amylose maize starch (41.9%)		46.0	
Control kit RS (43% s.s.)		41.0	
Kidney beans (dried-milled) (4.8% s.s.)		4.7	4.7
Regular maize starch (0.78% s.s.)		0.4	0.5

*Average of two determinations.
Cv, Cultivar; d.m., dry matter; RS, resistant starches.

All cooked samples were left to cool for some minutes until ready for consumption to mimic usual consumption patterns. Representative aliquots of cooled-cooked samples and bread samples were subsequently frozen by immersion in liquid nitrogen. The frozen samples then underwent freeze drying. Aliquots of the freeze-dried powder were analysed for their moisture content (ICC, 2003) and for their RS content according to the method of McCleary and Monaghan (2002) (standard AOAC 2002.02 and AACC 32-40). In this method samples are incubated in a shaking water bath with pancreatic α-amylase and AMG for 16 h at 37 °C, during which time non-RS is solubilized and hydrolyzed to glucose by the combined action of the two enzymes. The reaction is terminated by the addition of an equal volume of ethanol or industrial methylated spirits (denatured ethanol), and the RS is recovered as a pellet on centrifugation. This is then washed twice by suspension in aqueous industrial methylated spirits or ethanol (50%, v/v), followed by centrifugation. Free liquid is removed by decantation. RS in the pellet is dissolved in 2 M KOH by vigorously stirring in an ice-water bath over a magnetic stirrer. This solution is neutralized with acetate buffer and the starch is

quantitatively hydrolysed to glucose with AMG. Glucose is measured with glucose oxidase/peroxidase reagent (GO-POD), and this is a measure of the RS content of the sample. Non-RS (solubilized starch) can be determined by pooling the original supernatant and the washings, adjusting the volume to 100 mL and measuring glucose content with GOPOD.

Samples of raw and cooked banana, boiled and cooled (10 min) potatoes were treated as above and analysed for comparison together with reference standards from Megazyme (Ireland) (high-amylase maize starch, control kit RS, dried and milled kidney beans, regular maize starch).

Some freeze-dried samples were kept at room temperature and the RS was measured again according to the above-mentioned method after 3 days.

Results and discussion

RS can be found in many foods that people consume every day. Several studies indicate that its presence in food might be beneficial to human health. In particular, it might affect lipid and glucose metabolism, minerals absorption and gut microflora (Sajilata *et al.*, 2006). However, the amount of RS found in starchy foods made with natural ingredients is usually low, even if the levels measured differ according to the methods used.

In the paper by Englyst and Geoffrey (1996) values between 0.6 and 3.2 g/100 g, as eaten, are reported for different kinds of bread (white, wholemeal and rye), between 0.8 and 1.0 g/100 g for pasta of different shapes and between 0.1 and 6.2 g/100 g for breakfast cereals of different brands and made with different raw materials.

In a paper by Akerberg *et al.* (1998) bread made from 80% whole-meal barley flour plus 20% white wheat flour or 100% white wheat flour, contained low levels of RS, 1.5 and 0.2 g/100 g of starch, respectively. The steamed corn flakes contained amounts of RS of 2.3 g/100 g. The RS concentration in spaghetti, 5 g/100 g, is reported to be in accordance with Englyst data (Englyst *et al.* 1992). The two potato products analysed were either boiled, or boiled and stored at 5 °C for 24 h. The stored potatoes had a significantly higher concentration of RS (7 g/100 g, starch basis) than the potatoes tested immediately after boiling (4 g/100 g starch basis).

Previously, Englyst and Cummings (1987) reported RS values of 3 and 12 g/100 g (starch basis) for boiled and cool-stored potatoes, respectively. The intact, totally green bananas contained a substantial amount of RS, 72 g/100 g (starch basis).

In the work by Elmstahl (2002) who determined the amount of RS in starchy foods on the Swedish market according to the *in vitro* method of Akerberg *et al.* (1998), RS content expressed as percentage on total starch content, varied from 0.6% in wheat bread to 6.0% in rye bread. The extruded and puffed breakfast cereals were found to have a low RS content: 0.2% in oats and maize flakes and 1.2% in roasted puffed wheat grains, respectively. The RS fraction in spaghetti was higher (2.9%). The RS concentration in boiled potatoes was 2.0%.

Even though several authors have analysed cooked foods, very little information is given on the analytical protocol followed after cooking and before the analyses of RS were performed, so that it is sometimes difficult to compare results even within the same paper.

In our study, being aware of the fact that for foods that are consumed after cooking and, in general, after heat processing, it was important to determine the amount of RS in the food that might actually be ingested by consumers and because of the difficulties in handling and sampling that are encountered in the manipulation and analyses of cooked starchy foods, we introduced a preliminary preparation protocol to standardize the analyses of RS and obtain repeatable, reproducible and comparable results.

For the analytical measurement of RS itself we chose the method of McCleary and Monaghan (2002), which is a simple and relatively rapid *in vitro* test that has already been standardized (Association of Official Analytical Chemists, 2002; American Association of Cereal Chemists, 2008) and in which *in vivo* conditions are reflected as much as possible (McCleary & Monaghan, 2002). After several trials, we decided that the best results were obtained when the cooked sample was rapidly frozen in liquid nitrogen so as to block any change in the starch and it was immediately freeze dried to obtain a very homogeneous and powdery sample from which it was possible to weigh aliquots very accurately. In fact, by analysing in triplicate a group of 20 cooked and freeze-dried starchy foods of the same kind of those that were subsequently analysed, we obtained RS average values in the range of 0.9–3.6% d.m., with SDs within each sample, ranging from 0.008 to 0.16.

Following the preparation protocol described above, it was possible to obtain all the results that are reported in Table 1, where analysed foods have been divided in several groups, namely pasta, flour, whole grains, rice, bread, cornflakes. All these foods are representative of the cereals consumption within the Italian population. In fact, among starchy foods, cereal-based products are the most important sources of starch in the human diet, bread being the most important source of cereals in Italy (Brighenti *et al.*, 1998).

In the same table, for some of the samples, results are reported for the measurement of RS after a period of 3 days to further assess the repeatability of the McCleary and Monaghan method coupled with our protocol. Our results (Table 1) clearly show that quick freezing and freeze drying are able to ensure a good repeatability of the RS measurement.

With respect to the RS values found in all cereal-based foods, we can say that they ranged from 0.1% to 3.4% d.m. These values are undoubtedly lower than those found in boiled potatoes (3.9% d.m.) and especially raw ripe bananas (9.2%). It is interesting to note that cooking bananas lowers their RS content to a level around 1.2%. The values obtained for boiled potatoes are in general agreement with those found by Englyst and Cummings (1987) and by Akerberg *et al.* (1998).

The group that showed the highest RS values was that comprising cooked whole grains where values ranged from 0.8% to 2.7% d.m. Several cereal species were represented in this group (einkorn, emmer and barley). In contrast, the group that showed the lowest RS contents was represented by rice. Moreover, within this latter group, genetic differences and different processing conditions (white, brown and parboiled) did not significantly affect the measured RS values. Maize pasta, which was produced under the same conditions, but from flours of different cultivars, showed low values in the range of rice grains.

However, within the broad group of pasta samples we noticed that the level of RS present in traditional durum wheat semolina pasta and which is independent of pasta shape, can be increased when flours from other species, legumes in particular, are added to the durum wheat semolina. Within the emmer pasta group instead, values of RS differed according to the different pasta shapes obtained with different processing methods, showing that in these cases processing conditions affected the level of RS in the product.

The RS content in the five bread products included in the present study (Genzano, rosetta, ciabatta, wholemeal bread and mixed cereals bread), varied from 0.8% d.m. in the small rosetta, which is made with soft wheat flour only, to 2.5% d.m. in the five cereals and soy bread. So also in bread the raw materials used were the major determinants in the formation of RS. Moreover, according to the information coming from the only staling experiment (at room temperature) that we performed with the Genzano bread, storing the products for 5 and 30 days did not affect the RS values.

Polenta (maize flour cooked in salted water) in general showed low values for RS and they were independent of both cultivar and granviometry (see Bramata and Fioretto). The only exception was represented by Cultivar 6, which came from a special genotype typical of a geographical area in Central Italy.

As regards other products made with maize flours, we have reported the values of RS that we obtained in corn-flakes purchased from the market. It was interesting to note that they showed a wide range of RS values, from 0.2% to 2.2% d.m. In this group we can suggest that both the raw material used (as for the polenta flours we examined) and the processing conditions may have been different for the three brands and these differences may play a role in delivering the different RS values.

Conclusion

In conclusion, we can say that the analytical method coupled with the protocol described above, allows for the evaluation of RS and the comparison of heat-treated (bread) and cooked samples under standardized conditions. The RS, which is measured, can certainly be ascribed to the RS_3 fraction (defined above) and truly represents starch which is able to resist digestion by pancreatic α-amylase and AMG. Whether this can be considered as the true measure of the actual RS in foods consumed by humans will be a matter for further discussion.

Acknowledgements

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