

Recent research on resistant starch: analytical, technological and physiological aspects

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INTRODUCTION

Numerous publications had shown that some raw or even cooked starches were partly unavailable to most animal species including humans (1-3). Thus, even if resistant starch is a rather new concept, the reality of the resistance of part of the starches has been discovered a long time ago.

The term «resistant starch» has first been introduced by Englyst and co-workers (4) in 1982. Indeed these researchers found that many processed foods had higher apparent non-starch polysaccharides than the corresponding raw products. A detailed analysis revealed that the apparent increase was due to a glucan that could be dispersed in potassium hydroxide. Thus they first defined Resistant Starch as starch resistant to dispersion in boiling water and hydrolysis with pancreatic amylase and pullulanase (4). This fraction was constituted mainly of retrograded amylose which appeared to be highly resistant to *in vivo* digestion. The definition had been enlarged when *in vivo* experiments demonstrated that other fractions of starch could escape small digestion in humans.

In 1992, resistant starch (RS) had been defined as “the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (5). Therefore, it is the fraction of the starch which will not provide glucose to the organism but which will ferment in the large intestine to produce mainly gases and short chain fatty acids. Resistant starch is thus comparable to dietary fibers and is even often considered as dietary fiber (6,7).

Classification of RS

RS cannot be properly defined chemically due to the fact that the resistance of starch is related to hydrolysis conditions (nature of the enzyme(s), ratio starch/enzyme, characteristics of the hydrolysis). There is probably no starch fraction undigestible by any enzyme if the concentration is not limitant and the duration of the hydrolysis long enough. The physiological definition is probably much better but one has to keep in mind that RS, *in vivo*, will depend on the physiological state of the subject, the environment of the starch when ingested, and the other food components of the meal.

In 1987, Englyst and Cummings (8) mentioned a number of reasons explaining the uncomplete digestion of starch in the small intestine (1) Physically inaccessible starch (occurring mainly in partly milled grains and seeds), later identified as RS type I, (2) Native resistant starch granules (present in raw potato and banana), presently identified as RS type II, (3) Retrograded amylopectin, now excluded

from RS classification and (4) Retrograded amylose (found, for instance, in cooled, cooked potato, bread and corn flakes). This 4th category of RS, now identified as RS Type III was initially the only one to be (partly) quantified by the analytical methods (Englyst *et al.*, 1982 (4) or methods derived of the AOAC method for determination of dietary fibres) (9).

This classification proposed by Englyst is still valid (10). However, it should be mentioned that types I and II or I and III can coexist in the same food (11).

Analysis of RS

The analysis of resistant starch implies the performance of an enzymatic hydrolysis (α -amylase in most cases) which is usually supposed to mimic the hydrolysis of starch by endogenous enzymes in the upper part of the digestive tract (mouth, stomach and small intestine). The quantification of RS can be made by a direct analysis of the residual starch after the hydrolysis (9,12-14) or by subtracting to the total starch content of the sample, the amount of starch which had been digested (10).

After two collaborative studies (within an EC AIR-concerted action EURESTA) in 1992 (13) and 1993 (15), the conclusions were the following :

- Englyst (10) and Champ (13) methods give similar values for samples with a high level of RS. They both give an estimation of RS which does not take into account the potentially digestible starch found *in vivo* at the end of the small intestine.
- Faisant *et al.* (14) and Saura-Calixto (not published) proposed modifications to the method described by Champ (13). These methods are quicker and easier to be reproduced than Englyst's method.

Besides the practical aspects there is a fundamental problem which is the definition of what should be analyzed with the method. None of the methods, including «Englyst method» takes into account the whole amount of RS defined as “starch and products of starch degradation not absorbed in the small intestine of healthy individuals” (16).

Indeed, on the one hand, *in vivo* RS seems to consist of three more or less distinct fractions, of which the main fraction might be depending on the food or the starch : oligosaccharides (including glucose), crystallites (linear chains of α -glucans) and long chains which are probably pieces of starch granules. On the other hand, the *in vitro* RS consists of linear chains of α -glucans (16) (crystallites as observed *in vivo*) and starch granules in the case of native B-type starch (11).

RS content (10) of some foods are presented in Table 1.

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TABLE 1
Total and resistant starch content of some foods (10)

Sample	Dry matter (%)	g/100 g dry matter	
		RS	TS
White bread	54.5	1	77
Corn flakes	95.8	3	78
Instant potato	16.7	1	73
Boiled potato (hot)	22.8	5	74
Banana flour (unripe)	99.1	57	75
Boiled potato (cold)	23.8	10	75
Spaghetti	28.3	5	79
Peas (frozen, boiled 5 min)	18.3	5	20
Bean flakes	93.6	6	49

Industrial production of RS, functional properties of RS

In order to increase RS consumption, several approaches can be proposed to the consumer and the food industry. Dietary recommendations can suggest to increase the consumption of non refined foods such as legumes or multi-grain breads which can provide substantial amounts of RS. A second way to increase RS consumption would be to provide to the consumers foods enriched in RS by adding concentrates of RS (high amylose corn starch, for instance) or by promoting starch retrogradation during the cooking process and postcooking treatment. Food technologists and processors were quick to realise that processing techniques that increase the amount of resistant starch in foods would have potential nutritional and commercial value. As autoclaved cereal starches were the first to be characterised this led to the use of the expression 'man made fibre' to designate retrograded amylose. The main drawback to this solution could be the deterioration of the organoleptic properties of the food. There is a need to explore the new food products such as the different kind of products ready-to-eat, kept at 4°C or -20°C in order to know if there is a possibility of increasing RS consumption without completely modifying food habits of the populations.

Several groups already try to produce breads or biscuits enriched in high amylose starch (native or thermally treated) (17,18). Final concentration of the RS in the products could reach 14 g/100 g D.M. Moreover, one recent study (19) shows that RS can be used as texture agent. The authors claimed that the starch preparation forming a particle gel network could replace part or all the oil in dressings.

In vivo determination of RS

Several methods are available to assess physiologically resistant starch *in vivo* (i.e. starch fraction escaping small intestine digestion). RS can be quantified directly by collecting ileal samples in humans or indirectly by estimating the amount of starch fermented in the colon.

In humans, one direct method consists in working with ileostomized patients (persons who had undergone a colonic resection). A second direct method applicable in humans consists in the intubation of healthy volunteers and collection of the digestive content in the terminal ileum. The infusion of a marker is used to estimate water flow rate through the distal ileum and allows the calculation of the real amount of starch passing through the terminal ileum. The breath test can also be used to quantify the amount of starch entering the colon. This indirect technique is based on the measurement of

hydrogen in expired air. Indeed, the fermentation of carbohydrates entering the colon produces short chain fatty acids and gases such as CO₂, CH₄ and H₂. Part of these gases is absorbed, carried by blood and excreted through lungs in expired air (20). For each subject, the total amount of H₂ measured in breath after ingestion of an experimental meal is compared to the amount of H₂ expired after ingestion of a known amount (usually 10 g) of lactulose. This carbohydrate is not digested in the small intestine and is totally fermented in the colon. The 'calibration' with lactulose allows the quantification of carbohydrate fermented in the colon. The drawbacks of the technique are the followings: 1) fermentative pathways are different from one substrate to another and the comparison between the production of H₂ by starch and lactulose fermentation is difficult (21); 2) the proportion of H₂ absorbed by the colon and expired through the lungs depends on the use of H₂ by microflora and its kinetic of production (21). Therefore, the breath test cannot really be used to quantify starch malabsorption (21-23). It can however be useful to detect some clinical malabsorption or to make a screening of different products in very standardised experimental conditions.

Regarding all these methods used to assess starch digestibility in the upper part of the gut, it seems very difficult to have a precise determination of RS *in vivo*. All have drawbacks but they still are useful to estimate (and to study) RS in various foods.

Energy value of RS

The malabsorption of starch in the small intestine consequently decreases the amount of available glucose from a food and then represents a loss of energy. Part of this loss can be recovered through fermentations in the colon. RS entering the large intestine is fermented to a greater or lesser degree with the production of SCFA, gases (CO₂, H₂ and CH₄ (in part of the population)), heat and bacterial cells but only absorbed SCFA make a significant contribution to energy salvage. Overall energy absorption per g RS fermented had been estimated to be around 9.0 to 12.0 KJ/g (24) depending on the assumptions which have to be made for the calculations. However other authors estimated the energetic value of RS to be up to 15 KJ/g (25).

RS and the glycemic index

The extent and kinetic of digestion of starch in the small intestine influence both the amount of starch reaching the terminal ileum (i.e. RS) and the glycemic index (i.e. the passage of glucose into the blood). Therefore, the RS concept is also related to the way starch is hydrolysed in the upper part of the digestive tract but it gives an end point value. On the contrary, the glycemic index gives information on the kinetic of the early hydrolysis of starch.

Although some studies have demonstrated a good correlation between *in vitro* amylolysis rates and *in vivo* glycemia responses (26-28), no correlation exists between RS content and glycemic index (29-30). For example, cornflakes and hot potatoes have both a similar and high glycemic index but different RS content (4% and 1% of dry matter respectively). Liljeberg *et al* (28) observed very different glycemic index for breads containing all about 1% RS. Only some foods, such as legumes pasta yield low glycemic index in agreement with their relatively high RS content.

Several authors explored both acute and long term effect of RS added to a balanced diet on glycemia and insulinemia. They did not found any effect of RS compared to cellulose (31,32).

Actually, the glycemic index reflects the way the digestible fraction of starch is digested and absorbed, while RS content relates

the unabsorbed fraction without taking into account the kinetic phenomena.

RS and lipid metabolism

The incidence of RS consumption on cholesterol and triglyceride metabolism are uncertain. By substituting part of the digestible starch for a large amount of RS (40% of the food), different authors observed a decrease of plasma cholesterol and triglycerides in normal rats after several weeks adaptation (33-35). However, Gee *et al.* (36) used lower amount of substitution and did not observe any effect of retrograded amylose on cholesterolemia and triglyceridemia over two weeks in rats. In humans, Behall *et al.* (37) compared the influence of two starches, one containing a high level of amylose, the other a high level of amylopectin : they observed a lower basal plasma triglyceride and cholesterol with the high amylose starch than with the other. RS could therefore have long term effects similar to dietary fibers. As dietary fibers were shown to have an effect on postprandial metabolism when added as a supplementation to a meal (38), we tried to test a similar effect with RS. We compared postprandial metabolism with a normal meal to this meal supplemented with 30 g raw potato starch on 6 healthy individuals. No significant differences could be observed, but 4 out of the 6 subjects had lower cholesterol and triglyceride postprandial responses with the supplemented meal (39). This has to be considered as a case study, but the influence of RS on the postprandial metabolism should further be studied.

The mechanisms by which RS could interfere with lipid metabolism are still hypothetical. One could mention the role of a lower insulinic response with some food, a possible influence of RS in bile acids secretion (33,40) or also the role of metabolites produced by colonic fermentation on liver metabolism (33).

RS, colonic fermentation and physiology of the large bowel

The total digestibility of starches is in most of the cases close to 100%, except for high amylose maize starch which is about 90% (22,41). Therefore, almost all the starch reaching the colon is fermented.

Fermentative metabolism of starch was studied by *in vitro* experiments using rats or human faecal inocula. Several authors showed that a number of sources of starch were fermented *in vitro*, some very slowly (42) and that fermentation products included H₂, CO₂, acetate, propionate, butyrate and lactate (43,44). Weaver *et al.* (45) observed differences in kinetic and fermentative profiles between methane-producers and non-methane producers.

Despite variabilities, several authors observed that starch fermentation induced the production of a high proportion of butyrate (25 to 30 % of total SCFA) compared to other rapidly fermented substrates⁴⁵. When acarbose was used to inhibit small bowel digestion of starch, Scheppach *et al.* (46) observed an increase of 50% of fecal butyrate excretion.

Colonic flora is also able to adapt to RS ingestion during several days : amylolytic enzymes are induced and metabolic pathways seem to be modified during the adaptation (44,47).

While few starch can be detected in faeces, RS tend to increase fecal excretion. Shetty and Kurpad (48) showed that a supplementation by 100 g per day of raw maize starch increased by 30 % faecal mass without modifying the transit time. By inhibiting small bowel digestion with acarbose, Scheppach *et al.* (46) observed an increase by 68% of faecal mass. These observations were due to the large development of bacterial mass with the available substrate. However, such laxative properties could hardly be observed in more realistic experimental

conditions of feeding (49). Cummings *et al.* (50) showed that RS laxative effect was much less than that of wheat bran. Several indices let conclude that RS could participate in prevention of colonic diseases. RS induced a decrease of the level of ammonia, branched chain fatty acids and fecal enzymes involved in detoxification (51). Moreover, the reduced pH which accompanies fermentation of starches and other carbohydrates may, in part, be responsible for the reduced transformation of primary bile acids to the mutagenic secondary metabolites and the reduced activities of other bacterial specific biotransformations in the large bowel (51). Feeding rats with Hylon-VII (a high amylose maize starch) or Cerestar resistant starch (amylolysis residue of retrograded high amylo maize starch) induced changes in crypt cell proliferation (52). In a human feeding study with 14 healthy volunteers consuming 45 g Hylon VII daily, significant changes in colonic function and crypt cell proliferation were seen including an increase in stool output and in the relative amount of butyric acid in faeces. The concentration of deoxycholic acid in faecal water fell as did faecal water toxicity. Crypt cell production rates obtained from rectal biopsies showed a reduced labelling index, indicative of suppressed cell proliferation.

In conclusion a number of forms of RS can reach the colon and exert different effects. RS is largely fermented, produces mainly SCFA, and may be important in determining colonic epithelial cell health through effects on bile acids, butyrate production and moderation of nitrogen metabolism.

CONCLUSIONS

The definition of resistant starch adopted in 1992 is a physiological one : "resistant starch is the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals". As a result, RS as defined is not strictly resistant to the amylases and the term RS is often found by the biochemists as confusing.

Even if there is some underestimation of the RS content in some of the food, it seems that the amount of RS actually eaten by most europeans is very small (4.1 g/day/person). Many beneficial properties of RS have been shown during the past few years. However several questions have been raised. Is it necessary and useful for the health of most of the population to increase RS consumption ? Is RS more favourable than dietary fibre to improve some metabolic disorders like hyperlipidemia or diabetes. Should we focus our attention to the possible interest of butyric acid production by RS and the energetic aspect ?

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