

NUTRITIONAL IMPLICATIONS OF RESISTANT STARCH

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INTRODUCTION AND DEFINITION OF RESISTANT STARCH

EVOLUTION OF THE RESISTANT STARCH CONCEPT

Until recently, starch was generally regarded as completely digested and absorbed in the small intestine, at least after cooking. Some studies challenged this concept by demonstrating starch in human faeces (Van der Westhuizen *et al.* 1972; Wolf *et al.* 1977) or breath hydrogen excretion related to the consumption of starch in foods (Anderson *et al.* 1981). The observation by Englyst *et al.* (1982), in the process of developing a technique for measurement of dietary fibre, that enzyme resistant starch appeared together with the non-starch polysaccharides (NSP) of some processed foods provided the starting point for the present interest in resistant starch (RS). It was then directly demonstrated by experiments with ileostomates, that starch was passing right through the small intestine after ingestion of foods such as cereals, bananas and potatoes (Englyst & Cummings, 1985, 1986, 1987). Based on these and similar studies, Englyst and colleagues (Englyst & Cummings, 1990; Englyst & Kingman, 1990) concluded that the starch referred to in 1982 as RS was only part of the starch that escaped digestion and absorption in the human small intestine, and they extended the definition of RS to include all the starch that escapes digestion in the small intestine.

Research on dietary fibre has demonstrated the importance of fermentation of indigestible carbohydrates in the human colon. A main reason for the present great interest in RS is that it provides a substrate for the microflora of the large intestine, although possible effects in the upper intestinal tract have also been explored. *In vitro* studies using human faecal inocula had shown that starch from various sources was fermented (Englyst & Macfarlane, 1986; Wyatt & Horn, 1988). From a food technology point of view it is challenging that the RS content of foods can be varied within wide limits by choice of raw material and processing conditions.

DEFINITION OF RESISTANT STARCH

Resistant starch is the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992*b*).

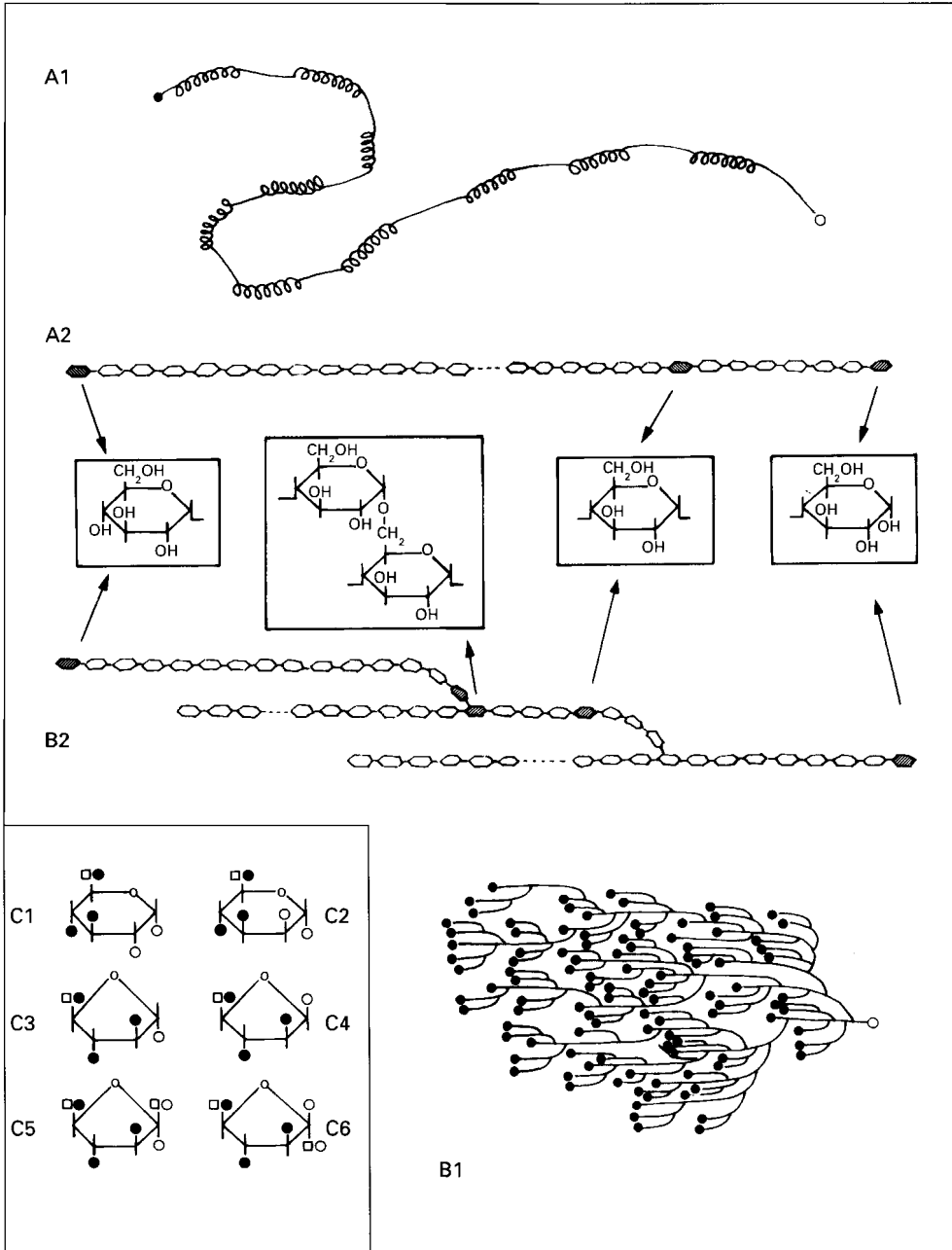


Fig. 1. Schemes of the linear, helicoidal amylose macromolecule (A1 and A2) and of the grape-like clustered amylopectin macromolecule (B1 and B2). Amylose in A1 shows one reducing (open circle) and only one non-reducing (closed circle) end-chain group. Amylopectin in B1 shows one reducing (open circle) but several thousands to several millions of non-reducing (open circles) end-chain groups. In the inset, oligosaccharides with three free hydroxy-groups (closed circles) are those allowing Con A coupling. C1 represents the α -D-glucose non-reducing end-chain group which can react especially with amylopectin (from Gallant & Bouchet, 1986).

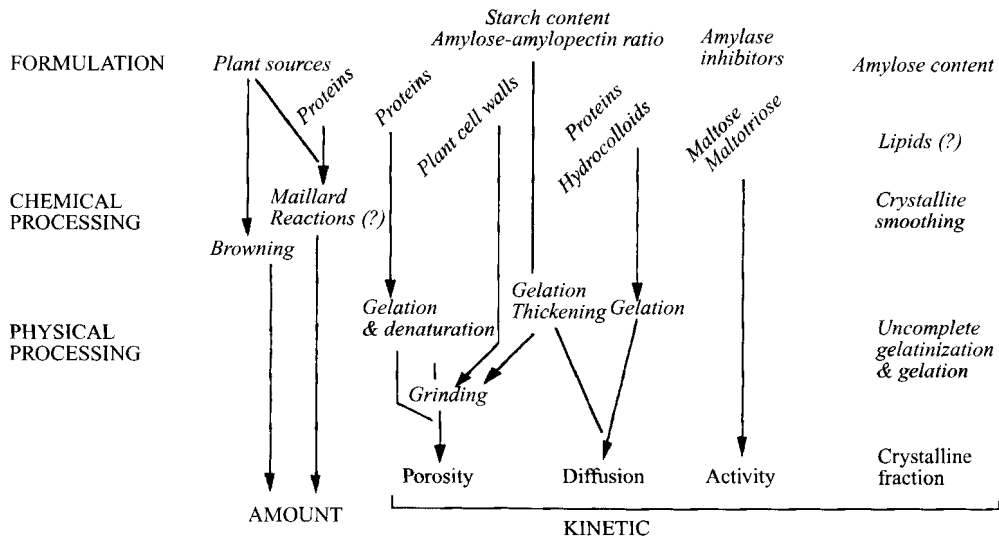


Fig. 2. Factors involved in the kinetics of starch hydrolysis by α -amylases (from Colonna *et al.* 1992). The effect of formulation and processing on the amount of resistant starch is illustrated as well as factors influencing hydrolysis kinetics, such as porosity, enzyme diffusion and activity, and crystallinity.

PHYSICAL AND CHEMICAL CHARACTERISTICS, AND ANALYSIS OF RESISTANT STARCH

STARCH – THE MOST ABUNDANT COMPONENT OF THE DIET

Starch is quantitatively the most important component of diets and a major source of dietary carbohydrate. It is composed of two principal macromolecules, amylose and amylopectin, the structures of which are illustrated in Fig. 1. For a review, see e.g. Gallant *et al.* (1992).

Amylose is a long, essentially linear polymer consisting of 1,4- α -D-anhydroglucose units with a few α -1,6-linked units. The 1,4-linked anhydroglucose units produce a gradual, natural twist of the amylose molecule in a helical conformation with six glucose residues per turn. In this structure, all the hydrophilic hydroxyl groups of the chain are on the external side of the helix, creating a hydrophobic cavity inside the helix, into which many small molecules can be bound, for instance the carbon chain of fatty acids (amylose–lipid complexes) and iodine (blue colour).

Amylopectin is one of nature's largest molecules (molecular weight 10^7 – 10^9) and has a branched structure with about 5% of the anhydroglucose units having 1,6- α -linkages. The structure is a clustering of many short 1,4- α -linked chains with an average length of 20 (range 12–70) glucose residues. The three-dimensional structure of amylopectin is not yet known in detail, but the main features are illustrated in Fig. 1.

Plants store starch in condensed granules of varying size and shape characteristic for each plant. Tuber starches, e.g. potato starch, are generally large ellipsoid or spherical granules, whereas cereal starches are small and polyhedral granules. Some cereals, e.g. wheat, also contain larger, lentil-shaped granules. Legume starches are generally kidney-shaped or ovoid.

The crystallinity of native starch granules varies from 15 to 45%, the remainder having an amorphous structure. Three crystalline forms, A, B and C, of starch granules have been identified from different diffractometric spectra. Cereal starches generally display the

A-form, whereas potatoes and some tropical tubers give the B-form. Most legume starches have the C-pattern, considered by some either as a distinct crystallographic pattern or a mixture of the A- and B-patterns.

The A pattern has water molecules located between the double helix-formed starch molecules (Imberty *et al.* 1988). In the B structure the double helices are packed densely in a hexagonal pattern with water molecules inside this structure only (Imberty & Pérez, 1988).

Native starch granules are slowly hydrolysed when exposed to amylases, B-type granules more slowly and less completely than A-type. The amorphous parts are most easily degraded and the granules become eroded. Most granules are first hydrolysed superficially, whereas extremely resistant granules, such as amylo maize, may be endocorroded, i.e. hydrolysed from the interior after penetration of enzyme through surface pores.

On heating above the gelatinization temperature, which varies with different starches, and with water in excess, the granules undergo swelling and partial solubilization, especially of amylose. This is due to breaking of hydrogen bonds between the starch molecules and intramolecular side chains. With lower water content as is often the case in foods, the crystallites melt at higher temperatures (> 100 °C).

Gelatinized starches are unstable and tend to reassociate upon lowering the temperature and at the water content of many foods. Gelation is the first step in this process. On ageing, the gels develop a B-type crystallinity. This is referred to as retrogradation. Repeated thermal treatments increase the retrogradation by increasing interchain amylose associations (for a review, see Colonna *et al.* 1992).

FACTORS AFFECTING ENZYMIC HYDROLYSIS OF STARCH

Colonna *et al.* (1992) reviewed factors affecting the enzymic hydrolysis of starch. The salivary and pancreatic amylases hydrolyse the α -1,4 bonds of amylose and amylopectin. These enzymes are able to bypass, but not to hydrolyse, the α -1,6 bonds of the branching points. The hydrolysis products are mainly maltose, glucose and dextrans containing the branching points. The intrinsic hydrolases of the small intestinal brush border membrane complete the hydrolysis to free glucose.

Amorphous or dispersed starch found in freshly cooked starchy foods is highly susceptible to salivary and pancreatic amylases. Some substances inhibit α -amylase (*EC* 3.2.1.1) activity *in vitro*: (1) proteins or glycoproteins present in legumes, but also in cereals, which are generally inactivated during cooking; (2) antinutrients such as tannins and polyphenols, which are more thermoresistant; and (3) hydrolysis products, especially maltose and maltotriose, that are further hydrolysed and absorbed, and thus of less importance *in vivo*.

Release of oligosaccharides from solid starchy substrates, such as starch granules or retrograded amylose, has more complicated kinetics. Colonna *et al.* (1992) used a mechanistic approach of hydrolysis by α -amylase in solution, and considered four successive phases: (1) the diffusion of the enzyme molecule towards its substrate; (2) the porosity of starchy substrates; (3) the adsorption of enzymes on the substrate; and (4) the catalytic event. These steps are influenced by a large number of properties in the raw material, as well as processing and storage conditions of foods, as shown in Fig. 2.

MAIN FORMS OF RESISTANT STARCH

There are a number of different reasons why starch in foods may be incompletely digested and absorbed during passage through the small intestine. Englyst & Cummings (1987) differentiated three main forms of RS: (1) starch that is physically inaccessible to digestive

enzymes owing to enclosure in food structures such as intact cells or partly milled or whole grains or seeds; (2) resistant B-type starch granules occurring in (uncooked) potatoes and green banana; and (3) retrograded amylose occurring in processed foods. These forms have been named RS 1, 2 and 3 respectively (Englyst & Cummings, 1990; Englyst & Kingman, 1990).

Although the three main forms of RS probably represent the main forms of indigestible starch in most foods, a number of other reasons for resistance should be kept in mind: amylose–lipid complexation, retrogradation of amylopectin, and the creation of new enzyme resistant glycosidic bonds by dry heating at high temperature (Asp & Björck, 1992).

CHARACTERIZATION OF RESISTANT STARCH OBTAINED *IN VIVO*

Before 1993 there were only a few studies characterizing RS (as defined above) obtained *in vivo* from humans (Champ, 1995). Starch recoveries in ileostomy effluents after consumption of various foods and diets were reviewed by Andersson (1992) and found to range from < 1% to 75% of the ingested starch, the latter figure relating to green bananas (Englyst & Cummings, 1986). Englyst & Cummings (1985, 1986, 1987) and Schweizer *et al.* (1990) identified both potentially digestible 'dietary starch' and 'resistant starch', defined as a fraction requiring solubilization with KOH or dimethylsulphoxide before hydrolysis, in ileostomy effluents. Low molecular weight dextrans and glucose were also identified.

The characterization of *in vivo* RS after ingestion of foods or reference materials containing various types of RS has been an important part of the work carried out within EURESTA and the physical characteristics of these reference materials are summarized in Table 1. Faisant *et al.* (1993*a–c*, 1994, 1995*a–c*) and Noah *et al.* (1995) showed three different fractions (Champ, 1995): (1) residues of starch granules and/or long chains of soluble starch molecules (degree of polymerization (DP) > 100); (2) a crystalline fraction with linear chains (DP about 26–38, expressed as weight average degree of polymerization); and (3) oligosaccharides with DP 5 or less together with free glucose. These fractions were found at the end of the ileum after ingestion of various sources of RS. The intermediate molecular weight fraction predominated after ingestion of retrograded or lipid complexed high amylose corn starch, constituting about 80% of the total RS. It was also the major fraction of RS found in dry beans cooked and stored at –20 °C overnight before being eaten (Noah *et al.* 1995). The high molecular weight fraction was most prominent after consumption of green banana flour (87%; Faisant, 1994), and 34% and 25% of the *in vivo* RS after bean flakes and potato flakes respectively (Faisant *et al.* 1993*c*). The small amount of RS following consumption of potato flakes contained mainly low molecular weight components.

Ekwall *et al.* (1995*b*) studied RS obtained *in vivo* from ileostomates after ingestion of diets containing autoclaved high amylose corn starch (*in vivo* RS content 44% of dry matter) or drum dried ordinary corn starch (*in vivo* RS 4%). They also identified three RS fractions. The main fraction in the effluent in both cases had an average DP ~ 80, ~ 5% of the RS was recovered in the low molecular weight fraction, and there were trace amounts of a high molecular weight fraction. A considerable part of the RS was available to enzymic degradation without solubilization, i.e. 'dietary starch' analytically.

Botham *et al.* (1995) reported a wide range of molecular weights in *in vivo* RS after a meal containing retrograded starch gel, with a main fraction containing amylose fragments with DP 70–80.

In conclusion, RS is composed of three main fractions, i.e. glucose and oligosaccharides, a crystalline fraction with intermediate molecular size, and a high molecular weight fraction

Table 1. Physical characteristics of EURESTA reference starch materials

Product	Origin	DSC					X-ray diffraction type
		Endothermic transitions			Enthalpy		
		T _i °C	T _{max} °C	T _f °C	J/g sample	J/g TS	
1 Raw potato starch	Roquette, Lestrem, France	44.4	62.2	80.6	16.7	24.6	B
2 Pregelatinized potato starch	Roquette, Lestrem, France	38.8	66.4	81.2	7.8	8.8	Amorphous
3 Bean flakes	Nestlé, Vevey, Switzerland	—	—	—	—	—	Amorphous
4 Raw green banana flour	INRA, Nantes, France	59.7	69.1	86.2	12.9	17.8	B
5 Cooked green banana flour	INRA, Nantes, France	38.6	50.9	64.6	3.5	5.1	Amorphous
6 High amylose corn starch (Hylon VII)	National Starch*	64.9	84.3	102.9	7.6	8.8	B
7 Extruded retrograded Hylon VII	CERESTAR, Vilvoorde, Belgium	121.3	140.4	159.8	5.2	6.0	B
8 Purified resistant starch (from sample 7)	CERESTAR, Vilvoorde, Belgium	121.3	144.9	158.7	5.4	5.9	B
		110.4	148.6	166.9	21.4	23.9	B

Differential scanning calorimetry (DSC) and X-ray diffraction analysis was performed as described by Leloup *et al.* (1992) at INRA, Nantes (by N. Faisant). T_i, initial temperature; T_{max}, maximum temperature; T_f, final temperature; TS, total starch.

* National Starch & Chemical Company, Bridgewater, NJ, USA.

containing residues of resistant granules as well as physically enclosed starch. The relative size of these fractions varies depending upon the source of the RS. Somewhat varying estimates have been obtained regarding the molecular size of the intermediate fraction. It is not clear whether this represents a true variation in DP of this fraction from various sources, or arises from methods used.

The presence of a low molecular weight RS fraction composed of oligosaccharides that are available for amylase digestion, and even free glucose, deserves special consideration. The digestion of starch in foods containing RS obviously continues throughout the small intestine. The low molecular weight fraction would then represent starch partly hydrolysed in the distal small intestine without being finally hydrolysed and absorbed. The failure of absorption in the distal small intestine may be simply due to lack of time, but the comparatively low activities of brush border hydrolases and limited capacity of glucose transport may also contribute to the malabsorption. Disaccharidase activities are maximal in the proximal and central parts of the small intestine; maltase (*EC* 3.2.1.20) and isomaltase (*EC* 3.2.1.10) activities decline to half maximal or less in the distal ileum (Asp *et al.* 1975). Finally, some continued hydrolysis after collection of the ileostomy evacuates cannot be excluded, but it should be noted that low molecular weight material was identified also in ileal content collected by intubation (Faisant, 1993*c*).

ANALYSIS METHODS FOR RESISTANT STARCH *IN VITRO*

The first method for analysis of RS was devised by Englyst *et al.* (1982) when discovering the enzyme resistant starch fraction in analysing foods for NSP: the difference between NSP glucose with and without solubilization with KOH or dimethylsulphoxide before the amylase/pullulanase (*EC* 3.2.1.41) hydrolysis of starch.

Johansson *et al.* (1984) and Björck *et al.* (1986) proposed a different method based on the measurement of starch remaining in a residue prepared for gravimetric measurement of dietary fibre (Asp *et al.* 1983). Heat resistant α -amylase (Termamyl) and pancreatin were used for starch hydrolysis. Residual starch was measured before solubilization, and total starch after solubilization with KOH. RS was calculated as the difference between total and residual starch. This type of method, as well as the original one of Englyst *et al.* (1982), includes milling and gelatinization steps. Of the three main forms of RS, therefore, these methods can be expected to measure only RS 3. The method based on determining starch in a dietary fibre residue has been simplified for the purpose of measuring RS by Saura-Calixto *et al.* (1993).

Berry (1986) published a method based on extensive α -amylolysis at 37 °C without gelatinization. Thus, resistance due to raw starch granules (RS 2) would be recovered, but still not RS 1. This method was slightly modified by collaborators of the EURESTA project (Method A) and tested together with the method of Björck *et al.* (1986) (Method B) in a first collaborative study within the project (Champ, 1992). Several methods were also used to determine total starch. Four different RS-containing materials were analysed: bean flakes, pregelatinized high amylose corn starch, Kellogg's corn flakes and raw potato starch. Method A gave higher values of RS in all materials. As expected, Method B did not detect any RS in the raw potato starch due to the gelatinization step included in that method. From the experiences obtained in this first interlaboratory study, further minor but important modifications of the Berry method were suggested by Goñi *et al.* (1996) and by the Unilever Research Laboratory (reported by Champ, 1992). These modifications, and an increased amyloglucosidase (*EC* 3.2.1.3) concentration to ensure a complete hydrolysis when analysing samples with a very high RS content, were included in the modification published by Faisant *et al.* (1995*d*).

Table 2. *EURESTA* reference materials: content of dry matter, total starch and various starch fractions (expressed as g/100 g as received) with the method of Englyst *et al.* (1992), provided by Dr H. Englyst, and with the method of Berry (1986) as modified by Faisant *et al.* (1995d)

Product	Dry matter	Total starch	Readily dig. starch	Slowly dig. starch	Resistant starch ¹	Resistant starch ²
1 Bean flakes	92.0	43.3	31.4	7.3	4.6	4.6
2 Raw potato starch	83.4	81.3	6.0	21.2	54.1	63.3
3 Raw green banana	99.2	72.8	3.0	15.7	54.2	47.3*
4 70% amylose corn starch	89.3	86.0	8.4	16.2	61.4	48.8
5 Extruded retrograded 70% amylose corn starch	91.2	89.9	47.2	15.3	27.4	29.8
6 Resistant starch purified	96.2	92.5	12.0	10.8	69.8	—

¹ Method of Englyst *et al.* (1992).

² Method of Berry (1986) as modified by Faisant *et al.* (1995d; * 1995a).

Englyst *et al.* (1992) devised a scheme for the classification and measurement of nutritionally important starch fractions including the three main forms of RS. The method is based upon analysis of starch fractions in foods as eaten, which is the basis for recovering even the physically enclosed RS 1. Whereas chewing would be the ideal way of disintegrating foods to mimic normal eating, mincing and standardized milling with glass balls in the presence of guar gum to increase the viscosity during the amylase digestion was chosen as a more convenient way in practice. The different starch fractions are then separated as readily digestible starch, slowly digestible starch and RS, with the possibility to determine the different forms of RS separately. The method was validated by them for a limited number of substrates in ileostomates. An *in vitro* RS assay that used chewing as the initial disintegration step was developed (Muir & O'Dea, 1992) and validated against *in vivo* studies in human ileostomates (Muir & O'Dea, 1993; Muir *et al.* 1995).

The Englyst method was first evaluated in a separate collaborative trial (Englyst *et al.* 1992), and in a second study the modified Berry method according to Champ (1992) and the Englyst method were further tested and compared (Dysseler & Hoffem, 1995a, b). Working group I (Champ, 1995; Champ & Faisant, 1995) drew some general conclusions from these method studies within the EURESTA project (Table 2):

- In general the two methods give very similar values for samples with a high level of RS.
- The modified Berry method is quicker and easier to reproduce than the Englyst method.
- Analysis of physically inaccessible starch is not solved by the modified Berry method.
- The Berry method as modified by Champ (1992) has only been validated against Englyst's method; few comparisons with *in vivo* data have been made.
- Englyst's method may reflect better the *in vivo* physiology than the other methods.
- There is a need for more *in vivo* studies (ileostomy and intubation) on real foods (as eaten).

- Further analytical studies have to be performed before any method can be recommended for labelling of RS.

RESISTANT STARCH INTAKE IN EUROPE

The RS intake in 10 different European countries was evaluated from national consumption statistics regarding starchy foods (Dysseler & Hoffem, 1995c). The calculations were based on literature data with the Englyst method (Englyst *et al.* 1992) or separate analyses of foods with the Englyst method or the modified Berry method (Champ, 1992). The mean RS intake according to these calculations would be 4.1 g/d, with variation from 3.2 to 5.7 between different European countries. Owing to differences in kind and completeness of data as well as in analytical methods, the data should be regarded as indicative only, and this survey did not allow any detailed comparison between countries. There is an obvious need for these intake data to be completed, and the main starch-containing foods analysed as eaten.

The present RS intake in Europe seems low but there is a considerable potential to increase the intake by new products with increased RS content, if RS proves to be beneficial to the consumer.

TECHNOLOGY OF RESISTANT STARCH PRODUCTION

Both the choice of raw materials and the methods and conditions for processing can affect the RS content of food ingredients and food products within wide limits. Disintegration of gross and cellular structures releases physically enclosed starch and thereby reduces the RS 1 content. Gelatinization, which is usually more or less complete in most starchy foods as eaten, with the exception of unripe fruits, would diminish RS 2 from RS granules. RS 3 is formed by retrogradation of amylose during processing, cooling and storage under moist conditions. The importance of amylose–lipid complexes, amylopectin retrogradation, chemical modification and heat treatments in dry conditions is difficult to assess at present (for review on the formation, structure and properties of RS 3 see e.g. Eerlingen & Delcour, 1995).

The great impact of food processing on RS warranted a separate group working on technological aspects, and aiming to provide suitable reference materials with different forms of RS for analytical and physiological studies (Würsch & Delcour, 1995).

FORMATION OF RESISTANT STARCH UNDER VARIOUS CONDITIONS

The RS content of common cereal foods like bread, breakfast cereals, pasta and rice is generally below 3%, potato 4–5%, potato flakes 3% (all figures on a dry matter basis, determined by the modified Berry method (Method A; Würsch & Delcour, 1995)). French fries contained 1.4% on a fat free basis, whereas Bravo *et al.* (1995) reported up to 32% RS in fried, freeze-dried and defatted potato chips.

Leguminous seeds generally have a comparatively high content of RS after processing for two reasons: the high resistance of cells towards disintegration in cooking, and the high content of amylose, > 30% (Tappy *et al.* 1986; Tovar *et al.* 1990, 1992). Cooking and freeze drying of milled and whole lentils (*Lens culinaris medicus*) made 9% of the starch resistant, and in peas 13%. Extrusion cooking of peas, on the other hand, gave only

1–1.5% RS (Würsch & Delcour, 1995). Englyst *et al.* (1992) reported the content of RS in various starch fractions of 34 different foods as determined by their method. Levels of RS in legumes ranged from 5 to 18% of the dry matter, corresponding to 12–40% of the starch.

RS in common foods can be increased by prolongation of the moist stage after cooking, by repeated cycles of heating and chilling, or by freezing. Storage of foods such as bread (30–40% water), on the other hand, does not seem to increase the RS content (Rabe & Sievert, 1992). Using a high pressure autoclave process, Escarpa *et al.* (1996) reported better control of gelatinization and increased RS yield in potato amylose and amylopectin mixtures.

Eerlingen (1994) and Eerlingen *et al.* (1993 *a, b*, 1994 *a–d*) made a series of studies on the formation of RS 3. Ordering of amylose chains resulting in RS formation occurs during rapid cooling (10 °C/min) to below 60 °C of an amylose solution heated up to 180 °C (Sievert & Würsch, 1993 *a*). Enzyme resistant starch formed from amylose fractions with different chain lengths differed in RS yield but not in RS quality (Eerlingen *et al.* 1993 *b*). In all cases, the isolated RS samples were of the B-crystalline type with similar chain lengths (number average degree of polymerization, DP_n , between 19 and 26, corresponding to about two times higher weight average degree of polymerization, DP_w). Storage of gelatinized starch for several days at temperatures between 0 and 68 °C also produced 4–6% RS with B-type crystallinity, whereas storage at 100 °C yielded about 10% RS with an A-type crystalline structure (Eerlingen *et al.* 1993 *a*).

Amylopectin, present in most starches, interferes with the ordering of amylose, thus inhibiting RS 3 formation (Sievert & Würsch, 1993 *a*; Eerlingen *et al.* 1994 *b*). The outer short chains of amylopectin can reorganize during storage of gelatinized high amylopectin maize starch, but the melting temperature of these crystallites is rather low, ~65 °C compared with 150 °C for crystallites obtained from amylose and constituting RS 3. Leloup *et al.* (1992) reported melting temperatures of around 125 °C and 46 °C for amylose and amylopectin respectively.

The importance of amylopectin retrogradation for *in vivo* RS formation is not fully explored. When amylopectin is extensively retrograded, as in stale bread, the rate of hydrolysis with pancreatic α -amylase at 37 °C *in vitro* is reduced, but the final extent of hydrolysis is not affected (Eerlingen *et al.* 1994 *b*; Würsch & Delcour, 1995). As pointed out by Englyst & Cummings (1987), amylopectin retrogradation may account for the high *in vivo* RS content found in cooked and cooled potatoes. The fact that the amylopectin from potato has longer outer chains than that from cereals could make retrograded potato amylopectin more resistant.

Added and endogenous lipids are other factors influencing RS formation through complexation with amylose. Such a complex would hinder double helix associations, thus inhibiting RS formation (Sievert & Würsch, 1993 *b*; Eerlingen *et al.* 1994 *a*). On the other hand, amylose–lipid complexes are more slowly digested and absorbed than free amylose (Holm *et al.* 1983), and may contribute to RS (Faisant *et al.* 1993 *c*).

Four products with high RS content were available for production of RS foods: raw potato starch, unripe banana, amylo maize starch, and retrograded amylo maize starch. In the case of potato starch and banana, heating conditions have to be carefully controlled to avoid gelatinization (Würsch & Delcour, 1995). Eerlingen *et al.* (1994 *d*) reported the production of a conventional straight dough bread with high RS content (up to 11% of the starch) and maintained texture and taste. The flour was partly replaced by raw or retrograded amylo maize starch (70% amylose) and gluten. Liljeberg and coworkers (Liljeberg & Björck, 1994; Liljeberg *et al.* 1995) reported 8% RS in a commercial pumpernickel bread. The long time–low temperature baking conditions used obviously favour RS formation. Porridge from high amylose barley and corn griddle cake (arepa)

prepared with high amylose corn varieties were other products with high amounts of RS. Such products would enable a considerable increase in the RS intake.

PRODUCTION OF REFERENCE MATERIALS

A number of reference materials were selected to include the three main forms of RS. It has been demonstrated that leguminous seeds can be processed with preservation of cellular structures (Tappy *et al.* 1986). This process was used to produce flakes from white beans (*Phaseolus vulgaris*) as a reference material by soaking, cooking and roller drying. Such material would be expected to contain physically enclosed starch (RS 1), but also retrograded amylose (RS 3), owing to the high amylose content (28–35%, Würsch & Delcour, 1995).

Three different materials were used as sources of RS granules (RS 2): raw potato starch, a flour from raw, freeze-dried green bananas, and a raw high amylose corn starch (Hylon VII). Pregelatinized potato starch and flour from cooked green bananas, containing marginal levels of RS, were also provided to serve as controls.

To provide reference material with high content of RS 3, the high amylose corn starch was extrusion cooked under conditions favouring retrogradation of amylose. Subsequently, the RS in this sample was enriched by treatment with pancreatic α -amylase to remove available starch.

The physical characteristics of these samples, measured by differential scanning calorimetry and X-ray diffraction, are shown in Table 1, and the contents of resistant and digestible starch in Table 2.

Food technologists and ingredient suppliers were quick to realize that processing techniques increasing the amount of RS in foods would have potential nutritional and commercial value. Autoclaved cereal starches were the first RS-containing materials to be characterized, and this led to the expression 'man-made fibre' to designate retrograded amylose. Two starches with a high content of RS are already commercially available (NoveloseTM, National Starch & Chemical Company, and Crystalean^R, Opta Food Ingredients, Inc.); they are probably both obtained from high amylose corn starch treated to promote retrogradation. These products can easily be incorporated into a number of foods such as breads or cookies. Optimal conditions for retrogradation depend on the nature of the starch, but appear to be in most cases $\sim 4^\circ\text{C}$ with a hydration level $> 70\%$ (Champ & Faisant, 1996).

PHYSIOLOGICAL EFFECTS IN THE UPPER GASTROINTESTINAL TRACT

By the definition mentioned above, RS is not absorbed in the small intestine of healthy individuals. Therefore, it is not digested to free glucose and does not provide glucose to the body, although partial enzymic degradation would in principle be possible. The presence of RS in the gut digesta could also influence gastric emptying and digestion of other nutrients, and satiety. The possibility of interference with the absorption of other compounds was also considered.

Glucose from rapidly digested starch is absorbed in the first part of the small intestine. That from more slowly digested starch can be expected to be absorbed more distally. When RS is present in the diet, starch digestion should theoretically take place throughout the whole length of the small intestine, so that glucose absorption may occur even in the terminal ileum.

In addition to the intrinsic properties of foods affecting starch digestibility, a number of physiological variables could be expected to affect the final starch digestibility, and thereby

the *in vivo* RS content. These have been called 'extrinsic factors' (Englyst *et al.* 1992) and include: the extent of chewing, the mouth to terminal ileum transit time, the amylase concentration in the gut, the amount of starch present, and any food components that might retard starch hydrolysis.

How much starch actually leaves the small intestine is of key importance in assessing the physiological effects of RS, and as a reference in the development of *in vitro* methods. Three different methods were used in human subjects – breath hydrogen and methane excretion tests, ileostomates, and intestinal intubation. Furthermore, a number of animal models were used and evaluated.

HYDROGEN AND METHANE BREATH TESTS

Hydrogen is formed in the body exclusively by bacterial fermentation in the gut. A fraction is absorbed and cleared in a single passage through the lungs. The major substrate is carbohydrate reaching the colon, and the breath hydrogen test has, therefore, been used extensively for the determination of malabsorbed carbohydrate, including RS (Anderson *et al.* 1981), and also to assess the mouth to caecum transit time.

Hydrogen that is not absorbed can be eliminated by routes such as methane production, sulphate reduction and acetogenesis (Gibson *et al.* 1990). Therefore, no simple stoichiometric relation exists between the amount of fermentable substrate (and hydrogen production) and breath hydrogen excretion. As reported by Christl *et al.* (1992), an increasing rate of hydrogen production results in reduced fractional disposal of hydrogen through the lungs.

Rumessen (1992) reviewed the hydrogen and methane breath tests for evaluation of resistant carbohydrates. The need for unabsorbable standards to compensate for the large interindividual variation was stressed. He concluded that measurement of breath methane might give supplementary information but this needed further evaluation.

Olesen *et al.* (1994) demonstrated that raw potato starch increased the breath hydrogen excretion with a dose–response relationship, although the response occurred later and had a longer duration than after wheat or oat bran. Raw potato starch even produced an elevated basal hydrogen excretion if measured after 12 h fasting. RS preparations with mainly retrograded amylose from the high amylose maize starch also tended to increase the breath hydrogen, but not significantly. There was no measurable increase in methane excretion after any of the resistant starch preparations. A far more pronounced breath hydrogen increase was found after ingestion of 200 g white wheat bread, indicating a considerable malabsorption of starch from ordinary bread, at least when eaten in such a high amount (Olesen & Gudmand-Høyer, 1995). A considerable breath hydrogen increase after white bread has previously been demonstrated (Anderson *et al.* 1981; Levitt *et al.* 1987).

In conclusion, RS results in measurable breath hydrogen increments due to fermentation. However, the response is quite prolonged, making very long follow-up periods necessary, and the results are semiquantitative only.

THE ILEOSTOMY MODEL

There are two different methods available for sampling digesta leaving the small intestine: experiments with ileostomates and intubation of healthy volunteers.

Use of the ileostomy model for the study of carbohydrate digestion and absorption and for effects of carbohydrates on sterol excretion was reviewed by Andersson (1992). The main advantage is direct and quantitative collection of ileal effluents over a defined period of time. A key issue is to avoid bacterial degradation during collection and handling of the

effluent. The almost quantitative recovery of dietary fibre components, and minimal degradation of bile acids and neutral sterols, confirm that this can be achieved in practice (for review, see Andersson, 1992). Furthermore, Cummings & Englyst (1991) found less than 5 mmol/l of short chain fatty acids (SCFA) in ileostomy effluent – a low although not zero figure which is compatible with the finding of a 100-fold increased bacterial count in the terminal ileum of ileostomy subjects, compared with the normal ileum (Finegold *et al.* 1970). The general opinion among EURESTA participants was therefore that bacterial fermentation in the ileum of ileostomy subjects is minimal and does not compromise results in practice (Cummings, 1992). There is an abundance of active pancreatic enzymes in the ileal effluents (for review, see Andersson, 1992), which means that some continued degradation of for instance starch in the collection bags before cooling is possible.

The relatively short oro-caecal time in ileostomates, compared with the total transit time where the colon is intact, means that balance experiments with accurate demarcation of the balance period can be performed within 24 h. A fasting period overnight is sufficient to ensure complete excretion of all food residues from the previous day (Andersson, 1992). The scheme, as developed in Cambridge, requires all the food under investigation to be given at breakfast. In this case the corresponding collection period can then be completed the same evening (Englyst & Cummings, 1985, 1986, 1987).

The key question remains as to whether the ileal function in ileostomates can be regarded as normal. There is a significant adaptation of the digestive and absorptive capacity in the distal ileum after establishment of the ileostomy. Adaptation to a varying extent also occurs in electrolyte and fluid absorption capacity. For this reason, it is recommended that investigations regarding, for example, starch absorption are based on groups of subjects (Andersson, 1992, 1995). No evidence of adaptation in starch absorption has however been obtained (Cummings, 1992; Andersson, 1992). Andersson (1995) compared two studies in which ileostomates had been tested in the early postoperative state (Andersson *et al.* 1984a), and more than six months postoperatively (Andersson *et al.* 1984b). The excretion of energy and carbohydrate (by difference) was similar.

For this type of study, the importance of selecting ileostomy subjects operated on for ulcerative colitis, with minimal ileal resection, has been stressed (Andersson, 1992; Cummings, 1992). Such subjects have bile acid losses of less than 1 g/d (Bosaeus & Andersson, 1987) and serum cholesterol concentrations in the normal range (Ellegård & Bosaeus, 1991).

Andersson (1992) reviewed the data on starch excretion in ileostomates, reporting results ranging from 2% or less of the ingested starch in oats and diets based on rice and white bread, to 12% from cooked and cooled potatoes, and 75% from green bananas.

Langkilde & Andersson (1995a, b) reported preliminary data on RS content in the EURESTA reference materials, as assessed *in vivo* using the ileostomy model. The results, expressed as g RS/100 g starting material were: raw potato starch 67.9, raw green banana flour 55.3, high amylose corn starch 43.7, and extruded retrograded high amylose corn starch 32.1. The pregelatinized potato starch control had only 0.8 and the cooked green banana flour 4.4 g RS/100 g. Bean flakes from a different batch have been investigated previously by Schweizer *et al.* (1990), who found between 9.0 and 10.9% RS in that material (corresponding to about 20% of the starch).

INTUBATION TECHNIQUES

The quantitative assessment of ileal flow can be made in man after intestinal intubation using the technique of constant perfusion of a solution containing a non-absorbable marker (Flourié, 1992). Flourié *et al.* (1988) used this technique for measuring starch malabsorption

from different diets. Volunteers are intubated with a triple lumen tube weighted by a mercury bag. When this has reached the colon, sampling of ileal contents takes place by aspiration 5 cm above the ileocaecal junction. A non-absorbable marker, polyethyleneglycol (PEG), is infused at a constant rate 25 cm proximal to the aspiration port, and the ileal flow is then assessed by estimating the dilution of the marker, which is presumed to be homogeneously mixed with the gut contents.

It can be argued that, with solid substrates such as RS, a liquid marker may not be suitable, and Flourié *et al.* (1988) found that PEG as a meal marker passed more rapidly than starch. However, the distance between the perfusion of the flow marker and the collection point (25 cm) could be optimized to minimize dissociation between liquid and solid phases and to obtain an optimal homogenization between the bolus and the marker. ^{14}C -polyethyleneglycol and ^{111}In have been used as meal markers to control the calculation of the RS flow at the end of the small intestine. Faisant *et al.* (1992) showed that solid phase markers of rare earth metals were suitable as starch tracers, but the safety for humans is not fully clear. Faisant *et al.* (1992) tested a number of solid phase markers, and ^{111}In was subsequently chosen as a meal marker to study one of the EURESTA reference materials, the raw banana flour (Faisant *et al.* 1995a, c). Using PEG as flow marker and ^{111}In as meal marker, Faisant *et al.* (1995a, b) found that 19.3 g (range 16.7–21.5) RS was recovered from the end of the ileum after ingestion of 30 g raw banana flour, corresponding to 64% RS. These values should be compared with 15.8 (13.3–19.2) g obtained in ileostomy studies using the same experimental meals (Langkilde *et al.* 1995). These limited data suggest that the intubation technique may provide somewhat higher estimates of RS than the ileostomy model, but more comparative studies are needed.

Another study with the intubation technique by Molis *et al.* (1992) showed 49.4 and 20.6% malabsorbed starch in lipid complexed and retrograded high amylose corn starch respectively. The *in vitro* content of RS, using the Berry method, was only 29.9 and 12.8% respectively, again indicating a higher than expected malabsorption using the intubation technique.

The advantage with the intubation technique is the possibility of studying individuals with an intact gut. It is, however, time consuming, invasive and expensive (Flourié, 1992). Furthermore, intubation reduces the intestinal residence time (Read *et al.* 1983). The importance of this effect for *in vivo* quantification of RS has not been investigated.

ANIMAL MODELS

There is a great need for appropriate animal models to study RS, as well as nutrition studies in general. For example, the numbers of subjects with ileostomies are decreasing owing to new surgical techniques creating continent pouches or rectal anastomoses. Animal models should have a digestive physiology and a habitual diet with a composition as close as possible to humans. The animal models should be evaluated in the same way as instrumental experimental techniques, and their advantages and weak points recognized.

Bach Knudsen (1992) reviewed five different animal models: antibiotic treated rats, caecectomized rats, colectomized rats, hydrogen excretion in rats, and ileum cannulated pigs regarding their usefulness for predicting starch digestibility in humans. The main advantages and disadvantages of these techniques are summarized in Table 3.

The antibiotic treated rat model was introduced by Eggum (1973), and has proved useful in studies on RS (Björck *et al.* 1986; Hansen *et al.* 1988). These authors used Nebacitin (a 1:2 mixture of Neomycin and Bacitracin) at 0.7% in the diet, which has been shown to reduce caecal and colonic microbial activity by 80–90%, allowing estimation of ileal digestibility by analysis of faeces (for review, see Bach Knudsen, 1992). However, there are

Table 3. *Advantages and shortcomings of animal models for measuring starch digestibility in humans (from Bach Knudsen, 1992)*

Animal model	Advantages	Shortcomings
Antibiotic treated rats	Low cost Easy to use and standardize High precision Can be adopted in most laboratories	Efficiency of Nebacitin must be checked (pH or ATP) Long term use may cause development of resistance to Nebacitin Preserved α -amylase in caecum may cause further degradation of starch Enlargement of caecum can cause difficulties in interpretation of results
Caecectomized rats	Eliminate 70–80% of the microflora without the use of drugs	Requires skilled persons to perform surgery Microflora not completely eliminated Digestibility of starch certainly overestimated
Colectomized rats	Eliminate 90% of the microflora without the use of drugs	Requires skilled persons to perform surgery Necessary to use markers to calculate digestibility
Hydrogen excretion in rats	Simple and non-invasive	Not suited as a quantitative model
Ileum cannulated pigs	Close in digestive physiology to humans Digestibility can be measured in the small and large intestine of the same animal Can be used to study the impact of food macrostructure	Requires skilled persons to perform surgery Stomach and small intestine of pigs more heavily colonized with micro-organisms than in humans Requires a marker technique to estimate digestibility in the small intestine Relatively expensive to use

still significant concentrations of SCFA present in caecal contents in spite of the antibiotic treatment (Bach Knudsen *et al.* 1982). In practice, measurement of caecal pH provides a simple way of ensuring efficient microbial suppression. Typically, pH is close to 8, as compared to 5–7 in rats not receiving any antibiotics (Björck & Asp, 1992).

The antibiotic treatment has a number of effects in the gut, similar to those found in germ free rats. Notably, the caecum is enlarged two to six times with 50-fold increase in total α -amylase activity, compared with normal rats (Boisen *et al.* 1985). The mean transit time through the gut has been reported to increase from 32 h in controls to 47 h in antibiotic treated rats fed a diet containing 4% dietary fibre. At a higher level of fibre intake (18%), the differences were less pronounced, i.e. 22 h and 25 h respectively. The prolonged retention of digesta together with the high amylase activity provide conditions for continued enzymic starch degradation in the colon. It is not known if degradation products are absorbed passively to any important extent.

For samples containing mainly the retrograded amylose type of RS, a number of studies (Björck *et al.* 1986, 1987; Björck & Siljeström, 1992; Tovar *et al.* 1992; Granfeldt *et al.* 1993) have shown a very good correlation between RS estimates using the antibiotic treated rat and the total starch remaining in the dietary fibre residue prepared according to Asp *et al.* (1983). This was true also for three of the samples used in the initial EURESTA ring test (bean flakes, retrograded high amylose corn starch and corn flakes; Champ, 1992). On the other hand, the fourth sample, raw potato starch, had 23% RS according to the rat model, but virtually none with the *in vitro* method owing to the gelatinization step included in it. The Berry method gave higher values for all the samples (Asp *et al.* 1992). When investigating the EURESTA reference samples, Ekwall *et al.* (1995a) found slightly lower *in vivo* RS estimates with the antibiotic treated rat model than *in vitro* with the Englyst or modified Berry methods for the processed samples (bean flakes, cooked green banana flour and extruded high amylose corn starch), while the antibiotic treated rat was surprisingly efficient in digesting the raw starches, especially that in raw green banana flour. Only about 10% of the raw banana starch passed through the gut of the antibiotic treated rats, but it should be noted that the sample had to be milled before incorporation in the diets, which might have damaged the starch granules to some extent. Of the raw potato starch, 42% was recovered in faeces, whereas Mathers *et al.* (1995) reported that 75% of raw potato starch was passing from the ileum in rats.

In conclusion, the antibiotic treated rat model seems to have good potential for ranking processed foods according to starch digestibility, but seems to underestimate the RS content especially in foods containing raw starch granules. The necessity of milling samples to get good control of the feed intake limits the possibility of assessing physically enclosed RSI.

Surgically modified rats, caeectomized or colectomized with an ileorectal anastomosis, have also been used in starch digestibility studies (Bach Knudsen *et al.* 1991; Hildenbrandt & Marlett, 1991). In addition to the ethical concern, such rats have considerably decreased intestinal transit time and reduced digestibility of nutrients.

Hydrogen excretion in rats has been tried to follow fermentation of indigestible carbohydrates. It was concluded, however, that this technique could not be used to quantify fermentable substrates escaping digestion in the small intestine, but rather related to the rate at which substrates were delivered to the microorganisms (Hansen, 1989; Bach Knudsen, 1992).

Ileal cannulation of pigs is a well established technique that has contributed much to our understanding of digestive-absorptive events (Bach Knudsen, 1992). One main advantage is that the pig is closer physiologically to humans than are rodents. Microbial activity in the longer small intestine is, however, much more extensive than in man.

Rat feeding trial

Gee *et al.* (1995) studied effects of RS on intestinal structure and function. The test materials were high amylose maize starch (Hylon VII), retrograded Hylon VII, 'Quenched' Hylon VII (gelatinized and rapidly cooled in liquid nitrogen to avoid retrogradation), and the extruded retrograded high amylose maize starch used as one of the EURESTA reference materials. These starches were given to normal rats as 20% of the diet for three weeks, and changes in the gut structure and function were followed. After the three weeks' feeding period, all the rats fed the RS-containing preparations had significantly higher faecal output. Liver mass and chemical composition were unchanged, and there was no evidence of changes in plasma lipid levels. Inconsistent effects were seen on crypt length in the jejunum. In the caecum crypt width was increased in the presence of RS. This may be associated with increased fermentative activity, evidenced by a fall in caecal pH of between 0.5 and 1.0 unit. Reductions in crypt cell proliferation rates were seen at various sites of the small and large intestine, whereas an increase was demonstrated in the caecum. It was concluded from these studies that the physiological effects of the consumption of RS were associated mainly with its fermentation in the large bowel. Rapid fermentation appears to favour an increase in crypt cell production rate in the caecum with a subsequent decrease at the colonic sites, whereas slower, persistent fermentation has less marked effect on the large bowel (Gee *et al.* 1995).

EFFECTS ON GLYCAEMIC RESPONSE, SATIETY, AND THERMOGENESIS

Since RS is not absorbed in the small intestine, it would not be expected to influence the postprandial hyperglycaemia after a meal, unless it interferes with the absorption of digestible starch or other nutrients. As pointed out by Raben *et al.* (1994*a, b*), a possible effect on satiety could be either negative due to decreased amount of absorbed carbohydrate, or positive if the RS had effects like some kinds of dietary fibre, for example on gastric emptying.

A joint study was performed within EURESTA on the effect of raw *v.* pregelatinized potato starch on postprandial glucose and hormonal responses, and on subjective sensations of hunger and satiety in healthy male volunteers (Raben *et al.* 1994*a*). The raw potato starch meal, containing 27.1 g RS and 13.6 g digestible starch, resulted in significantly lower postprandial glucose, lactate, insulin, gastric inhibitory polypeptide, glucagon-like peptide-1 responses and a reduction in the satiating power of the meal, compared with the meal containing 46.5 g digestible starch (Raben *et al.* 1994*b*). De Roos *et al.* (1995) confirmed that 30 g RS 2 or RS 3/d for a week had little influence on appetite and food intake.

In the same experimental setup, Tagliabue *et al.* (1995*a, b*) and Heijnen *et al.* (1995*b*) found that the increase in postprandial energy expenditure was significantly greater after the test meal containing pregelatinized starch. The increase obtained after raw potato starch corresponded to its content of digestible starch, supporting the conclusion that RS has no thermogenic effect and does not influence the thermogenic response to digestible starch. Previously Ranganathan *et al.* (1994) reported that highly resistant starch (Lintner *i.e.* acid extracted high amylose corn starch) gave a slight but not significant glucose response when compared with cellulose. There was no thermogenic effect with the resistant starch or cellulose, nor any effect on the metabolic rate.

Granfeldt *et al.* (1995) studied the glycaemic response to arepa breads prepared from

ordinary (25% amylose) 'dent', or high amylose (70% amylose) corn, in which 4% and 30% respectively of the starch was resistant (Granfeldt *et al.* 1993). The two products were given to healthy volunteers in equivalent amounts, either regarding total starch or digestible starch. In both cases, the glycaemic response after the dent product was significantly higher than after the high amylose product. This shows that the reduced glycaemia after the high amylose product was not simply related to a decreased proportion of digestible starch, but rather to a reduced enzymic accessibility of the digestible starch in the presence of RS (Granfeldt *et al.* 1995).

EFFECTS ON PLASMA LIPIDS

Dietary fibre of the soluble, viscous type has a well documented effect in lowering the low density lipoprotein cholesterol level. The possibility of such effects of RS has been studied in animal and human experiments, and by measuring effects on sterol excretion from the small intestine.

De Deckere *et al.* (1993) found that high amylose corn starch lowered the blood cholesterol level in rats by 10–15%, which is in line with several other studies (Sacquet *et al.* 1983; Mathé *et al.* 1993). De Deckere *et al.* (1993) also found significant reductions in triacylglycerol levels. In a recent study De Deckere *et al.* (1995) tested different types of RS in rats. They found no effect on total serum cholesterol but again reductions in the triacylglycerol concentration. An earlier study by Demigné & Rémésy (1982) showed reductions in both cholesterol and triacylglycerols by feeding raw potato starch to rats, which was confirmed in a more recent study from the same group (Morand *et al.* 1994).

Thus, several animal studies have shown that RS may reduce serum lipid levels. In order to investigate mechanisms involved in this effect, De Deckere and colleagues studied faecal sterol excretions in rats (De Deckere *et al.* 1995; Verbeek *et al.* 1995). There was no effect of RS on the liver cholesterol content, but the serum total bile acids concentration increased, suggesting an increased enterohepatic bile acid pool. There was no effect of RS on caecal content or faecal excretion of neutral sterols. Total bile acid excretion, on the other hand, increased and this may be a mechanism for the cholesterol reducing effect. There was also an increased ratio of primary over secondary bile acids. In a human ileostomy study, fully reported by Ekwall *et al.* (1995*b*), Langkilde & Andersson (1992) found a significantly lower cholic acid excretion after retrograded high amylose corn starch than after the pregelatinized ordinary corn starch used as a control, but there was no effect on cholesterol or chenodeoxycholic acid excretion.

With this background, possible effects of RS on plasma lipids in man have been investigated. In a pilot study by Olesen *et al.* (1995) there was no significant effect on blood cholesterol concentration by 50 g/d of raw potato starch or high amylose corn starch, but it was stressed that the baseline values fluctuated considerably, masking any minor effects. A more comprehensive study recently performed by Heijnen *et al.* (1996) in healthy volunteers indicated that there was no significant effect of supplementing 30 g/d RS 2 or RS 3 to the diet on fasting levels of serum total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, or triacylglycerols. Ranganathan *et al.* (1994) observed, within an acute study, that a high amylose corn starch had no effect on blood non-esterified fatty acids (NEFA) and NEFA oxidation comparable with cellulose. However, a lowering of blood NEFA was observed by Faisant *et al.* (1994) in two subjects with subnormal levels of fasting NEFA. This observation has to be confirmed in a much higher number of subjects.

In conclusion, RS does not seem to have major effects on plasma lipid levels in healthy,

normolipidaemic humans. The effects demonstrated in animals probably require higher doses of RS than is feasible in practice in a human diet.

EFFECTS ON MINERAL BALANCE

Resistant starch type 2 (resistant granules), but not retrograded RS (type 3), increased apparent calcium absorption in rats by enhancing the solubility of these minerals in ileal and caecal digesta, probably by decreasing the luminal pH through fermentation (Schulz *et al.* 1993). To study possible effects on mineral balance in man, Heijnen *et al.* (1995a) gave RS, 30 g/d, either raw or pregelatinized high amylose corn starch, in supplements to 24 apparently healthy male volunteers. The apparent absorptions of Ca, Mg and P were not affected by RS supplementation. The authors mentioned three possible reasons for the discrepancies between animal and human experiments in mineral absorption: (1) a possible difference in fermentation efficiency between man and rat; (2) the use of a different kind of RS in the rat study, that may be less resistant to fermentation; and (3) the fact that rats consumed on average 17 g RS per kg body weight daily, compared with only 0.4 g in the human study (Heijnen *et al.* 1995a).

PHYSIOLOGICAL EFFECTS IN THE LARGE BOWEL

Before the inception of the EURESTA programme, there were few studies on the effects of RS on large bowel function (for review, see e.g. British Nutrition Foundation, 1990; Livesey, 1992; Annison & Topping, 1994). Indirectly, the fact that faecal bulking of foods containing dietary fibre is more pronounced than is expected from their content of NSP indicates a 'carbohydrate gap' (Stephen 1991) that could be filled by RS. Cummings & Macfarlane (1991) postulated that 8–40 g enzyme resistant starch could enter the large bowel daily on a British diet.

The studies on large bowel effects of RS within EURESTA were summarized by Cummings *et al.* (1995). Owing to the insolubility and absence of hydrophilic and viscosity increasing properties of RS, physiological effects could be expected to originate primarily from its fermentation in the large bowel. Some early studies show that starches from a number of sources are fermented at various rates, and that fermentation products include hydrogen, CO₂, acetate, propionate, butyrate and lactate (Englyst & Macfarlane, 1986; Macfarlane & Englyst, 1986; Wyatt & Horn, 1988). The possibility that RS might be an especially good substrate for butyrate production, first suggested from *in vitro* studies by Englyst *et al.* (1987), increased the interest in starch as a fermentation substrate in the human colon.

Animal studies mainly in rats had shown a different extent of fermentation of RS from various sources (e.g. Faulks *et al.* 1989), and evidence for effects on cell proliferation were present (Demigné & Rémésy, 1982; Gee *et al.* 1991). The use of breath hydrogen excretion as an indirect measure of starch fermentation is reviewed above. Starch infusions into the colon were used by Flourié *et al.* (1986), who demonstrated lowering of pH and production of SCFA as a result of the fermentation.

The working group on large bowel effects of RS agreed that the priorities were to determine the amount of RS reaching the large intestine, and to study its fermentation using both *in vitro* and *in vivo* methods. Secondly, the impact of fermentation on colonic luminal events, epithelial cell physiology, and metabolism at other sites in the body required further investigation, e.g. regarding effects on faecal composition including bile acids, SCFA and nitrogen metabolism, with special emphasis on factors of importance in colonic carcinogenesis (Cummings *et al.* 1995). Two joint initiatives were set up:

development of a simple standardized *in vitro* method for fermentation in the laboratory using human faecal inocula, and studies on the impact of RS on cellular proliferation in the gut.

FERMENTATION AND RING TEST FOR ITS MEASUREMENT *IN VITRO*

Previous studies of carbohydrate fermentation by human faecal flora *in vitro* have used different conditions – medium, buffers, inoculum size and fermentation time – which make comparison between studies difficult (Cummings *et al.* 1995). A standard *in vitro* test was therefore devised within EURESTA and tested in eight laboratories with 40 individual faecal inocula. The results are summarized by Cummings *et al.* (1995) and reported fully by Edwards *et al.* (1996).

The starches studied in this ring test were pregelatinized potato starch (0% RS), raw potato starch (54% RS), highly refined retrograded amylose (60%) and glassy pea starch (5.8% RS). Faeces from healthy adult donors were processed within 1 h of passage and made into a 32% slurry with phosphate buffer pH 6.5. This was then diluted in equal volumes with phosphate buffer containing the starch to be tested. Anaerobic conditions were maintained as far as possible. In general, the agreement between laboratories was regarded as good for both SCFA production and starch remaining at various time intervals during the 24 h experiment, with clear discrimination of fermentation rate between the various starches under investigation. Retrograded amylose was slowly fermented, which may have implications for its action in the human colon. There were no differences between methane and non-methane producers in starch fermentation nor in the stoichiometry of fermentation products (Cummings *et al.* 1995).

A number of studies point to the SCFA pattern from RS breakdown being different from that of NSP. The preferential production of butyrate in the fermentation of RS, demonstrated *in vitro*, has been confirmed in human faeces (Scheppach *et al.* 1988; Van Munster *et al.* 1994) but not in animal studies (Mathers & Smith, 1993; Berggren *et al.* 1995). The yield of SCFA from starch is relatively high, approaching 60% on a weight basis. As a consequence, the energy from RS may be higher than from NSP which gave a lower yield of SCFA (Cummings *et al.* 1995).

ANIMAL MODELS

As mentioned above, animal models (rats and pigs) have been used to quantify the starch flow to the large bowel, i.e. for determining the amount of RS. The fermentation pattern has been studied by SCFA analyses of caecal contents and faeces. Starch reaching the caecum is usually readily fermented, and the retrograded type of RS (RS 3) can usually not be detected in faeces (Björck *et al.* 1987; Goodlad & Mathers, 1992; Key & Mathers, 1993). Although it has been reported that starch fermentation yields increased proportions of butyrate, Mathers & Smith (1993) found that with high intakes of raw potato starch the proportion of butyrate declined, possibly because of an effect of prolonged caecal transit time (Mathers & Dawson, 1991).

The fate of absorbed carbon from digestion and/or fermentation of starch was investigated by feeding rats a single dose (20 mg) of either ¹⁴C-retrograded or ¹⁴C-gelatinized bean starch. At 3 and 18 h after dosing with the retrograded starch, most of the radioactivity incorporated into the animal's tissue was recovered in carcass, pelt and liver. The radioactivity was mainly incorporated into protein, especially into glutamic acid and aspartic acid (Abia *et al.* 1993, 1995, 1996; Cummings *et al.* 1995).

CELL PROLIFERATION

In a human feeding study with healthy volunteers consuming 45 g raw high amylose corn starch, the faecal output of butyrate increased, both in absolute amounts and as a proportion of total SCFA (Van Munster *et al.* 1994). The concentration of deoxycholic acid in faecal water fell as did faecal water toxicity as measured by exposing colonic cell cultures to faecal water extracts; the latter could be a consequence of reduced pH due to the fermentation. Crypt cell production rates obtained from rectal biopsies showed reduced labelling index, indicative of suppressed cell proliferation (reviewed by Cummings *et al.* 1995).

BOWEL HABIT

The major end products of starch fermentation are SCFA and bacterial cells. Livesey (1995) estimated from unpublished data on rats (Mathers) and humans (Englyst & Cummings) that bacterial dry matter production was ~ 250 g/kg starch fermented. This would be expected to contribute to the faecal bulk because of the high intracellular water content of bacteria.

A number of studies on the effect of RS on bowel habit have been reported but findings are somewhat inconsistent. There was little effect on colonic function and no significant difference in faecal bulk when comparing a diet with breakfast cereals providing 10 g/d RS *v.* another cereal providing only 1 g/d (Tomlin & Read, 1990). Shetty & Kurpad (1986), on the other hand, demonstrated an increased faecal bulking by increased starch intake. A number of studies have shown no effect on transit time (for review see Cummings *et al.* 1995). Obviously, more studies are needed on the faecal bulking effect of various types of RS in man.

BILE ACID METABOLISM

Van Munster *et al.* (1995) demonstrated, using an *in vitro* human faecal incubation system, that addition of fermentable substrate in the form of lactulose or RS (Hylon VII) can inhibit the conversion of primary into secondary bile acids and decrease the concentration of soluble deoxycholic acid. This secondary bile acid has been shown to be cytotoxic. In the study by Van Munster *et al.* (1994) in which 45 g/d of raw high amylose corn starch with about 60% resistant starch was given to healthy volunteers for 2 weeks, the fraction of secondary bile acids was decreased from 93% to 82%, and the cytotoxic bile acids in the caecal water fraction decreased significantly (Cummings *et al.* 1995).

CALCULATING THE ENERGY VALUE OF RESISTANT STARCH IN MAN

Livesey (1995) reviewed the energy value of RS in man, noting the fact that whereas the amounts of energy made available from NSP and sugar alcohols have been reasonably well defined, the amounts from RS have received little attention so far. The working group on energy chose a model established for unavailable carbohydrate (NSP and RS) (British Nutrition Foundation, 1990):

$$E = (1 - A - B - C) \times D \times G \times H.$$

In this model, *E* was the energy value, *A* was the efficiency of microbial mass production, *B* was the efficiency of combustible gas production, i.e. H₂ and CH₄, *C* was the heat of fermentation (kJ/kJ of starch fermented), *D* was the extent of fermentation of RS in the

large intestine, G was the yield of ATP per kJ SCFA produced in fermentation as a fraction of the ATP that would have been produced had the energy absorbed been glucose, and H was the heat of combustion of resistant starch, 4.2 kcal or 17.5 kJ/g. The aim was to establish reasonable numerical values for the unknowns for RS.

Based on a number of studies, e.g. Livesey *et al.* (1990), reviewed by Livesey (1995), the efficiency of microbial biomass production (A in the model) was estimated to be approximately 0.3 kJ/kJ RS fermented. Regarding B , no quantitative experiments have been reported measuring how much energy might be lost as combustible gases. Livesey (1992) and Livesey *et al.* (1993) reviewed all types of carbohydrates and sugar alcohols investigated so far, concluding that this loss is $\leq 5\%$ of all the carbohydrate energy fermented. A similar figure for RS is supported by a number of *in vitro* experiments reviewed by Livesey (1995).

As mentioned previously, human studies (Raben *et al.* 1994*a, b*; Heijnen *et al.* 1995; Tagliabue *et al.* 1995*a, b*) have indicated that the resistant fraction of raw potato starch is not thermogenic. These studies, however, consider only the non-fermentative phase of intestinal degradation. A number of preliminary studies, reviewed by Livesey (1995), indicate that the thermogenic effect of the fermentative degradation of carbohydrates is of the order of 3 kJ/g. While the range of RS investigated and the number of observations in adults are limited, it did not appear that RS would be more thermogenic than expected, and that the terms C and G would together account for about 20% of the energy in starch fermented, i.e. 3 kJ/g.

There is evidence that RS does not alter the absorption of other energy yielding nutrients. With increasing intake by rats of RS from raw potato starch, Mathers *et al.* (1995) reported a progressive increase in dry matter lost from the terminal ileum, which paralleled the increase in RS. In ileostomates fed RS from raw green banana, raw potato and retrograded high amylose corn starch, the ileal losses of starch contributed 87, 85 and 114% respectively of the increase in dry matter lost from the ileum (Langkilde *et al.* 1995). These data indicate that the model does not need inclusion of a term for changes in macronutrient absorption due to RS ingestion (Livesey, 1995).

The numerical values suggested for the constants, i.e. $A = 0.30$, $B = 0.05$, $C + G = 0.20$, are similar to those proposed for unavailable carbohydrates in general (British Nutrition Foundation, 1990; Livesey, 1992). Substituting these values in the model gives a value of 8.8 kJ/g for fully fermentable RS. This value is expected to decrease with decreasing fermentability, D .

CONCLUDING REMARKS AND FURTHER RESEARCH NEEDS

RS has been established as one of the main sources, together with NSP and oligosaccharides, of carbohydrate substrate for the colonic microflora, and thereby is one determinant of large bowel function in man. Physical enclosure, ungelatinized starch granules of the B-type, and retrogradation are the main reasons for resistance. The rate and completeness of fermentation of RS varies depending on the source and heat treatments used. This creates the possibility of influencing the site of RS fermentation in the large bowel through appropriate choice of raw material and processing conditions.

Methods for the determination of RS are designed to estimate the residue after treatment of the sample with enzymes simulating normal starch digestion in the small intestine. Two main approaches for determination of total RS have been evaluated. A critical step is simulation of the normal disintegration of foods by chewing in order to recover physically enclosed starch.

Some animal experiments have shown cholesterol lowering properties, but such effects have not been confirmed in normolipidaemic man with doses of RS that can be readily incorporated in a diet and tolerated. However, hyperlipidaemic subjects have not yet been studied. RS did not seem to affect postprandial energy metabolism and had no effect on mineral balance in humans. The caloric value has been estimated as ~ 2 kcal/g. There is evidence for the production of a comparatively high proportion of butyrate from the fermentation of starch. This and other SCFA may have health promoting effects on colonic epithelial cells through effects on the conversion of bile acids, nitrogen metabolism and faecal bulk.

The effects of different types of RS should be further explored, regarding site of fermentation, fermentation products, effects on cell proliferation, colonic nitrogen metabolism and other factors of importance for colonic health, especially cancer risk.

The current intake of RS in different population groups seems to be rather low (~ 4 g/d) but this should be investigated further. In view of the various physiological effects discussed above, production of foods designed to contain RS with optimal physiological properties seems to have a considerable health benefit potential.

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APPENDIX

In 1990, the Commission of the European Communities approved a concerted action project within its Food-Linked Agro-Industrial Research programme, entitled 'Physiological Implications of the Consumption of Resistant Starch in Man' (FLAIR Concerted Action No. 11, COST 911). The project, named by the acronym EURESTA, involved up to 40 research groups from 11 European countries and lasted four years.

Proceedings from the second plenary meeting held in 1991 have been published (Asp, 1992a), giving a state of the art description at the beginning of the project. The present review focuses on the work carried out by participants in the project since then, and is based especially on a final publication from the project (Asp *et al.* 1995), containing working group reports and summaries of presentations at the last plenary meeting in 1994. The review has been organized according to the working group structure of the project.

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