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Resistant starch—a review of the physical properties and biological impact of RS_3

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Abstract

Resistant starch (RS) encompasses forms of starch, which are not accessible to digestive enzymes. By far, retrograded starch, and particularly retrograded amylose, are the most thermally stable forms. Retrograded amylose is especially useful as a source of thermally stable RS_3 for commercial food applications, since it survives most food processes.

Retrograded starch has been studied extensively for understanding the behavior of gels and certain staling processes in foods. Characterization of retrograded starch has been done as it interferes with the total dietary fiber (TDF) assay, with an emphasis on the negative impact of retrogradation. Until recently, little is known on the nutritional and commercial value for retrograded starch as an RS_3 product.

Early studies into the digestibility implied that retrograded amylose was non-nutritive, but more recent studies show that amylases, in fact, slowly attack the structure. Consequently, glucose and other oligosaccharides are released from retrograded starch over a sustained period through the normal digestive process. Modulation of glucose release and uptake in humans can be an important consideration in the use of resistant starch in food products for certain target groups, such as diabetics and athletes.

Sources of resistant starch are reviewed, with a focus on the principles behind the production of a food ingredient highly concentrated in retrograded amylose. Applications include a product for modulating the glucose response of diabetics, and effects on an extruded cereal product.

Keywords: Total dietary fiber; Resistant starch; Glucose

1. Introduction

Resistant starch (RS) was first recognized as a complicating factor in the determination of total dietary fiber (TDF) levels by the Prosky Method (Englyst, Trowell, Southgate & Cummings, 1987). Since the definition of dietary fiber specifies "non-starch polysaccharides" (Asp, Furda, Schweizer & Prosky, 1988), it is clear that any form of starch, which interferes with the assay, is not a traditional fiber. Subsequent research has shown that RS is not rapidly digested like ordinary starch, and that this feature imparts biological benefits. Some of the benefits are like traditional fiber, and others are unique to resistant starch. This article reviews the physical nature of resistant starch, its occurrence in food, its physiological benefits and potential use.

RS has been defined as the fraction of starch, which escapes digestion in the small intestine, and may be digested in the large intestine (Englyst, Kingman & Cummings, 1992). This is similar to the traditional definition, except for the qualification that fiber is non-starch in origin. A number of factors contribute to starch's resistance to digestion, which have led to four categories, each with similar resistance properties. They are as follows.

RS_1: physically inaccessible to digestion by entrapment in a non-digestible matrix;
RS_2: ungelatinized starch;
RS_3: retrograded starch;
RS_4: chemically modified starch.

RS_3 is of particular interest, because of its thermal stability. This allows it to be stable in most normal cooking operations, and enables its use as an ingredient in a wide variety of conventional foods.

2. Formation of resistant starch

Starch occurs in many plant tissues as a granule. Granules are usually between 1 and 100 μm in diameter, depending upon the plant source. Starch is comprised of two molecular types: amylose, the straight chain polyglucan comprised of

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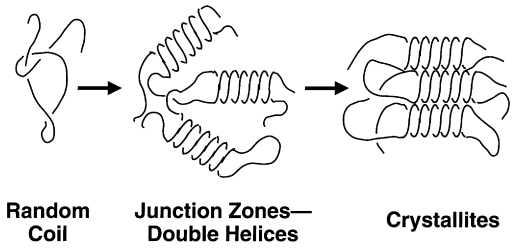

Fig. 1. Schematic of amylose retrogradation.

approximately 1000, α-D-(1 → 4) linked glucoses; and amylopectin, the branched glucan, comprised of approximately 4000 glucose units with branches occurring as α-D-(1 → 6) linkages (Zobel, 1988). Within the granule, starch is tightly packed in a radial pattern, and is relatively dehydrated. This compact molecular structure limits the accessibility of digestive enzymes, various amylases, and accounts for the resistant nature of raw starch granules, RS_2. In the diet, raw starch is consumed in foods like banana.

Starch granules are disrupted by heating in an excess of water in a process commonly known as *gelatinization*, which renders the molecules fully accessible to digestive enzymes. Some sort of hydrated cooking operation is typical in the preparation of starchy foods for consumption, rendering it rapidly digestible. Typically, starch hydrates at temperatures ranging from 40 to 120°C, depending on the source of the starch and its amylose content. Upon cooling, starch undergoes a relatively slow re-association process commonly termed *retrogradation.* During retrogradation (Colonna, Leloup & Buléon, 1992; Morris, 1990), starch molecules re-associate, and can form tightly packed structures stabilized by hydrogen bonding. The association process can be driven further by dehydration. These structures are thermally very stable, and can only be rehydrated at 80–150°C, depending upon the extent and nature of the retrogradation. Amylose forms a thermally stable RS_3 complex, resistant to amylases (Jane & Robyt, 1984). It is found in foods such as cereal products and stale breads, and can be manufactured as a food ingredient.

In the formation of RS_3, the starch granule is completely hydrated. Amylose is leached from the granules into solution as a random coil polymer. Upon cooling, the polymer chains begin to re-associate as double helices, stabilized by hydrogen bonds (Wu & Sarko, 1978). The individual strands in the helix contain six glucose units per turn in a 20.8 Å repeat. Upon further retrogradation the double helices pack in a hexagonal unit cell. Schematically, the process is shown in Fig. 1.

High yields of stable, retrograded amylose were obtained by Sievert and Pomeranz (1989) when high amylose corn starch (amylomaize VII or *ae*-VII hybrid) was thermally cycled between 134 and 4°C in an excess of water. Enzyme resistance correlated with the enthalpy of the retrograded starch peak measured by DSC. The peak ranges from 120–165°C, and is apparently associated with the melting of amylose double helices.

Isothermal formation of RS is favored at 100°C (Eerlingen, Crombez & Delcour, 1993a). Higher temperatures are optimal, but not accessible to 1 atm operations. The thermal cycling of Sievert and Pomeranz (1989) may not be necessary to the extreme low temperature of 4°C, but it seems that cycling to 134°C is advantageous for the formation of extremely stable RS.

Amylopectin interferes with amylose retrogradation (Berry, 1986). Retrograded amylose yield may be increased by the removal of amylopectin with a debranching enzyme, such as pullulanase (Berry, 1986).

The degree of polymerization (DP) of amylose also affects the yield of RS_3 which rises with DP up to 100 and remains level above that (Eerlingen, Deceuninck &

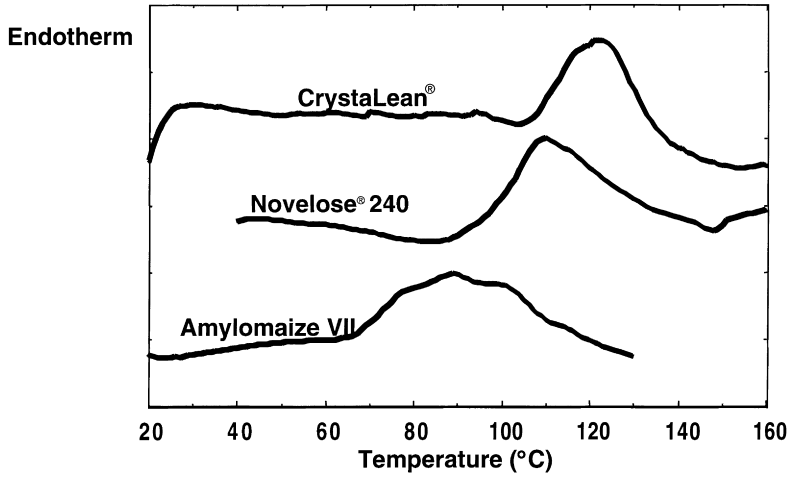

Fig. 2. DSC thermograms of commercial RS ingredients. Amylomaize VII is native granule of the *ae*-VII hybrid of corn and is a product of Cerestar, Inc. Hammond, IN. Novelose® 240 is a RS_2 product of National Starch and Chemical Company, Bridgewater, NJ. CrystaLean® is a RS_3 product of Opta Food Ingredients, Inc., Bedford, MA.

Table 1
Digestion of resistant starch and non-resistant starch with pancreatin

	Percent digested	
	2 h	24 h
Maltodextrin (5 DE)	95.9	99.1
CrystaLean®	39.6	53.8

Delcour, 1993b). This is supported by Gidley, Cooke, Darke, Hoffmann, Russell and Greenwell (1995) as minimum DP of 10 is necessary to form the double helix, and the maximum is about 100 glucose units. This indicates why amylopectin is so unfavorable to the formation of thermally stable resistant starch. Not only are the branches hindered in movement, but the typical lengths in them, 20–40 glucose units, are far from the optimum of 100 (Eerlingen, Jacobs & Delcour, 1994).

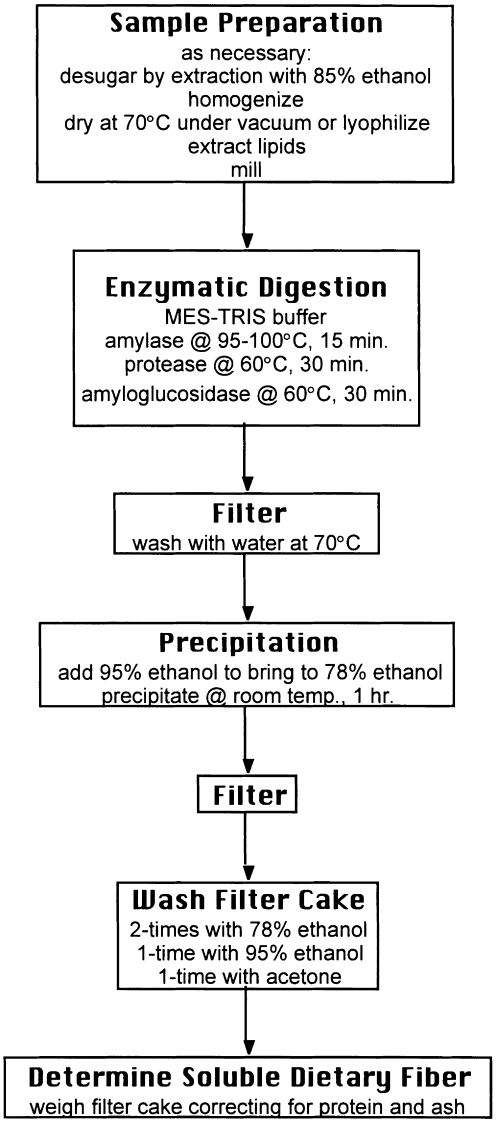

Fig. 3. AOAC/AACC method for total dietary fiber (TDF). AOAC method 991.43 and AACC method 32-07.

In the commercial development of an RS product, it is advantageous to start with a native starch high in amylose. The naturally high level of amylose in the *ae*-VII hybrid of corn makes it particularly suitable. The thermal stabilities of various commercial products are compared by DSC in Fig. 2. When analyzed in a 3-times excess of water, the thermal transition can be related to the *melting* of the hydrogen bonded starch structures. In the amylomaize VII (*ae*-VII) hybrid of corn (Amylomaize VII, Cerestar, Inc., Hammond IN), containing approximately 70% amylose, the transition associated with gelatinization occurs between 65 and 120°C. This is the native RS_2, and thermally more stable than raw common corn starch.

The level of resistance in an RS_2 product can be increased by using hybrids, which have amylose levels higher than 70%. Also, starch granules may be fractionated by size to obtain higher amylose levels and higher levels of RS_2 resistance (McNaught, Maloney, Brown & Knight, 1994). Novelose® 240 (National Starch and Chemical Company, Bridgewater, NJ) is a thermally modified RS_2 based on the same *ae*-VII hybrid of corn (Shi & Trzasko, 1997). The modification renders the native granule more stable by holding the starch at elevated temperature (60–160°C) in the presence of limited water (10–80%).

RS_3 products have been proposed based on the *ae*-VII hybrid (Chiu, Henley & Altieri, 1994; Iyengar, Zaks & Gross, 1991). CrystaLean® (Opta Food Ingredients, Inc., Bedford, MA) is a commercial, highly retrograded RS_3 based on the *ae*-VII hybrid. It is produced by first fully hydrating and disrupting the starch granules, followed by an enzymatic debranching of the amylopectin to yield a low DE maltodextrin mixture, which is almost entirely a straight chain. Then, the mixture is treated through thermal cycles to achieve a high level of retrogradation prior to drying. DSC analysis shows that the endothermic peak is shifted significantly higher than either of the RS_2 counterparts to 105–145°C, which enables it to withstand most normal cooking operations.

Slow glucose release from retrograded amylose has been demonstrated in vitro by digestion with pancreatin at 37°C, to simulate the processes in the small intestine. Table 1 compares the rate of digestion of CrystaLean® with that of maltodextrin, an easily digested, starch hydrolysate, and shows that RS_3 is digested slowly, at approximately half the rate of maltodextrin.

3. Analysis

The concept of resistant starch is clear to most as the fraction of starch not readily digestible as *normal* starch. Quantification can be mired in definitions and analytical methods, however. Results from various analytical methods help to understand some of the physical characteristics.

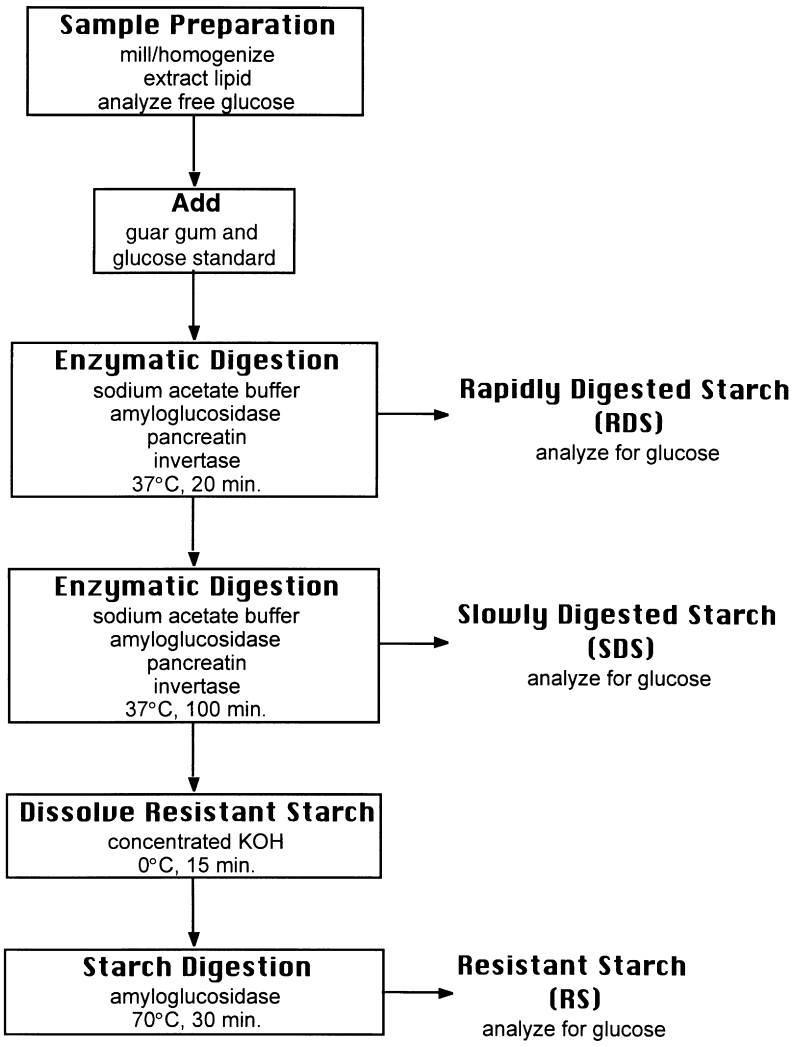

Fig. 4. Englyst method for resistant starch.

Since there is no regulatory definition of RS in the US, it is typically quantified as part of the TDF by the Prosky Method (Englyst et al., 1987). The fate of RS in an assay must be considered in the meaningful quantification of non-digestible and slowly digestible polysaccharides. One must be aware whether the intent of the assay is to provide a legal (regulatory) quantification of fiber, to provide a quantification of non-starch polysaccharides, or to truly reflect the in vivo resistant starch. The thermal responses of the three samples shown in Fig. 2 mirror the RS measurements by various methods. In general, the portion of crystalline structure melted in the assay is available to digestive enzymes, and measured as non-resistant starch.

The predominant assays for the regulatory determination of TDF are the AOAC and AACC methods. They are identical. Both old (AOAC 985.29 and AACC 32-05) and new (AOAC 991.43 and AACC 32-07) methods, the latter referred to as the Prosky Method, extract lipid, digest carbohydrate and protein enzymatically, and arrive at the remaining non-digestible fiber content gravimetrically. The difference between the old and new methods is in the buffer system and washing solutions. The subtle change from a phosphate (old) to a MES-TRIS buffer, and changes in pH for the enzymatic digestions affect the TDF results with respect to RS. Shifting the washing to a more aqueous medium effectively removes oligosaccharides from the filter cake prior to the gravimetric determination of TDF. The new method (Fig. 3) provides lower estimates of TDF than the old method. Refinements have been proposed (Lee, Vincent, Prosky & Sullivan, 1996) to the basic scheme. Instead of determining the undigested carbohydrate gravimetrically, the digested carbohydrate is measured as glucose by a method like HPLC, and fiber is determined by difference.

Adhering to the formal definition of dietary fiber as non-starch polysaccharides requires several refinements to the AOAC/AACC methods. They are intended to fully digest the starch by rigorously solublizing it in concentrated KOH or DMSO (Englyst, Wiggins & Cummings, 1982; Faisant, Planchot, Kozlowski, Pacouret, Colonna & Champ, 1995).

Since the amylase digestion in the AOAC/AACC methods is conducted at 95–100°C, as shown in Fig. 2, the high temperature melts some of the RS (either RS_2 or RS_3), leading to an underestimate of the RS available in vivo. Since RS has physiological benefits, improvements to get correct estimates include the use of porcine pancreatin at 37°C instead of fungal α-amylase at 95–100°C (Berry, 1986; Eerlingen & Delcour, 1995). This gives increased estimates of non-digestible carbohydrate, and accounts for the total polysaccharides passing the small intestine.

Englyst et al. (1992) have proposed an analytical method for rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch, with further fractionation into RS_1, RS_2 and RS_3. The sample is prepared at 100°C, which can disrupt the RS content or promote RS_3 formation. Next, the enzymatic digestions with protease, amylase and glucoamylase are accomplished at 40°C, which is close to that in the in vivo process. This method correlates to findings in healthy human ileostomates (Englyst, Kingman, Hudson & Cummings, 1996), and is truly indicative of physiologically important RS. A schematic of the method is given in Fig. 4.

Appropriate assay procedures are necessary when determining TDF when significant amounts of RS are present. The elevated temperature (95–100°C) used in the Prosky Method for regulatory purposes, is not favorable for RS determination, unless thermally stable RS, preferably RS_3, could be detected. Even small adjustments in the enzyme digestion conditions between the old and new AOAC/AACC methods affect the TDF determination. Ideally, resistant starch measurements should be done at physiological temperatures, but these methods are not currently accepted for regulatory declaration of fiber content. For research purposes, methods, which mimic in vivo conditions, need to be used.

4. Physiological effects

There is ample justification through nutritional studies that RS behaves physiologically like fiber, and should be retained in the TDF assay. RS assays as insoluble fiber, but has the physiological benefits of soluble fiber. Additionally, RS exhibits a level of slow digestibility, and can be used as a vehicle for the slow release of glucose.

Observations in animal systems are consistent between research groups, but the magnitudes of some findings differ. This is due to the lack of specificity in the definition of RS. Many times the apparent RS level in diets is confounded by the analytical method used for quantification, and it is sometimes unclear whether researchers use RS_2 or RS_3 in their studies.

Numerous studies in rats (de Deckere, Kloots & van Amelsvoort, 1995; Gee, Faulks & Johnson, 1991; Gordon, Topp, Shi, Zallie & Jeffcoat, 1997; McIntyre, Albert, Folino, Muir, Gibson & Young, 1994; Ranhotra, Gelroth, Astroth & Eisenbraun, 1991; Rickard, Folino, McIntyre, Albert, Muir & Young, 1994; Younes, Levrat, Demigné & Rémésy, 1995) have shown that RS escapes digestion in the small intestine, and is slowly fermented in the large intestine. The general behavior of RS physiologically is similar to soluble, fermentable fiber, like guar. The most common conclusions include increased fecal bulk and lower colonic pH. Additional observations suggest that resistant starch, like soluble fiber, has a positive impact on colonic health by increasing the crypt cell production rate, or decreasing the colonic epithelial atrophy in comparison with no fiber diets. There is indication that resistant starch, like guar, a soluble fiber, influences tumorigenesis, and that serum cholesterol and triglycerides are reduced. Overall, since resistant starch behaves physiologically as a fiber, it should be retained in the TDF assay.

Further studies in humans support the findings of the rat model system (see Muir, Young & O'Dea, 1994). In the small intestine, RS may be slowly absorbed, but, more importantly, is associated with an increased malabsorption of starch. This results in decreased postprandial glucose and insulin responses. This effect, to be discussed later, has significant implications for the use of RS in food formulations for people with certain forms of diabetes. The interference with starch absorption also implies long-term benefits in controlling hyperlipidaemia. In the colon, RS increases fecal bulk, lowers colonic pH and the portion fermented by the intestinal microflora produces a wide range of short-chain fatty acids (SCFA), primarily acetate, propionate and butyrate (Kritchevsky, 1995; Muir et al., 1994; Phillips, Muir, Birkett, Lu, Jones & Young, 1995; Silvester, Englyst & Cummings, 1995). SCFA production has a positive impact on bowel health, including increased absorption of magnesium and calcium, epithelial proliferation, balance of bacterial species, and bacterial metabolism of bile salts. Whether through the action on bile salts or through dilution effects, RS is thought to provide a degree of protection against bowel cancer.

The findings in human subjects liken the physiological effects of RS to some fermentable dietary fibers (Kritchevsky, 1995; Muir et al., 1994; Phillips et al., 1995; Silvester et al., 1995). Since RS remains mostly undigested until it is partially fermented in the colon, the metabolism of RS occurs 5–7 h after consumption (Muir, Lu, Young, Cameron-Smith, Collier & O'Dea, 1995), in contrast to normally cooked starch which is digested almost immediately. A decrease in colonic and serum ammonia levels is also supported in humans (Silvester et al., 1995).

The work on resistant starch in humans is in its early stages, and some of the literature findings for rats are not yet supported. For example, the cholesterol lowering effect in rats is not found in human studies with normolipidemic subjects (Heijnen, van Amelsvoort, Deurenberg & Beynen, 1996).

The energy contribution of resistant starch is reported to range almost the entire gamut from 0 to 100% digestible.

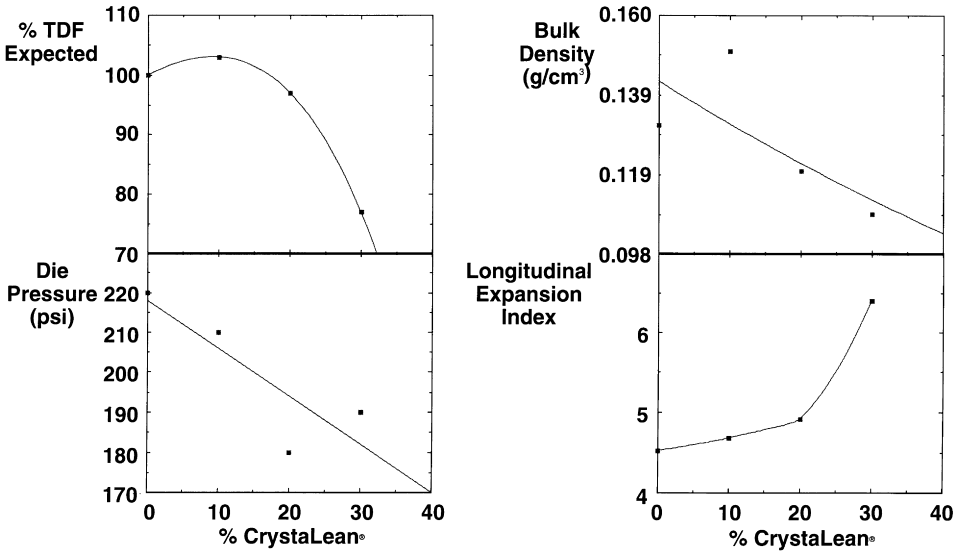

Fig. 5. Extrusion parameters of an expanded, corn-based breakfast cereal. The graph in the upper left shows the percent of RS surviving the extrusion process. The graph in the lower left shows the effect of RS on die pressure, which relates to a decrease in the specific mechanical energy with increasing RS. The two graphs on the right represent the physical nature of the extruded product, showing that overall bulk density increases with RS level, and that most of this is realized in the longitudinal expansion of the product.

Most of the studies indicate that 30–70% of RS is metabolized (Behall & Howe, 1995; Behall & Howe, 1996; Cummings, Beatty, Kingman, Bingham & Englyst, 1996; Ranhotra et al., 1991; Ranhotra, Gelroth & Glaser, 1996), while the balance is excreted in the feces. The variability is largely due to inconsistent definitions and assay procedures, and to effects caused by the malabsorption of other ingested starch.

The slow digestion of RS has implications for its use in controlled glucose release applications, particularly for diabetics. Digestion over a 5–7 h period reduces postprandial glycemia and insulinemia, and has the potential for increasing the period of satiety (Raben, Tagliabue, Christensen, Madsen, Holst & Astrup, 1994; Reader, Johnson, Hollander & Franz, 1997). In a human study (Reader et al., 1997) using a commercial RS_3 ingredient (CrystaLean®), the maximum blood glucose level and the area under the curve responses for serum glucose and insulin were significantly lower after consuming RS_3 than after other carbohydrates (simple sugars, oligosaccharides, and common starch). The RS_3 was incorporated into a bar type product. The glycemic response of subjects with type II diabetes mellitus was compared to the responses in subjects fed a common candy bar or a nutrition bar. All three bars had similar macronutrient contents. The study showed that the RS_3-containing bar decreased postprandial blood glucose, and may play a role in providing improved metabolic control in type II diabetes (non-insulin dependent). There may also be a benefit for diabetics by lowering lipid levels and prolonging satiety. Another study with CrystaLean® in dogs (Murray et al., 1998) supported the result that the postprandial area under the curve for glucose and insulin was reduced, and that RS in enteral formulas may improve gastrointestinal tract health status due to fecal bulking, potential dilution of toxins and greater production of short-chain fatty acids.

RS has also been suggested for use in probiotic compositions to promote the growth of such beneficial microorganisms as Bifidobacterium (Brown et al., 1996). Since RS almost entirely passes the small intestine, it can behave as a substrate for growth of the probiotic microorganisms.

5. RS_3 in an extruded cereal

Traditional food fibers have a detrimental effect on the texture of many items, such as baked goods and extruded products. Fibers tend to strengthen the physical structure of a food and limit its ability to expand. Expansion of air cells is an important property in the development of texture during baking and extrusion.

RS_3 allows for the addition of high levels of material which assays as TDF, but does not interfere with structure, especially for extruded products (Zallie, Altieri, Chiu & Henley, 1996). When tested in an expanded, corn-based breakfast cereal formula, RS_3 was shown to have no detrimental effect on the structure. Fig. 5 shows the results of these extruder trials using the RS_3 product, CrystaLean®. The die temperature in these trials was 160°C, sufficient to *melt* the double helical structures in an excess of water (see Fig. 2). The RS_3 is relatively stable, however, because the process is run under a limited water condition of 16%. The upper left graph of Fig. 5 shows the percentage of RS surviving the process. Up to a 20% use level, essentially 100% of the RS survives and at a 30% use level approximately

22% of the RS is lost, presumably due to high shear damage in the extruder.

More importantly, the addition of TDF through an RS ingredient does not have a detrimental effect on structure, as can be seen in the bulk density of the product. If RS interfered with cell expansion in the extruder, a net increase in bulk density would be observed, and, in fact, the opposite is observed. Most of the decrease in bulk density is attributed to the increased longitudinal expansion of the product. It was also observed that the die pressure decreases with increasing level of RS_3. Hence, the addition of RS_3 to a conventional puffed corn breakfast cereal retains the textural properties of the product while increasing the TDF level, and decreasing the energy consumption of the process.

6. Summary

RS offers an exciting new potential as a food ingredient. It has been shown to possess physiological benefits similar to soluble fibers, and, in addition, to be used as a mechanism for sustained glucose release. Quantification of RS is problematic, since it is not a distinct chemical entity, but rather a set of physical states, which alter the rate of digestion of conventional starch. The most important aspect of an analytical procedure in the quantification of RS is the assay temperature. The traditional Prosky Method for quantifying TDF is conservative in assaying RS. With this quantification, physiological studies suggest that RS provides sufficient benefit to remain as part of the TDF assay. As definitions of RS become refined, the understanding and use of RS should increase.

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