Chapter

Resistant Starch

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Abstract

Not all starch that is ingested into the human body is digested into D-glucose – the portion that defies this process is referred to as resistant starch (RS) where chemically and mechanically, five different forms have been identified. Regardless of the form, an extensive breadth of health benefits has been associated with the consumption of RS. These include the potential of RS becoming part of weight and diabetes management plans as well as improved colon health and prevention of colon cancer. Therefore, in the past decade, there has been a significant amount of research into how RS concentrations can be increased in various food systems, which have had varying degrees of success; however, are limited to either enzymatic, thermal, or acidic alterations to starch. In a similar fashion, chemical methods of RS measurement have also received a considerable amount of change and enhancement over time, though with most of them to some extent attempting to replicate human carbohydrate digestion.

Keywords: resistant starch, crystallinity, butanoic acid, health benefits, digestion, glycemic index

1. Introduction

Resistant starch could be the next "super food," its wide range of health benefits make it a very appealing ingredient in food formulations. It was first discovered in the early 1980s and since then large amounts of research have been devoted to RS and its applications in the food industry. The objectives of this chapter are to introduce and explain the different types of RS, identify the wide range of health benefits associated with the consumption of RS as well as the current mechanisms of increasing RS concentrations.

2. Starch digestion

The digestion of starches (carbohydrates) begins as soon as the food product enters the oral cavity where the act of chewing (mastication) breaks down the chunks of food into smaller particles [1]. These particles have a larger surface area to volume ratio allowing an effective and penetrating coating of saliva, secreted by the salivary glands in a response to chewing. Saliva (pH 6.8), while mostly water, is approximately 1% a combination of electrolytes and enzymes [1]. One of these enzymes is a digestive protein known as α-amylase, which initiates starch hydrolysis by randomly cleaving the $\alpha(1 \rightarrow 4)$ linkages found in starch [2].

Once the food is of a small enough size and sufficiently coated in saliva, it is then swallowed and passes through into the stomach via the pharynx and esophagus.

The environment of the stomach has a very low pH, around 1.0, due to the presence of hydrochloric acid (HCl). This low pH environment deactivates the α-amylase introduced in the mouth and as such no further carbohydrate digestion occurs in the stomach [3]. Other digestive enzymes including proteases and lipases are introduced initiating the degradation of proteins and lipids [4].

From the stomach, this mixture of acid, enzymes and partially digested food (known as chyme) enters the first section of the small intestine call the duodenum. Here, secretions from the pancreas and the gall bladder raise the pH up to around 7.8 allowing further α-amylase to be introduced from the epithelial cell walls lining the small intestine. The breakdown (hydrolysis) of starch therefore continues in the small intestine creating shorter and shorter chains of carbohydrates with varying lengths until maltose or dextrins are reached. **Figure 1** showcases the pathway that is carbohydrate digestion. Maltase, another pancreatic enzyme is introduced to

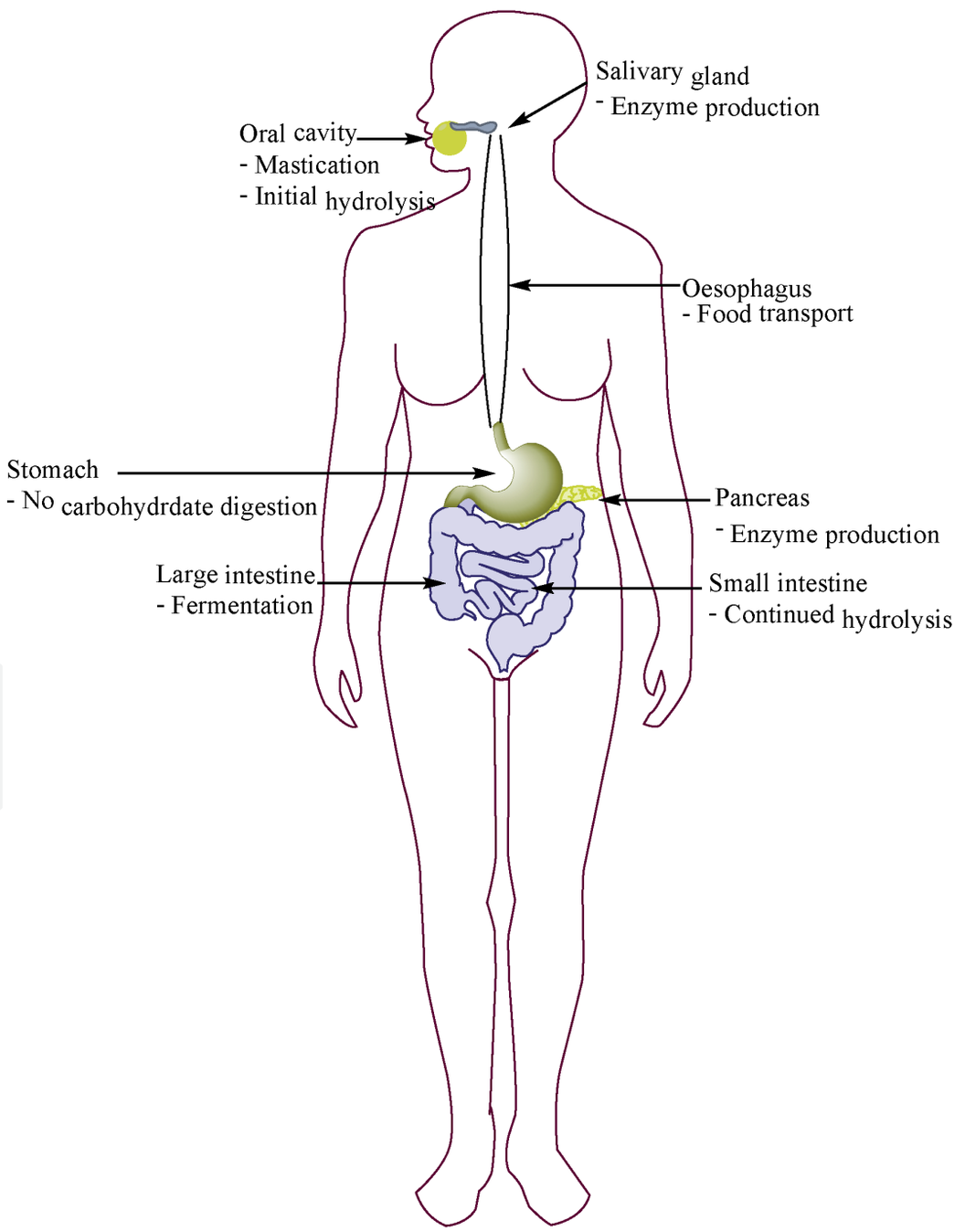

Figure 1.
A summary of different locations and organs, where carbohydrate digestion takes place.

cleave the α(1 → 4) linkage of maltose as α-amylase cannot complete this process. α-amylase cannot break α(1 → 4) linkages if they are near or adjacent to already cleaved bonds, hence the requirement for the additional enzyme, maltase [3, 5]. Once glucose is produced, monosaccharide is absorbed through the wall of the small intestine into the bloodstream via a number of complex pathways.

The extent and rate of carbohydrate digestion in the human body is highly dependent upon the chain length as it is ingested, hence, the number of glycosidic bonds present and their form. Small structured carbohydrates like maltose, glucose and fructose often present in sweet foods like confectionary and fruits are digested and absorbed relatively quickly as little or no enzymatic digestion is required [6]. Larger, more complex carbohydrates on the other hand such as oligosaccharides and starch can take a significantly longer time to digest – based on this information, starches can be classified into three main forms based on their rate of digestibility [7]:

- Rapidly digestible starch (RDS)
- Slowly digestible starch (SDS)
- Resistant starch (RS)

RDS is strongly correlated with high glycemic index foods as it is mainly amorphous starch that may have been either completely or partially gelatinized. These include baked goods likes white breads and cookies that are digested fairly quickly, in less than 20 min [6]. SDS is frequently found in weight loss and healthy eating programs as digestion takes significantly longer, between 20 and 110 min. The result of this is a more consistent and controlled release of glucose into the bloodstream over a longer period of time, which also has an impact on sustained satiety (feeling of "fullness") [7].

3. Resistant starch

The term resistant starch (RS) was first coined by Englyst et al. in 1982 [8]. RS as a definition refers to the proportion of starch that is ingested though is not digested by human enzymes. This portion of starch therefore passes through the small intestine into the colon undigested, where it has been shown to act as a carbon substrate for beneficial bacteria, making RS a form of dietary fiber. At present, five different forms of RS have been classified (RS1 – RS5) grouped based on how they are resistant to digestion (**Table 1**).

RS1 is best described as physically inaccessible starch as a physical barrier is present which prevents enzymes from gaining access to the starch. This barrier is often a plant cell wall where RS1 is frequently found in grains and millet seeds [9]. R2S is commonly referred to as raw starch, or, native starch where the starch granule is completely intact and as such has undergone no form of pressure or thermal processing. Native forms of starch typically have higher degrees of crystallinity resulting in this resistant nature [9].

In comparison, RS3 has intentionally undergone a process of gelatinization and retrogradation (recrystallization) which is usually done hydrothermally. This heating process with subsequent cooling, allows additional starch to crystallize and hence RS to form. RS3 has a number of applications in food manufacturing as it has the ability to form during food processing, unlike both RS1 and RS2 [6]. RS3 is formed most efficiently when the amylose portion of the starch is higher than

Form	Description	Food sources	Reference
RS1	Physically inaccessible starch	Whole or partially milled seeds, legumes and pasta	[11]
RS2	Raw, ungelatinized starch	Green bananas	[12]
RS3	Recrystallized amylose	Foods that have been cooked then cooled, including potato and pasta salads	[13]
RS4	Chemically modified starch	Not naturally occurring	[12]
RS5	Amylose-lipid complex	High amylose foods	[9]

Table 1.
Different forms of resistant starch with common food sources.

usual, allowing for efficient packing and stacking upon cooling. RS4 has reduced digestibility through chemical modification including etherization, esterification, or cross-linking. Finally, RS5 has only recently appeared in literature over the past 5 years and forms when the amylose portion of the starch complexes with a lipid, such as a free fatty acid to form a helical structure [10].

With ongoing research it would not be surprising if additional forms of RS are indeed discovered and this current classification of RS be altered or revisited in the future. Usually, in most staple, day to day foods, the RS content of foods is low (<2%), which has been one of the main driving factors in the increase in research over the last decade investigating methods of how to increase this.

4. Health benefits

It is clear that the consumption of RS is positively associated with large and broad range of health benefits in disease prevention as well as treatment, often working complimentary. Briefly, in this section of the chapter, some of the more researched and significant health benefits of RS to date will be discussed, while it is noted that this is very much an active and broad research area [11].

A number of the health benefits associated with RS can be explained by microbial fermentation within the human colon, making RS a prebiotic. In order for a substance to be classified as a prebiotic, it must [12]:

1. resist digestion from both enzymes and acid produced by mammals

2. act as a carbon source for selected bacteria in the large intestine

3. contribute positively to the health of the host via fermentation

4.1 Short-chain fatty acids

Through fermentation in the large intestine, a range of short-chain fatty acids (SCFAs) are produced. The SCFAs are manufactured in varying quantities where it largely depends on the type of bacterium and the source of starch though almost always include a combination of acetic (usually the largest amount), propionoic, lactic, and butanoic acids [13]. As a synergistic combination, these acids assist in maintaining the low pH environment of the colon preventing the growth of pathogenic bacteria and enhancing the proliferation of beneficial, probiotic strains.

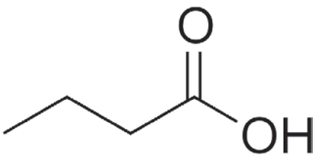

Figure 2.
The molecular structure of butanoic acid.

Butanoic acid (**Figure 2**) in particular has been shown to demonstrate a range of other health benefits including acting as a preferred carbon source for the cells of the colon (colonocytes). This improves the overall health of the colon by enhancing the strength of the epithelial layer, increasing blood flow, and reducing inflammation [14]. Several other studies have also found that increased quantities of butanoic acid may also be linked with a reduced risk of colon cancer. Butanoic acid has been shown to limit the growth of abnormal, fast growing tumor cells by stopping the G1 phase in cell replication [12]. Colon health is also enhanced by the ability of RS to hold water which increases defecation rates which in turn decreases the accumulation of mutagenic compounds.

4.2 Glycemic effects

Foods that have large amounts of RS are digested at a much slower rate when compared to similar foods containing larger concentrations of either SDS or RDS [15]. The effects of these slower digestive processes can be observed in human and animal test subjects with the measurement of blood glucose levels and subsequent insulin responses at different time periods post ingestion. It has been shown and would be expected that foods high in RS would be associated with low glycemic index foods [12]. Therefore, RS has the potential to become part of treatment plans and management programs for weight loss as well as diabetes type two [6].

MacNeil et al. [16] conducted a study where humans suffering from type two diabetes consumed baegels that were supplemented with varying amounts of RS2. Interestingly, when the RS replaced a portion of the total wheat flour, a reduction in postprandial glucose and insulin levels were observed. Although, when the RS2 was added in addition to the wheat flour these reductions were not seen – a phenomenon also observed by Luhovyy et al. [17] in cookies. Behall and Hallfrisch [18] facilitated the formation of RS3 by adding high amounts of amylose into bread formulations finding that when the amylose content made up 50% of the formation, significant reductions in postprandial glucose and insulin were observed – which was attributed to the straight chained nature of amylose, allowing for efficient stacking and hence crystallization upon cooling.

Evidence is also present to suggest that the consumption of RS can affect the regulation of satiety hormones including glucagon-like-peptide-1 (GPL-1) and peptide YY (PYY). Both hormones play a role in the stimulation of insulin secretion and facilitate a feeling of satiety through the central nervous system, working synergistically with leptin [15]. Zhou et al. [19] and Hoffmann [20] conducted similar studies measuring hormone levels after an increased intake of RS and saw increases in both GLP-1 and PYY while Hoffmann [20] additionally saw a decrease in levels of ghrelin.

It is evident that RS has the potential to have a multi-tiered approach when it comes to the management of weight as well as carbohydrate related conditions including diabetes, in addition to all of the secondary diseases that are commonly

associated with obesity. RS therefore has significant potential from a health point of view to be incorporated into various food systems not only from a glycemic perspective but a lower bowel health perspective as well.

5. Increasing resistant starch concentrations

Given the range of health benefits that are associated with the consumption of RS, a great deal of research has been conducted around how concentrations can be increased in various food systems. In this chapter, we will focus on three main methods of achieving this, with, heat, enzymes and acid.

5.1 Thermal treatments

It was mentioned briefly at the start of this chapter that RS3 has a number of applications due to its ability to form during food processing. This is typically best achieved with starches that have higher percentages of amylose, the straight chained form of starch as when the starch is cooled, they have a higher ability and chance to stack together and hence crystallize via hydrogen bonding, compared to that of amylopectin [21]. Indeed, some of the most positive results have come from when researches that have exposed starch to a number of hydrothermal cycles, that is, heating and cooling at pre-determined temperatures and times, more than once [22]. For instance, Liu et al., [23], exposed buckwheat and sorghum starches to annealing and saw buckwheat RS increase from 3.3 to 4.8% and from 3.5 to 4.2 for sorghum. As expected, they also say an inverse relationship between RS and RDS – as RS increased, the RDS proportion decreased.

5.2 Enzymatic treatments

Debranching enzymes such as isoamylase and pullulanase are capable of cleaving the $\alpha(1 \rightarrow 6)$bonds, commonly found in amylopectin and amylose to a smaller extent. By cleaving these branching points, the result are more straight chained forms of starch, similar to that of amylose – increasing RS concentrations in the same mechanism mentioned previously regarding thermal treatments [24]. Pullulanase in-particular, has received a considerable amount of recent research [24–26]. Shi et al. [26] studied investigated the effects of varying pullulanase concentrations on the digestion of waxy (high amylopectin content) maize starch. The native maize starch possessed an RS concentration of 1.2% while after an exposure to a pullulanase concentration at 20 enzyme U/g, produced a dramatic increase to 37.7% RS. They also saw an increase in the apparent amylose content, which would be expected as the sample, which in its native form is nearly 100% amylopectin, begins to have its $\alpha(1 \rightarrow 6)$bonds cleaved. Interestingly, they also found a crystalline shift after the enzymatic and thermal treatment, where the maize moved from having an A-type crystal to having a combination of both B-type and V-type. The V-type, as measured with X-ray diffraction, refers to a complexation of amylose with a lipid, otherwise known as RS5 [10], though while still a form of resistant starch, this would indeed hinder the formation of the intended RS3.

5.3 Acidic treatments

The action of acidic on RS formation appears to have a very similar mechanism to that of the debranching enzymes – the ability of a low pH environment to hydrolyse glycosidic linkages increasing the crystalline forming capability. At this point, treating starches with acid appears to be the most ineffective method of increasing RS concentration, when compared to hydrothermal or enzymatic exposure rounds.

Acid hydrolysis has been observed in various starches using scanning electron microscopy, where pores form on the outside of the starch granule after an extended exposure. These pores would then act as an access point for hydronium ions to enter the granule, reducing the proportion of amorphous starch present [27]. However, this does not appear to be the case with all starches, with Miao et al. [28] finding that the RS concentrations in maize starch first decreased before increasing with time of exposure, though increases were negligible.

6. Measurement of resistant starch

An effective means of measuring RS has proven to be a complex task, where a number of both *in-vivo* and *in-vitro* methods have been developed since the early 1980's, with many iterations and alterations since then. A great deal of development was done during the mid-1990s, one of which was by Englyst, Kingman and Cummings [29] in 1992, that effectively separated RDS, SDS, and RS. A method that is still commonly used to gain a broader understanding of digesting [24, 30], often relating to glycemic index studies. The design of this measurement model was to replicate human digestion using a range of additives such as proteases, HCl and guar gum to replicate the stomach. Starch digestion is achieved with an enzyme mixture of invertase, heat stable α-amylase, pullulanase, pancreatin and amyloglucosidase (AMG) where aliquots are taken at different time periods of digestion. Aliquots from 20 min represent RDS, while 120 min of digestion refer to SDS and anything left after 240 min is defined as RS. Glucose concentrations are determined using glucose oxidase and the starch contents are calculated using the following equations:

$$\text{RDS (\%)} = \frac{(G20 - FG) \times 0.9}{TS}$$

$$\text{SDS (\%)} = \frac{(G120 - G20) \times 0.9}{TS}$$

$$\text{RS (\%)} = \frac{TS - (RDS + SDS)}{TS}$$

One of the more commonly used methods nowadays was developed by McLeary and Monaghan [31] in 2002 and was the result of a comparative study between the different methods used at the time to measure RS in food systems. McLeary and Monaghan [31] experimented with a range of factors, including:

- Concentration of AMG
- Concentration of pancreatic amylase
- The effect of including proteases
- Incubation conditions such as time, temperature, pH and agitation

In this method (AOAC Method 2002.02/AACC 32–40.01) 100 mg samples of the food are weighed out and exposed to an enzyme cocktail of both AMG and α-amylase for 16 h at exactly 37°C. In this step, the non-resistant starch is hydrolysed to glucose, before the reaction is then halted with the addition of ethanol in a repeated washing process. If the researcher requires to measure total starch content so, they can now

take aliquots from the ethanol for subsequent quantification and addition to resistant starch contents. After the ethanol washing, a pellet contain the RS is obtained at the bottom of the centrifuge tube. The RS is dissolved with agitation over an iced bath with the addition of KOH before introducing AMG for the final time, to hydrolyse the remaining starch into D-glucose. Subsequently, the RS and total starch contents can be determined with the glucose oxidase peroxidase (GOPOD) reagent.

Clearly, the different methods of RS measurement in foods are complex and have the potential for a great deal of variability if users are not trained adequately. In addition, they can also be rather time intensive and expensive. The methods described here are by no means all of the methods researched – for a greater discussion on the topic, the reader is referred to Berry in 1986 [32], Bjork in 1986 [33] and Champ in 1992 [34].

7. Conclusions

In conclusion, it is clear that resistant starch has a large amount of potential to be incorporated into food systems in order to convey a wide range of health benefits from reducing the risk of colon cancer to managing weight loss and diabetes type two. A range of methods have been in trial in attempts to increase RS concentrations in from various sources of starch, including thermal, enzymatic and acidic treatments. Great success has been observed when a holistic approach has been adopted, manipulating each factor to optimize RS formation, rather than depending on one method individually. A great deal of future research lies in this area of optimizing RS concentrations at an economical level, as enzymatic treatments can add significant financial costs when done on a large scale.

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