Carbohydrates

Integrated Research on Glycobiology and Glycotechnology

Volume II

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Edited by **Sydney Marsh**

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Preface

This book was inspired by the evolution of our times; to answer the curiosity of inquisitive minds. Many developments have occurred across the globe in the recent past which has transformed the progress in the field.

This book has been compiled for those interested in the study of carbohydrates. The book has many topics for those who are involved in glycobiology and related fields, as it encompasses the fundamentals of carbohydrates in metabolism, the influences of environment and fungi in plant carbohydrates and application of carbohydrates in microbes. This comprehensive book can serve well as an uncomplicated introduction to different disciplines of carbohydrate investigators and glycobiologists.

This book was developed from a mere concept to drafts to chapters and finally compiled together as a complete text to benefit the readers across all nations. To ensure the quality of the content we instilled two significant steps in our procedure. The first was to appoint an editorial team that would verify the data and statistics provided in the book and also select the most appropriate and valuable contributions from the plentiful contributions we received from authors worldwide. The next step was to appoint an expert of the topic as the Editor-in-Chief, who would head the project and finally make the necessary amendments and modifications to make the text reader-friendly. I was then commissioned to examine all the material to present the topics in the most comprehensible and productive format.

I would like to take this opportunity to thank all the contributing authors who were supportive enough to contribute their time and knowledge to this project. I also wish to convey my regards to my family who have been extremely supportive during the entire project.

Editor

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Carbohydrate Metabolism

Food Structure and Carbohydrate Digestibility

Suman Mishra, Allan Hardacre and John Monro

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1. Introduction

Carbohydrate is almost universally the major dietary source of metabolic energy. Nearly all of it is obtained from plants, and nearly all of it requires digesting before it is available for metabolism. While digestion is aimed at breaking down molecular structure within carbohydrate molecules, there is a raft of further plant structural impediments to be overcome before most plant carbohydrates are available for digestion.

Starch, for instance, represents energy stored, not for animals, but for the plant that made the starch. It is a reserve available to carry a plant between seasons, to sustain it during periods when photosynthesis is limited, to prepare it for times of intense energy use such as flowering, and to support its progeny in seeds before autonomous growth. But so accessible is free starch as a form of energy that plants have taken special measures, many of them structural, to protect it physically from all sorts of opportunist consumers – animals, fungi and bacteria - and from the effects of existing in a hydrating entropic environment. All these structural barriers have to be overcome before the carbohydrate becomes available for digestion, and can be used as a source of food-derived energy.

In the human diet, lack of available carbohydrate is associated with under-nutrition and its attendant problems, while at the same time a surfeit of available carbohydrate is associated with obesity, metabolic syndrome, and diabetes – scourges of the developed world. Therefore, as food structure can have a critical role in determining the proportion of carbohydrate that is made available by food processing and digestion (Bjorck et al. 1994), it is of fundamental importance to nutrition and health.

This chapter discusses the importance of carbohydrate digestibility to human health, various forms of plant and food structure that have an impact on carbohydrate digestibility, and how food processing methods of various types overcome them.

2. The nutritional importance of carbohydrate digestion

Digestibility, energy and the glycemic response

The nutritional importance of available carbohydrate currently extends far beyond its role as a major source of sustenance for humans. Thanks to modern agriculture, transport and food technology, and to the market-driven economy in which appetite-driven food wants, rather than nutritional needs and survival, have come to determine the types of foods available to consumers, energy intakes have far exceeded energy requirements. As a result, the "developed" world is now facing an obesity crisis. Carbohydrate digestibility has gained new importance, not only because of its contribution to obesity, but also because a secondary consequence of obesity is the metabolic syndrome for which a defining feature is glucose intolerance – an impaired ability to control blood glucose concentrations after a carbohydrate meal.

It is now evident that the adipose tissue of obesity is not a passive fat storage tissue, but is physiologically active and intimately involved in glucose homeostasis. It plays a key role in glucose intolerance and Type 2 diabetes by producing factors, including free fatty acids, that induce insulin resistance (Saltiel & Kahn 2001). Resistance to insulin leads to a reduced rate of clearance of glucose from the blood, and the resulting increased concentration of glucose in the blood leads to generalized damage throughout the body, from chemical bonding (glycation) of proteins, increased oxidative stress, and damage to numerous biochemical processes (Brownlee 2001). In response to increased blood glucose and to the rate of blood glucose loading, insulin production increases, with its own damaging effects (Guigliano et al. 2008). Ultimately, exhaustion of the capacity of the pancreas to produce adequate insulin means that the insulin resistance of Type 2 diabetes evolves into the insulin insufficiency of Type 1 diabetes. The generalized, cumulative, systemic damage of prolonged and/or repeated exposure to high blood glucose concentrations manifests itself as a raft of disorders associated with long-term diabetes – kidney failure, circulatory problems, neuropathy, heart disease, blindness and so on – that are imposing enormous costs in suffering and resources (Zimmet et al. 2001).

In the context of the pandemic of obesity and glucose intolerance in the modern world, new ways of manipulating the rate and extent of digestibility of carbohydrate are being sought. The rate of starch digestion is important because the degree to which blood glucose loading exceeds blood glucose clearance determines the acuteness of the net increase in blood glucose concentrations, and consequently, the intensity of the insulin response required to remove the glucose overload and restore normal blood glucose concentrations. The rate of digestion also determines how sustained will be the supply of glucose by continued digestion in the gut, and therefore, how prolonged its contribution to delaying the urge to eat again will be.

Carbohydrate digestibility and colonic health

The extent of digestion during transit through the foregut is important because it determines the proportion of starch that is available to the colon as polysaccharide for fermentation, which has a role in colonic health (Fuentes-Zaragoza et al. 2010) and probably also in appetite control through the colonic brake feedback mechanism (Brownlee 2011). Undigested food residues, including both food structures and the carbohydrates and other nutrients that they have protected from digestion, are now recognized as being not simply gastrointestinal refuse, but a valuable feedstock for the colonic ecosystem. Through both fermentation of the residues and through the ability of a proportion of them to survive colonic transit, they play an essential part in maintaining gut health and function, as well as good health in general (Buttriss & Stokes 2008).

It is increasingly recognized that events in the colon influence the body as a whole, through products of colonic fermentation, through effects on the immune system mediated by the colonic epithelium, and through neuronal and hormonal feedback from the colon to upstream regions of the digestive tract (Wikoff et al. 2009). Short chain fatty acid products of colonic fermentation, propionic acid in particular, may play a direct role in blood glucose control by suppressing the release of plasma triglycerides, which contribute to insulin resistance. Colonic fermentation also appears to have indirect effects on hormones from the pancreas and adipose tissue that are involved in the regulation of energy metabolism (Nilsson et al. 2008).

Recent research suggests that obesity is associated with a colonic microbiota that is more effective in scavenging energy from undigested food polysaccharides than the microbiota from lean individuals (Turnbaugh et al. 2006). Although the daily increments in energy gain may be small, over time they accumulate in expanding adipose tissue. Recovering undigested energy by colonic fermentation could make the important difference between starving and surviving in an energy-depleted environment where food is scarce and of poor quality, or under the precarious conditions in which we evolved. However, in the present developed world of plenty, it may contribute to the difference between remaining trim and being overtaken by creeping obesity.

3. Forms of food structure affecting carbohydrate digestion

Food structure can take a number of forms that can affect the availability of carbohydrate in a number of different ways and at a number of different levels – molecular, cellular, plant tissue and food.

3.1. Molecular level

In the case of short chain sugars, such as the disaccharides sucrose, maltose and lactose, the structural constraint on digestion to monosaccharides lies solely within the glycosidic linkage between monosaccharide units, and is easily overcome by disaccharidases of the gut brush border (Wright et al. 2006). But even then, the rate at which the monosaccharide units traverse the gut wall, and so the extent to which absorption is completed during small intestinal transit, depends on the ability of membrane-bound transporters to recognize the structure of monosaccharides. Glucose transporters (SGLT1, GLUT 2) achieve active ATP-driven facilitated transport against a gradient, whereas the transporters that recognize fructose as a structure carry out less effective absorption by facilitated transport, which may result in overflow of fructose into the terminal ileum and colon, leading to intestinal discomfort from the resulting osmotic and fermentative effects (Gibson et al. 2004). Similarly, the structural specificity of lactase means that decline in lactase activity leads to the severe gastrointestinal problems of lactose intolerance.

Starch

Starch presents a different challenge for digestion from that of the common food disaccharides. Although it consists solely of α-D-glucose units, it may have a degree of polymerization of thousands or millions, and the glucose units may be α(1-4) linked into long linear amylose chains, or shorter amylose chains may be connected at α(1-6)-linked branch points. Most starch (~70%) is branched (amylopectin) and has a molecular weight of 50-500 million, and a degree of polymerization in the millions, depending on the plant species (French 1984; James et al. 2003; Thomas & Atwell 1998). The long regular string of glucose units in both amylose and amylopectin provides the opportunity for interactions between starch chains, leading to the buildup of pseudo-crystalline regions, which may sterically inhibit amylase access.

a. Native starch and Starch granules (RS2)

Above the scale of amylose and amylopectin molecules, the starch is organized during growth in plants into granules that impose further restrictions on enzyme access (Ayoub et al. 2006; Gallant et al. 1997). Starch granules characteristically consist of concentric rings of alternating amorphous and pseudo-crystalline structures laid down during granule growth (**Figure 1**). The amorphous starch corresponds to regions that are rich in branches at (α(1-6) glycosidic bonds, while in the pseudo-crystalline regions the starch is highly organized as closely packed short branches, approximately 10-20 glucose subunits in length (Gallant et al. 1997; Ratnayake & Jackson 2007; Waigh et al. 2000). The high degree of organization of the pseudo-crystalline region is revealed by the typical Maltese cross birefringence pattern of

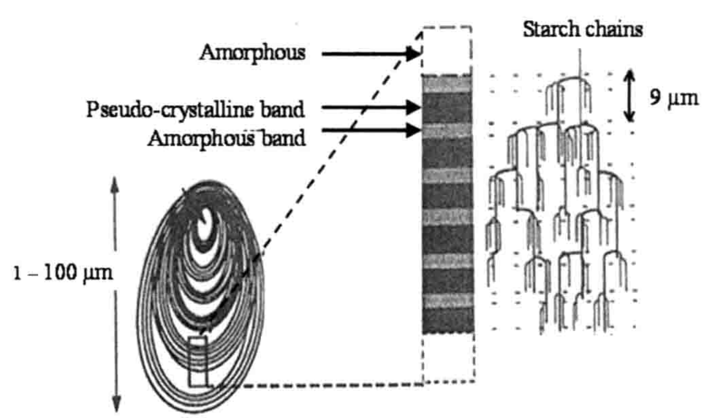

Figure 1. Schematic view of the organization of starch within a native starch granule

native starch when viewed in polarised light. The pseudo-crystalline regions are far more resistant to digestion by α-amylase than the amorphous regions (Donald 2004), and the highly organized starch granule as a whole may be relatively resistant to digestion, thanks to protein and lipid at the granule surface, which together form a coating resistant to water and digestive enzymes (Debet and Gidley, 2006).

Although covered with a resistant coating, almost all types of starch granules have been shown to bear surface pores that are entrances of channels that reach the near centre (hilum) of the granule (Huber & BeMiller 2000). The pores may be well developed in maize and nearly absent and much smaller in potato and tapioca (Juszczak et al. 2003). They may play an important role in digestion by allowing penetration of water and enzymes into the centre of the granules (Copeland et al. 2009) and leaching of glucose outwards, so the native starch granules often appear to be digested from the inside out (Gallant et al. 1997; Oates 1997; Planchot et al. 1995; Tester & Morrison 1990). However, digestion remains relatively slow while the starch is organized in its native (ungelatinized) state.

b. Gelatinized starch

Gelatinization is the loss of the pseudocrystalline structure of the starch granules and is characterised by a loss of the maltese cross pattern in polarised light and rapid water absorption and digestion in the presence of amylase. It involves a dramatic loss of structural organization of starch granules in response to temperatures above about 60°C in conjunction with excess moisture, or by processing at temperatures above 120°C at high shear, even at low moisture levels, such as during extrusion processing (**Figure 2**).

Various techniques used to study the gelatinization process suggest that the profound change in structure during gelatinization in moderate heat and in the presence of excess water is due principally to water invasion and swelling of the amorphous regions of the starch granule (Donald 2004). Because the molecules of the amorphous regions have connecting bonds with the semi-crystalline regions, as the amorphous regions swell they force the molecules of the pseudo-crystalline regions to dissociate. As the swelling and dispersion progresses, the starch becomes increasingly accessible to digestive enzymes, and the glycemic impact of the starch rises dramatically. Starch granule pores may assist by allowing water to invade deeply into the granule interior.

Starches differ in their susceptibility to gelatinization, and have been classified as those that swell rapidly, those that have restricted swelling associated with surface lipids and proteins (Debet & Gidley 2006), and a third group of granules that contain high amounts of amylose (high semi-crystalline content), which do not swell significantly at temperatures below 100°C.

c. Retrograded starch (RS3)

Retrogradation of starch is a form of structural change that has a large effect on digestibility. It occurs as the linear portions of starch molecules that have been dispersed during gelatinization randomly re-crystallize, without the organizing guidance of the living plant, when the gelatinized starch is cooled. Both amylose and amylopectin will retrograde.

However, amylose chains being less branched than amylopectin, will tend to re-crystallize almost irreversibly and again become nearly resistant to amylase digestion, while retrogradation of branched amylopectin is less complete and more reversible, and digestion by amylase is retarded less.

d. Modified starches (RS4)

As starch is a long digestible polymer covered in exposed hydroxyl groups, there are many ways that it may be modified. It may be partly depolymerized by enzymes or acid, substituent groups may be added (e.g. acetylated), it may be oxidized, cross-linked, pre-gelatinized and retrograded. Most modifications to starch are designed to change its functional properties as a food ingredient by altering its rheological characteristics (Taggart 2004; Whistler & BeMiller 1997). All the chemical/processing modifications involve structural change at the molecular level and many alter the digestion characteristics of the starch. Where chemical modification of starch causes resistance to digestion, type 4 resistant starch (RS4) is formed (Sajilata et al. 2006).

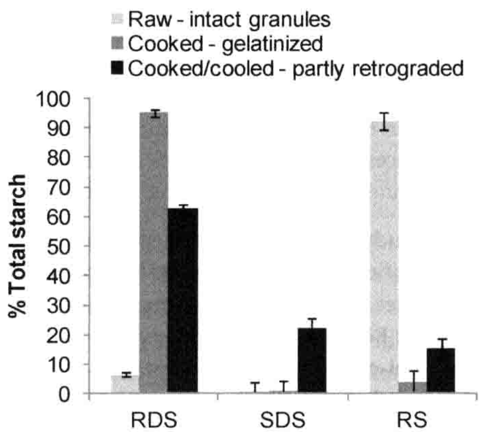

Figure 2. Effects of food structure at the molecular level - dependence of starch digestibility *in vitro* on its molecular form: rapidly digested (RDS), slowly digested (SDS) and digestion-resistant starch (RS) in potatoes digested raw (pseudo-crystalline, intact starch granules), freshly cooked (starch dispersed after gelatinizing), and cooked-cooled (starch partially recrystallized by retrogradation). (Mishra and Monro, unpublished)

e. Occluded starch (RS1)

In the mature endosperm of most cereals, the thin cell walls are largely obliterated and the endosperm becomes a protein matrix containing embedded starch granules (Eliasson & Wahlgren 2004; White & Johnson 2003). The density and occluding effect of the protein reduces water uptake during cooking by preventing the swelling of the starch granules and as a result reduces the rate of digestion. In species with hard endosperm, such as certain wheat and maize varieties and in rice, the protein matrix is almost continuous, whereas in wheat and maize cultivars with soft endosperm, and in cereals such as oats, rye and sorghum (Earp et al. 2004), there are many discontinuities that create pathways for water and enzyme penetration into the endosperm. As a result, soft endosperm variants hydrate more quickly and present a greater internal surface area of starch for water absorption and digestion.

In pastas based on high-protein durum wheat, a relatively slow rate of digestion and low glycemic impact has been attributed to protein coating the starch granules, inhibiting both gelatinization and amylase access to starch (Colonna et al. 1990; Jenkins et al. 1987). Microscopy has revealed that protein-starch conglomerates survive in cooked pasta (Kim et al. 2007). Because of the protein occlusion of starch, carbohydrate digestion in pasta may be enhanced by cooperative protease activity (Holm & Bjorck 1988). In fatty or oily tissues such as nuts, the hydrophobic nature of the fat may also be a factor protecting the starch from hydration, gelatinization and subsequent digestion.

f. Complexed starch

Complexing of starch with other macromolecules may involve a change in structure that is associated with reduced digestibility. Amylo-lipid complexes, formed when starch is gelatinized in the presence of lipid, are regarded as crystalline (Eliasson & Wahlgren 2004). The rate of digestion of amylose-lipid complexes is less than digestion of amylose, but greater than digestion of retrograded amylase (Holm et al. 1983).

3.2. Cell and tissue level

So far we have been discussing structural factors at the sub-cellular level that may affect carbohydrate digestibility. At the multicellular level, many sources of food carbohydrate are swallowed in the form of plant tissue fragments in which cell walls, and multiple overlying layers of cells, may act as partial barriers to both digestive enzyme penetration into, and carbohydrate diffusion out of the fragment or particle. In fruits, cereal kernels, nuts and pulses, tissue structure may influence the availability of carbohydrate and other nutrients (Mandalari et al. 2008; Palafox-Carlos et al. 2011; Tydeman et al. 2010a; Tydeman et al. 2010b).

Cereals

Seeds have evolved as dry, mechanically resistant structures that protect the embryo and the starchy endosperm from insect and animal attack until germination. In addition, in many mature grains such as rice, maize, the hard wheat varieties and some legumes, the molecular structural organization of starch and the protein that surrounds it results in a very hard endosperm that fragments into particles when crushed. Although the surface of such kernel particles is available for attack by digestive enzymes, penetration into the dense particles, especially when uncooked, is slow, and a high proportion of the starch may reach the colon. To obtain the digestible energy available in such grains, they must be subjected to processes such as grinding, flaking and cooking before being consumed. However, the dependence of digestibility on particle size provides a means by which the rate of starch availability, and the amount escaping foregut digestion to act as a substrate for colonic bacteria, may be influenced. The progressive decrease in rapidly digestible starch and increase in inaccessible (resistant) starch with increasing particle size is a very clear trend (**Figure 3**).

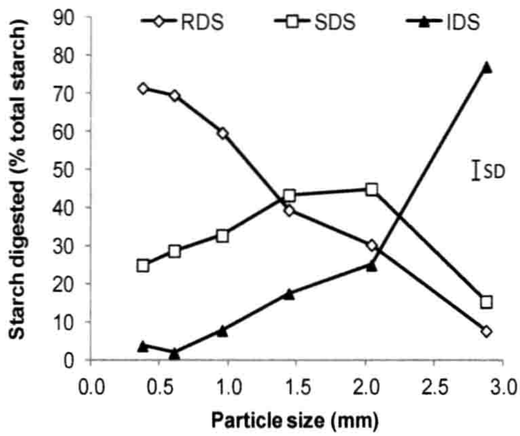

Figure 3. Effect of tissue structure on digestibility: Effect of particle size in chopped kernel fragments of the wheat cultivar 'Claire' on the *in vitro* digestibility of starch; RDS = rapidly digested (0-20 min), SDS = slowly digested (20-120 min), IDS = inaccessible digestible starch (undigested until residue homogenized) (Monro and Mishra, unpublished).

Pulses

In pulses, the starch-containing reserve tissues of the cotyledons differ in structure from the endosperm of cereals, in that the cells of the storage tissue are living and the walls retain an organized structure separating cells and contributing to tissue support in species in which the cotyledons become "seed leaves" after germination (Berg et al. 2012). In contrast, cereal endosperm cell walls are thin and usually disintegrated, and the structural integrity of the kernel is maintained by the starch/protein concretion of the endosperm, combined with the tough surrounding testa or seed coat (the bran in wheat). The differences in structure between the pulses and cereal products are reflected in the patterns of carbohydrate digestion from them (**Figure 4**).

The thick and resistant cell walls of pulses and may retard the gelatinization of starch by confining it within the cell lumen (Tovar et al. 1990; Tovar et al. 1992). When the starch is densely packed within resilient clusters of intact cells with robust cell walls, swelling is constrained. In addition, an encapsulating layer of gel from unconstrained starch in the outer cell layers of pulse fragments may create a barrier that impedes water penetration. However, partly because they are pectin rich compared with cereals, when processing is harsh or prolonged, the cell walls of pulses will degrade enough for the cells to separate. Then the starch becomes free to swell and disperse, and digestion is more rapid.