Notes on Factors to Consider Regarding Nutritional Definition of Starch Sept. 11, 2008

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Why define "starch" for use in nutritional labeling as something other than a plant storage polysaccharide?

Summary Points:

- AAFCO's 2007 proposed definition of starch for use in the nutritional labeling of animal feeds described it in part as "The non-structural storage polysaccharide of plants". This designation as a plant-derived carbohydrate is consistent with current ISO and FAO definitions.
- Starch is historically defined as a plant storage polysaccharide. Primary uses for starch analyses seemed related to identifying plant starch as a contaminant (plant starch in meat) or for food processing characteristics (plant starch can form gels, other carbohydrates may not).
- Present enzymatic-colorimetric assays for "starch" measure all carbohydrates from which glucose is released after purified enzymes (α-amylase and amyloglucosidase) specifically hydrolyze glucose – glucose α-(1-4) and α-(1-6) linkages (the same bonds that small intestinal enzymes are capable of hydrolyzing). They do not specifically measure plant starch.
- Plant starch, glycogen in animal tissues such as liver, glycogen in microbes such as yeast, maltodextrins (breakdown products of starch) typically produced during baking, and maltose/isomaltose all contain glucose α-(1-4) and/or α-(1-6) linkages that are hydrolyzed to release glucose in the "starch" assay.
- There is no practical way to distinguish animal or microbial glycogen from plant starch particularly if gelatinized starch is present in the sample. There is debate over the appropriate method to extract maltodextrins. Unless samples are pre-extracted to remove maltodextrins, measured starch values corrected for free glucose contain starch, glycogen, maltodextrins, and maltose/isomaltose. Maltodextrins and maltose /isomaltose are typically not present or are present in small amounts in animal feedstuffs. Exclusion of glycogen from the nutritional definition of starch would force the exclusion of certain matrices (animal/pet feeds containing yeast or liver?) from analysis and labeling for starch content.
- Starch, glycogen, maltodextrins, and maltose/isomaltose all have potential to be hydrolyzed by small intestinal enzymes; maltodextrins and maltose/isomaltose are breakdown products of starch that has been partially digested by small intestinal enzymes. Nutritionally, these α-(1-4) and α-(1-6) linked glucose carbohydrates all fall into the same category for enzymatic digestibility, though they may vary in their rate of digestion. But rate does not dictate classification, as even plant starch varies widely in its rate of digestion depending on whether it's been gelatinized (baked or steam flaked products), or is uncooked (ground grains). These carbohydrates (if resistant starch is excluded) do not appear to have other than nutritional effects on the animals (they are not "prebiotics").
- If these α-(1-4) and α-(1-6) linked glucose carbohydrates digest similarly and the intent is to describe a nutritional entity, why partition them?
- **Modified Proposed AAFCO Definition for "Nutritional" or "Dietary" Starch (Aug 3, 2008 AAFCO, Lab Methods & Service Committee):** An alpha-linked-glucose carbohydrate of or derived from plants, animals and microbes from which glucose is released after gelatinization through the use of purified α-amylases and amyloglucosidases that are specifically active only on α-(1-4) and α-(1-6) linkages. Its concentration in feed is determined by enzymatically converting the alpha-linked-glucose carbohydrate to glucose and then measuring the liberated glucose. This definition would encompass plant starch, glycogen, maltodextrins, and maltose/isomaltose.

Definitions

Derived from:
Hodge, J. E., and E. M. Osman. 1976. Principles of food science. Part I Food chemistry. O. R. Fennema, Ed. Marcel Dekker, Inc. New York and Basel.
and
BeMiller, J. N. 2007. Carbohydrate chemistry for food scientists. 2nd ed. AACC International, St. Paul, MN.

Glycogen: A branched polymer of glucose (a polysaccharide) similar to amylopectin, but with a much higher degree of branching. Found in animal tissues (liver, oyster), and in microbes (yeast). Glycogen is made solely of glucose linked by α-(1-4) (in the straight chains) and α-(1-6) (at the branch points) bonds.

Maltodextrins: Produced from the partial hydrolysis of starch using acids or enzymes, and containing 2 to 20 glucose units.

Maltose / Isomaltose: Disaccharides produced by partial hydrolysis of starch with acid or enzymes. Maltose contains an α-(1-4) linkage, and isomaltose contains an α-(1-6) linkage. Because these are breakdown products of starch, these are considered to be part of the maltodextrins group, but as disaccharides, they can also be considered to be sugars (FAO definition).

Starch: The non-structural storage polysaccharide of plants, made solely of glucose linked by α-(1-4) (in the straight chains) and α-(1-6) (at the branch points) bonds. It is comprised of amylose, a primarily linear polymer, and amylopectin, a more branched polysaccharide. Alternate, more specific chemical definition: A storage polysaccharide of plants comprised solely of α-D-glucopyranosyl units linked 1,4 with branches attached by 1,6 linkages."

Background

Below are notes by topic that give the basis for statements made in the Summary Points.

Glycogen

Southgate, D. A. T. 1976. Determination of food carbohydrates. Applied Science Publishers, Ltd., London.

P 60. "The analyst must at this stage recognize that the values for starch, like so many of the other food carbohydrates, are largely defined by the method of analysis used."
P 88. …glycogen in meat… it can be measured as glucose after hydrolysis with amyloglucosidase…"

Approved methods of the American Association of Cereal Chemists (2000) 10th Ed., AACC International, St, Paul, MN, Method 76-11

Possibly recognizing the inability of the method to differentiate among these α-glucans, the American Association of Cereal Chemists method 76-11 "Starch – Glucoamylase method with subsequent measurement of glucose with glucose oxidase" indicates, "This method is applicable to starch determination in complex media, including all starchy products (food and feed, digestive contents) and glycogen." Note that glycogen was not designated separately from starch in the title of the assay.

AOAC method 958.06 "Starch in Meat"
Recognizing the inability to differentiate plant starch from animal glycogen, this method specifically indicates that it is "Not applicable to liver products."

Bernard, C. 1877. De la matière glycogène considèrèe comme condition de dèveloppement de certains tissus, chez le foetus avant L'apparition de la fonction glycogènique du foie, *In:* Leçons sur le diabète et la glycogenèse animale. Paris, pp. 331-381.
Demonstrated that glycogen is hydrolyzed by saliva. He measured the production of gas from a preparation of glycogen derived from dog liver after adding saliva + yeast used for making beer, or yeast alone. More gas evolved from the saliva + yeast treatment. See email below indicating that yeast used for beer can utilize sugar and maltose, but utilize maltooligosaccharides only slowly. Maltose is released from glycogen by the amylase in saliva.

Dr. Martin Stokes, Rumen Microbiologist and Brewer of Beer, University of Maine, personal communication, 7/31/08
(The point: yeasts can use the disaccharides and monosaccharides, but can only very slowly use the maltooligosaccharides, so Bernard's results could not have been solely from the action of the yeast. MBH)
> Can the yeast used for beer making make use of maltooligosaccharides
> and starch, or only sugars? (re: Bernard's results)
"Most yeasts can only use sugars, including the disaccs maltose, and sucrose. I presume they have a maltase to release glucose and an invertase to generate the glucose and fructose, which they then ferment to alcohol, CO2, and ATP. Some brewing yeasts can very slowly attack short chain maltooligosaccharides (small dextrins) that are produced during the mashing process of brewing. Alpha and beta amylases at 145 to 154 degrees F during mashing make a mix of maltose and dextrins, the balance depends on the temperature and the pH as these two enzymes have different pH and Temp optima. Cooler mashes tend to make more maltose producing a thinner higher alcohol beer. Higher mash temps produce a larger percentage of dextrins and less maltose. So from the same amount of barley starch you get less maltose and less alcohol, with more dextrins that produce body and mouth feel in the beer. Essentially you get a more chewy beer. Now, in a beer that is not pasteurized and still contains live yeast and some unfermented oligosacc dextrins, like homebrew, if you store a bottle for along time, say 12 months, at cool room temp (45 to 65F) it becomes very fizzy. This is because of slow fermentation of short chain oligosacchs. The beer dries out, has less body if you ever get to drink any, and sprays all over the kitchen when you try to open it. If you chill it to 32 and open it very carefully you can pour it into a glass but it turns to foam as soon as it hits the glass. You get a couple of ounces of beer and pints of foam. This assumes that the bottle has not exploded in storage."

Pham, T.-H., Mauvais, G., Vergoignan, C., De Coninck, J., Cachon, R., & Feron, G. 2008. Gaseous environments modify reserve carbohydrate contents and cell survival in the brewing yeast *Saccharomyces cerevisiae*. *Biotechnol. Lett.* 30, 287-294
Describes the use of amyloglucosidase to analyze for glycogen in yeast cells.

Maltodextrins / Maltooligosaccharides

Southgate, D. A. T. 1976. Determination of food carbohydrates. Applied Science Publishers, Ltd., London.
P48. "Dry heating of starch leads to the production of dextrins."

P48-49. "…many foods also contain acid or enzymatically degraded starches. These 'glucose syrups' are widely used in the food processing industry and in the production of confectionary. The molecular species present….the mixtures include some free glucose, maltose, maltotriose and maltotetrose, and these will usually be considered as falling within the *free sugar* fraction. However, for analytical purposes many of the procedures for the measurement of starch can be used for these products and it is convenient to consider them in this chapter."(Chapter on starch analysis)

Cummings, J. H. and A. M. Stephen. 2007. Review: Carbohydrate terminology and classification. European Journal of Clinical Nutrition 61 (Suppl 1): S5–S18

P. S8. "Food oligosaccharides fall into two groups: (i) maltodextrins, which are mostly derived from starch and include maltotriose and α-limit dextrins that have both α-1-4 and α-1–6 bonds and an average DP8 *(DP = degree of polymerization, indicates that the α-limit dextrins have 8 linked glucose molecules on average; DP of <20 is more widely accepted. MBH).* Maltodextrins are widely used in the food industry as sweeteners, fat substitutes and to modify the texture of food products. They are digested and absorbed like other α-glucans …"

(That maltodextrins are added to human foods is relevant to animal feeds particularly if these foods become part of the by-product stream that becomes animal feeds. MBH).

Gibson, G. R. and M. B. Roberfroid. 1995. Dietary Modulation of the Human Colonic Microbiota: Introducing the Concept of Prebiotics. J. Nutr.125: 1401-1412.

P. 1401. "In contrast, prebiotics are nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacterial species already resident in the colon, and thus attempt to improve host health."

(The point here is that prebiotics are nondigestible to the host animal, but can be utilized by microbes, so, starch, glycogen, maltodextrins, maltose/isomaltose which have the potential to be digested by small intestinal enzymes are not prebiotics. Resistant starch could be a prebiotic, but is not digested by small intestinal enzymes. MBH)

The Sugar Association Website: http://www.sugar.org/consumers/sweet_by_nature.asp?id=277

What is maltodextrin?

A maltodextrin is a short chain of molecularly linked dextrose (glucose) molecules, and is manufactured by regulating the hydrolysis of starch. Typical commercial maltodextrins contain as few as three and as many as nineteen linked dextrose units.

While the singular term "maltodextrin" is permitted in an ingredient statement, the term "maltodextrin" can be applied to any starch hydrolysis product that contains fewer than 20 dextrose (glucose) units linked together. This means that the term "maltodextrin" stands for a family of products, not a single distinct ingredient.

Additionally, today's commercially important maltodextrin products are produced from corn, potato or rice. Unlike the other starch sweeteners, the undefined term "maltodextrin" can be used in an ingredient list no matter the original source of starch.

Maltodextrins are used in a wide array of foods, from canned fruits to snacks. Maltodextrins may also be an ingredient in the single-serve, table-top packet of some artificial sweeteners.

Dr. Ruurd Zijlstra, Associate Professor, Ingredient Evaluation and Feed Processing**, University of Alberta, personal communication**

A question: do you know of any data that suggests that a-1,4 and a-1,6 linked maltooligosaccharides should be nutritionally distinct from starch in monogastrics (i.e., they have other than nutritional effect on the animal -- like fructooligosaccharides)?

Interesting question, but I would doubt if there any research data on this or that such effects exists, because it is a small fraction in most regular feedstuffs, and I'm not sure if any regular feedstuff contains high amounts so that these possible effects can be tested. Using regular feed analyses, does it end up as rapidly-digestible starch or as sugars?

It likely would be very comparable to rapidly-digestible starch. Perhaps it might already serve as a substrate for microbes in stomach and duodenum/jejunum in swine, but obviously, competition with regular glucose absorption would be severe.

ISO 15914 (E) Animal feeding stuffs – Enzymatic determination of total starch content, 1st Ed. 2004-02-01. International Organization for Standardization, Geneva, Switzerland.

Recommends the use of 40% ethanol for extraction of maltooligosaccharides.

McCleary, B.V., T. S. Gibson, and D. C. Mugford. 1997. Starch (Total) in cereal products, amyloglucosidase – α-amylase method. J. AOAC Int. 80, 571-579.

Recommended use of 80% ethanol to pre-extract and remove sugars and maltooligosaccharides for starch analysis.

Digestive Enzymes:Hydrolysis of Starch / Glycogen / Maltodextrins

The Merck Index, 12th Ed., 1996, editor S. Budavari, Whitehouse Station, N.J.: Merck and Co., Inc., p102, #640

"Amylase. *(Includes)* Enzymes catalyzing the hydrolysis of α-1→4 glucosidic linkages of polysaccharides such as glycogen, starch, or their degradation products. ***Endoamylases*** attack the α-1→4 linkage at random. A single type of endoamylase is known, I.E., α-amylases (α-1,4-glucan 4-glucanohydrolases), so named, because the reducing hemiacetal group liberated by the hydrolysis has α optical configuration and mutarotates downward. The more common α-amylases include those isolated from human saliva, human, hog, and rat pancreas…and barley malt. ***Exoamylases*** attack the α-1→4 linkages only from the non-reducing outer polysaccharide chain ends. Those breaking every glucosidic bond (α-1→4 and α-1→6) to produce solely …glucose are known as glucoamylases *(Glucoamylases are identified as enzyme EC 3.2.1.3. This designation includes amyloglucosidase. MBH)*. Those breaking every alternate bond to produce maltose are known as β-amylases (α-1,4 glucan maltohydrolases). Exoamylases are exclusively of vegetable or microbial origin."

Sherman, H.C. 1932. Chemistry of food and nutrition (fourth edition). The MacMillan Company, New York.

(In this section, dextrins = maltodextrins. MBH)

P 20. "Dextrins… are formed from starch by the action of enzymes, acids, or heat. The term *dextrin*, even if used in the singular, must be understood as belonging to a group rather than an individual substance. Small amounts of dextrin are found in normal, and larger amounts in germinating, cereals…

The digestion of dextrin has already been mentioned in connection with that of starch, both saliva and pancreatic juice forming dextrin during the digestion of starch…"