Starch and Sugar Analysis
An Update

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Topics

- AAFCO / AOAC: Carbohydrate Labeling
- Sugar & Fructan Analysis
- AOAC: Starch Analysis of Animal Feeds
- Comparability of "Sugar" Methods
- Digestibility: How do we get where we want to be?

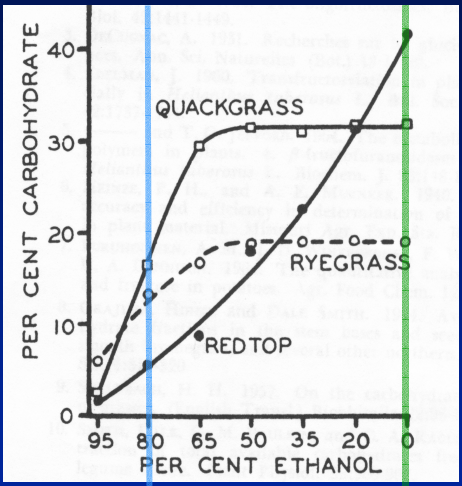
Labeling: Considerations

- AAFCO receiving pressure to allow labeling for carbohydrates in feeds
- Must / will cover all animal species
- Which carbohydrates?
  - Nutritionally relevant & defined
  - Verifiable by AOAC / other recognized method
  - Regulatory analyses consistent with those used for diet formulation?

Which Measures Are Relevant?

- Ethanol or water-soluble carbohydrates?
- Nonstructural carbohydrates or nonfiber carbohydrates by difference?
- Analytes or empirically measured fractions?

Empirical Analyses



Mono-, di- & oligo-saccharides

.....+ fructans

As percentage of water increases, more & larger carbohydrates are extracted.

Difference in values depend on composition of the feedstuff.

Composition of extract will vary by feedstuff.

Error related to nutritional relevance will vary.

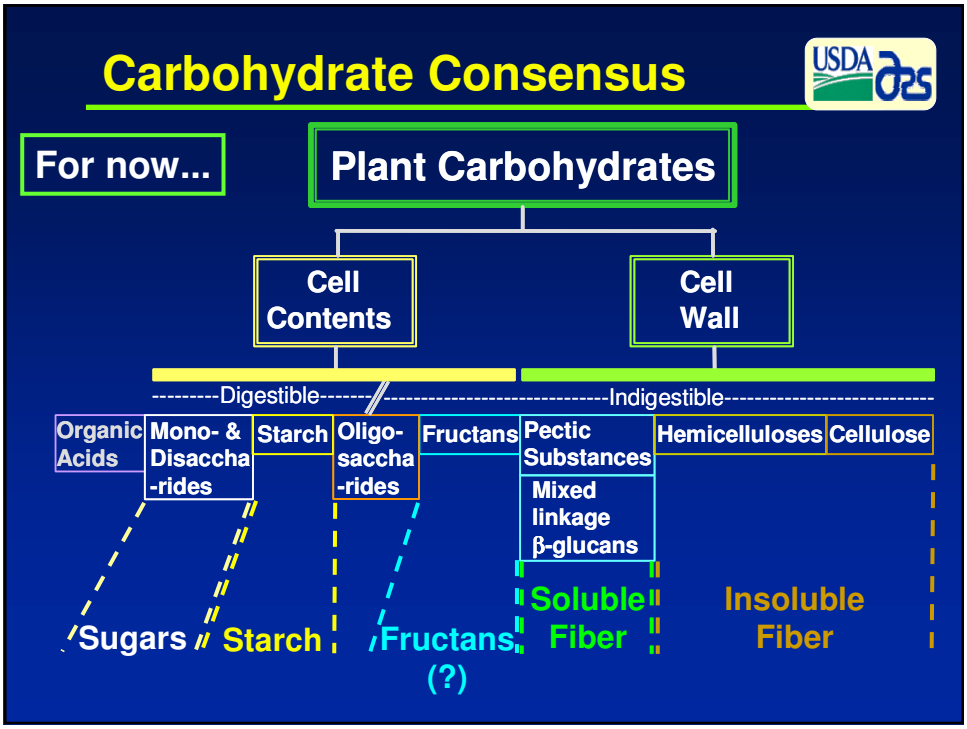
Smith and Grotelueschen, 1966

Carbohydrate Consensus

Class (DP)	Components
Sugars (1-2)	Mono- & disaccharides, polyols
Oligosaccharides (3-9)	Malto- & other oligosaccharides
Polysaccharides (>9)	Starch & non-starch polysaccharides

FAO Food and Nutrition paper – 66, 1997; USDA

Carbohydrate Consensus



Proposed AAFCO Definitions

Starch

The non-structural storage polysaccharide of plants, an alpha-glucan with the glucose released after gelatinization through the use of purified amylases and amyloglucosidases that are specifically active only on a-(1-4) and a-(1-6) linkages. Its concentration in feed is determined by enzymatically converting the starch component to glucose and then measuring the liberated glucose.

Proposed AAFCO Definitions

Sugars

The sum of all free disaccharides and monosaccharides such as sucrose, lactose, maltose, glucose, fructose and galactose or others digestible by enzymes found in the animal's digestive tract.

Fructans

Polysaccharides and oligosaccharides in which fructose is the major constituent and glucose is the minor constituent. Glucose content is 33% or less.

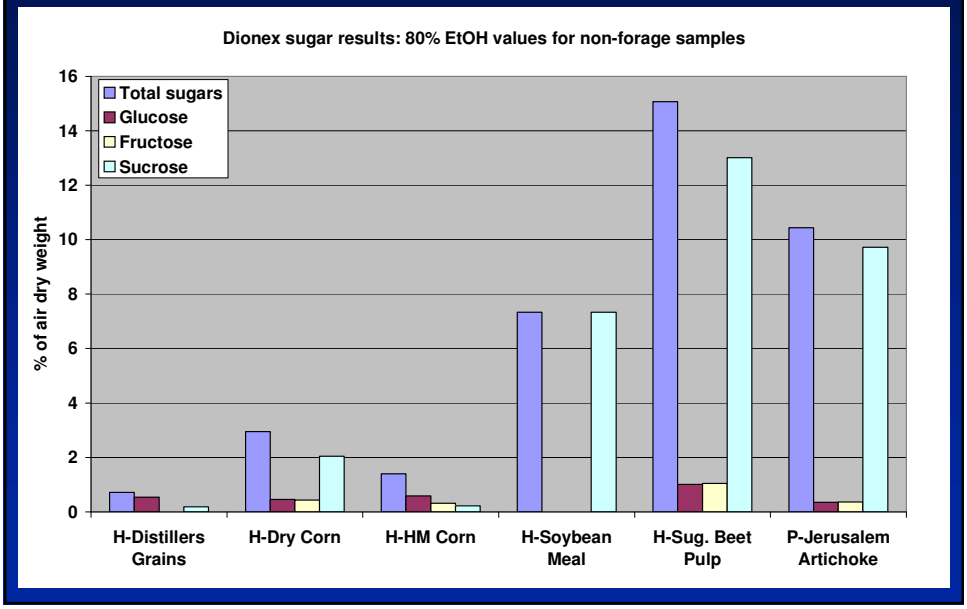
Analysis Approaches

- **Starch:** enzymatic / colorimetric. Need a new AOAC method for animal feeds.
- **Sugars:** HPLC, HPIC
- **Fructans:** Not yet.

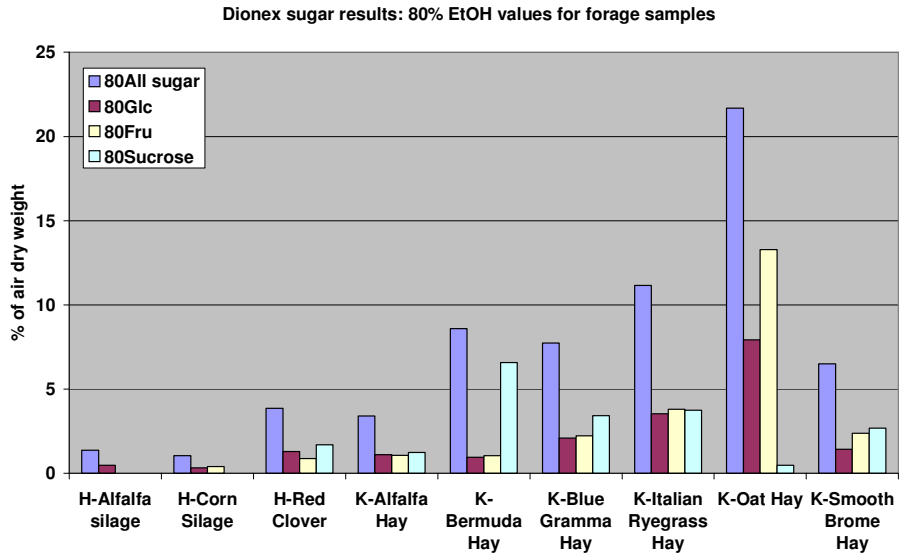
Sugars & Fructans

- Collaboration: USDA-ARS Logan, UT
- Comparison of “gold standard” HPIC and current sugar methods
- Effect of extraction method: water, 50% or 80% ethanol (solubility, preservation) – will not use denatured alcohol.
- Hydrolysis & measurement of fructans
- Diverse forage & feed samples
- Maltose, lactose

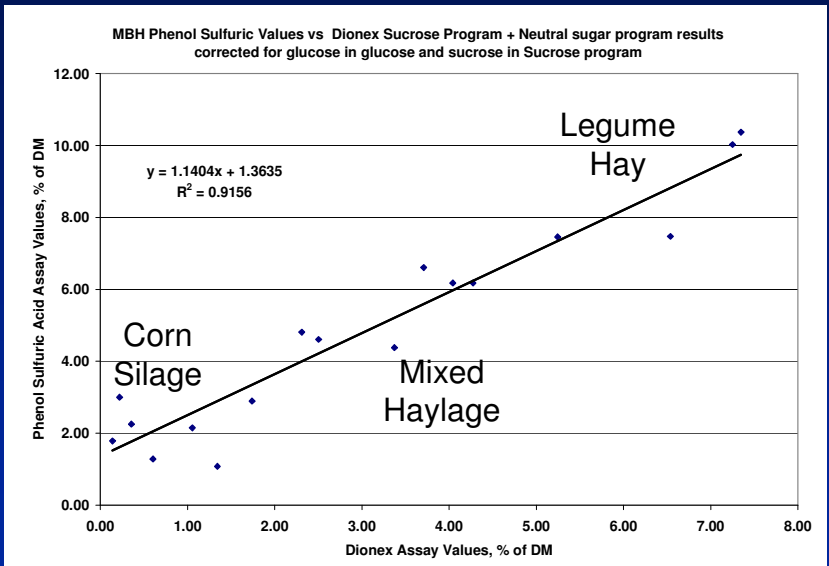
Sugars



Sugars



Empirical Analysis vs Analytes



Hall and Taysom, unpub.

Phenol-Sulfuric Acid Assay

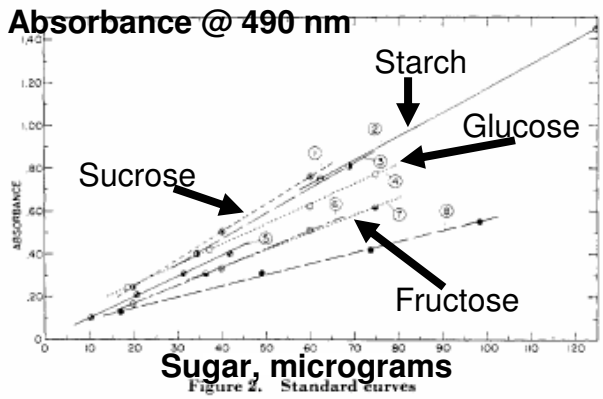


Figure 2. Standard curves

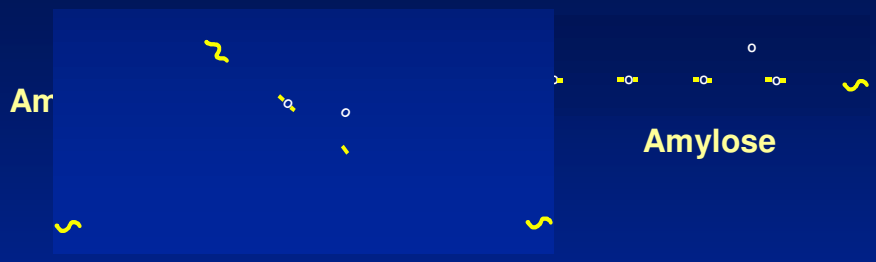
1. Sucrose, Beckman Model DU, 490 mμ, 100 mg. of phenol
2. Potato starch, Beckman Model DU, 490 mμ, 100 mg. of phenol
3. Dextran from *Leuconostoc mesenteroides* strain NRRL 512. Beckman Model DU, 490 mμ, 103 mg. of phenol
4. D-Glucose, Evelyn, filter No. 490, 80 mg. of phenol
5. L-Rhamnose, Coleman Jr., 480 mμ, 40 mg. of phenol
6. Raffinose, Beckman Model DU, 490 mμ, 100 mg. of phenol
7. D-Fructose, Beckman Model DU, 490 mμ, 200 mg. of phenol
8. 2-Deoxy-D-ribose, Coleman Jr., 490 mμ, 80 mg. of phenol

- **Different sugars respond differently.**
- **Selection of the sugar used as a standard alters the standard curve.**

Comparability: Sugars

- 80% Ethanol & Reducing Sugars
- Different extractions, different detection methods, different interferences, variation in the analyses (?)
- At low concentrations?

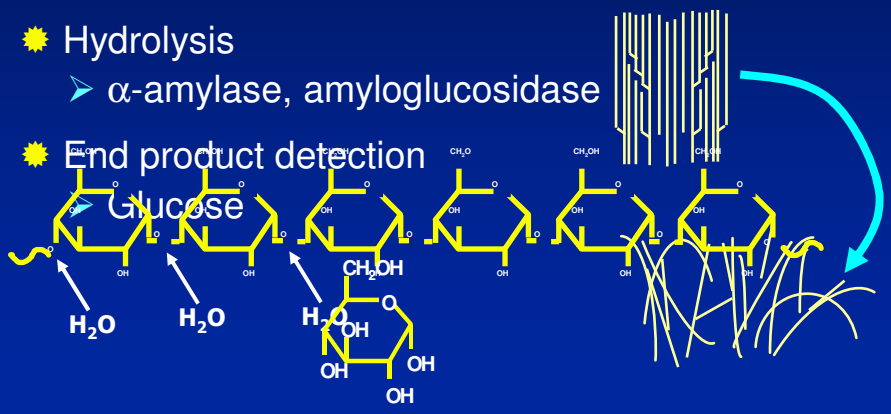
Starch: Definition



- α-(1-4) glucan with α-(1-6) branch linkages
- With AAFCO & Industry support, planning an AOAC collaborative study for analysis of starch in animal feeds.

Starch: Enzymatic Analysis

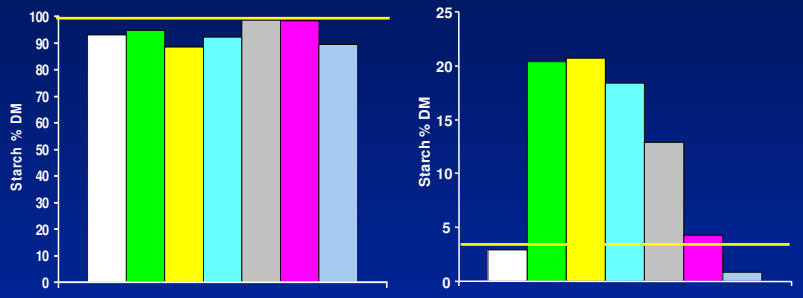
- Gelatinization
  - Disrupting the hydrogen bonding/ crystalline structure of starch chains
- Hydrolysis
  - α-amylase, amyloglucosidase
- End product detection
  - Glucose



Starch: Enzymatic Analysis

- Repeatability: ± 2%
- Sources of error:
  - Glucose source (purity, DM)
  - Non-amylase enzyme activity
  - Inadequate gelatinization
  - Incomplete hydrolysis (enzyme, grinding & sample)
  - Detection of non-starch end products
  - End product disappearance/destruction
  - Accuracy of glucose std curve, etc…..

Starch: Interfering substances



Corn Starch

Confectioner's Sugar

Enzymes or acids can hydrolyze sucrose.

Hall et al., 1999

Starch: Low Recovery

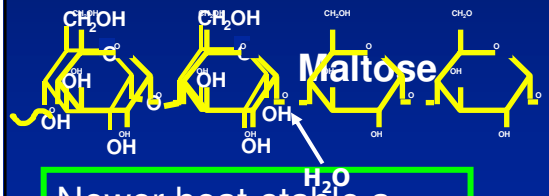
- Heating in distilled water with α-amylase
- Hydrolysis in pH 4.75 Na acetate buffer with amyloglucosidase

	% Starch DM basis	
Wheat starch, raw	96.6 + 0.6	Holm et al., 1986

Adjusting values for recovery is a bad idea.
What are your assumptions?
Is low recovery acceptable & "normal"?

Starch: Low Recovery

- Isomerization of reducing end glucose
  - Neutral to alkaline pH + heat: reducing end glucose isomerizes to fructose. An issue with hydrolysis with gelatinization at neutral pH?



Newer heat-stable a-amylases can function at more acidic pH.

	Autoclave pH 4.5	7.0
Glucose	96.7	93.2
Maltulose	0	4.6
Maltose/ isomaltose	2.6	1.7

Dias and Panchal, 1987

Starch: Bach Knudsen, 1997

Danish Institute of Agricultural Sciences

- Volumetric additions. Final volume can be determined by weight.

Starch Assay

- Carry a reagent blank, control starch sample and control glucose sample through the assay.
- Analyze for free glucose in samples to which no enzymes have been added.
- Analyze sucrose with the assay to verify that no glucose is released by enzymes or run conditions.
- Avoid neutral/alkaline conditions with partially hydrolyzed samples.

Digestibility Characteristics

- Starch digestibility/degradation
  - Enzymatic and fermentation methods
- Measurement affected by particle size.
- Measurement affected by starch and feed characteristics.
- Relationship to in vivo rates?
- What alters rates in vivo?

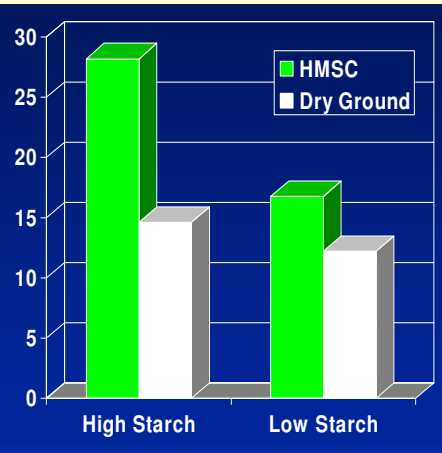
What system will the values be used in?

Qualitative or quantitative values?

Numeric and qualitative?

Starch: Rates Subject to Change?

Rates of Total Starch Fermentation, %/h



- **Ruminal starch fermentation rates were decreased at lower starch levels in the diet.**
- **Change greater for rapid than slow rate.**
- **Starch enzyme activity ~68% on low starch diet?**

Oba and Allen, 2003 Starch $P<0.001$, Corn $P<0.001$,Starch x Corn $P<0.01$

Digestion: Numeric & Qualitative

- In vitro or in situ results are independent of ration interactions that will vary and can matter.
- Currently, digestibility values are probably relative (higher or lower), not true/innate for the material (affected by lab, method,…..)
- What is the relationship of the measure to how the feed digests in the animal and our ability to predict it? (biology & models; correlations vs. absolute value; right answer for right reason)

Goals for Digestibility Measures

- Are we satisfied with what we have? Predicting normal or abnormal outcomes?
- What are correct/acceptable **ANIMAL** methods to get digestibility data & samples needed for method validation? TMR extrapolated to individual feeds?
- How precise / accurate can values be or need to be (analytical variation, application/sensitivity)? Are other "easier" measures correlated?
- Are qualitative values that **show direction** of response in context useful?
- Continue to work to understand interactions.

Who do we need in the discussion:

-- To decide on the questions and methods to address the practical issue of how to link animal and in vitro methods, and get the methods we need/want?

Scientifically sound basis and perhaps pragmatic.