

CHEMISTRY OF HIGH FRUCTOSE CORN SYRUP (HFCS) USPHS Training Symposium

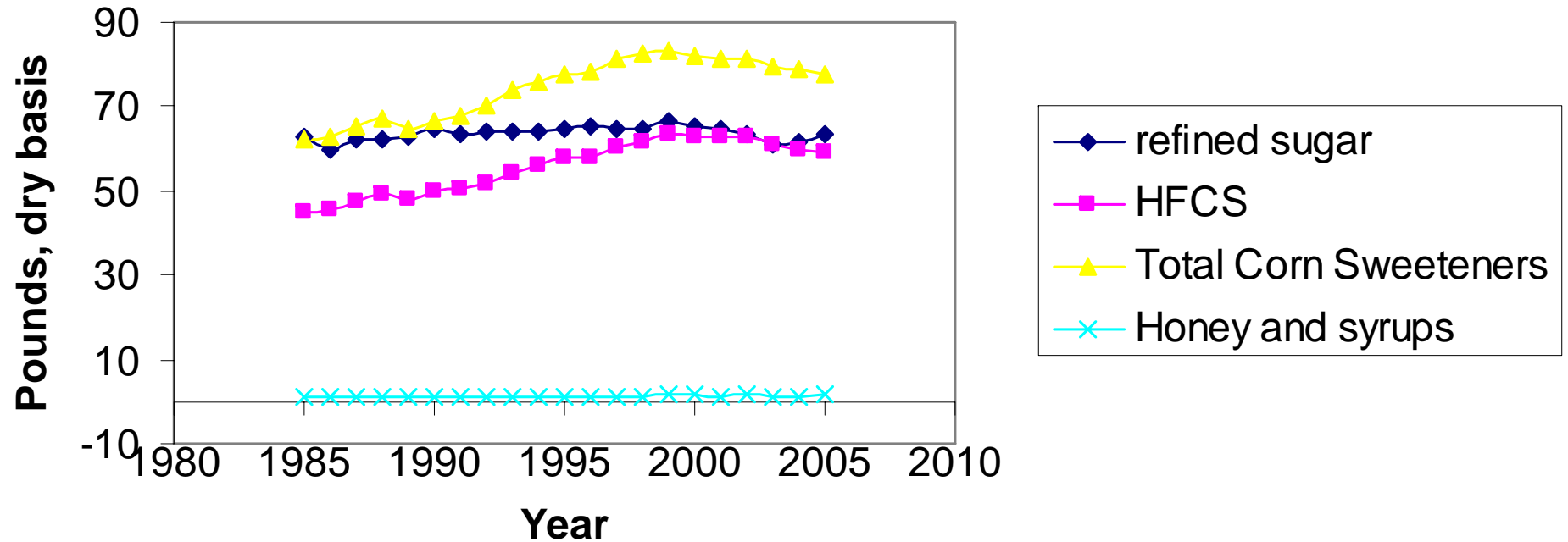
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- Industrial manufacture
- Impurities from manufacturing method
 - Maltooligosaccharides
- Impurities formed from shipment / storage conditions
 - Hydroxymethylfurfural (HMF)
 - Hydrolysis products of HMF:
Levulinic acid, Formic Acid

U.S Per Capita Sweetener Deliveries for Food and Beverage Use



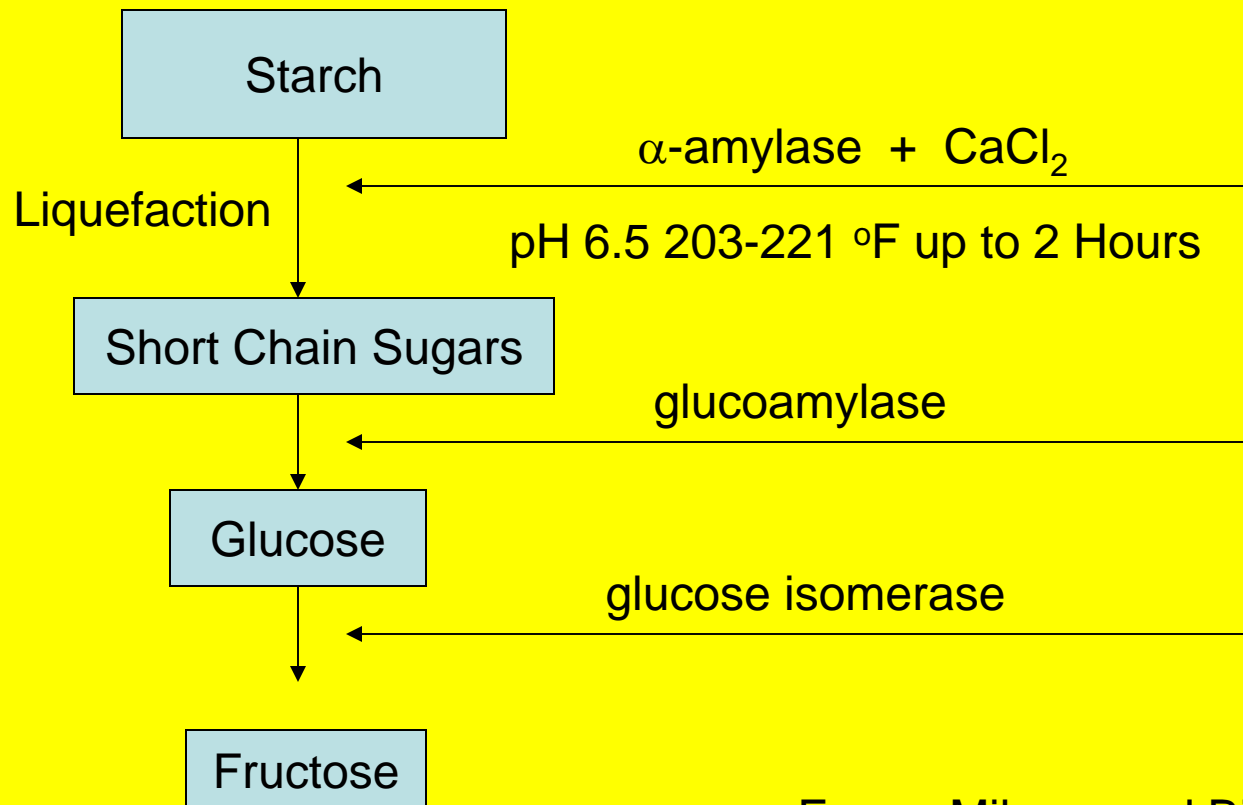
Source: USDA Economic Research Service, www.corn.org/percapsw.htm

High Fructose Corn Syrup Types and Usage

- HFCS 42: soft drinks, beer, other beverages, confectionary products
canned goods, HFCS 55
- HFCS 55: ice cream, yogurt, processed foods, feed for honey bees for crop pollination
- HFCS 90: used to make HFCS 55, exported

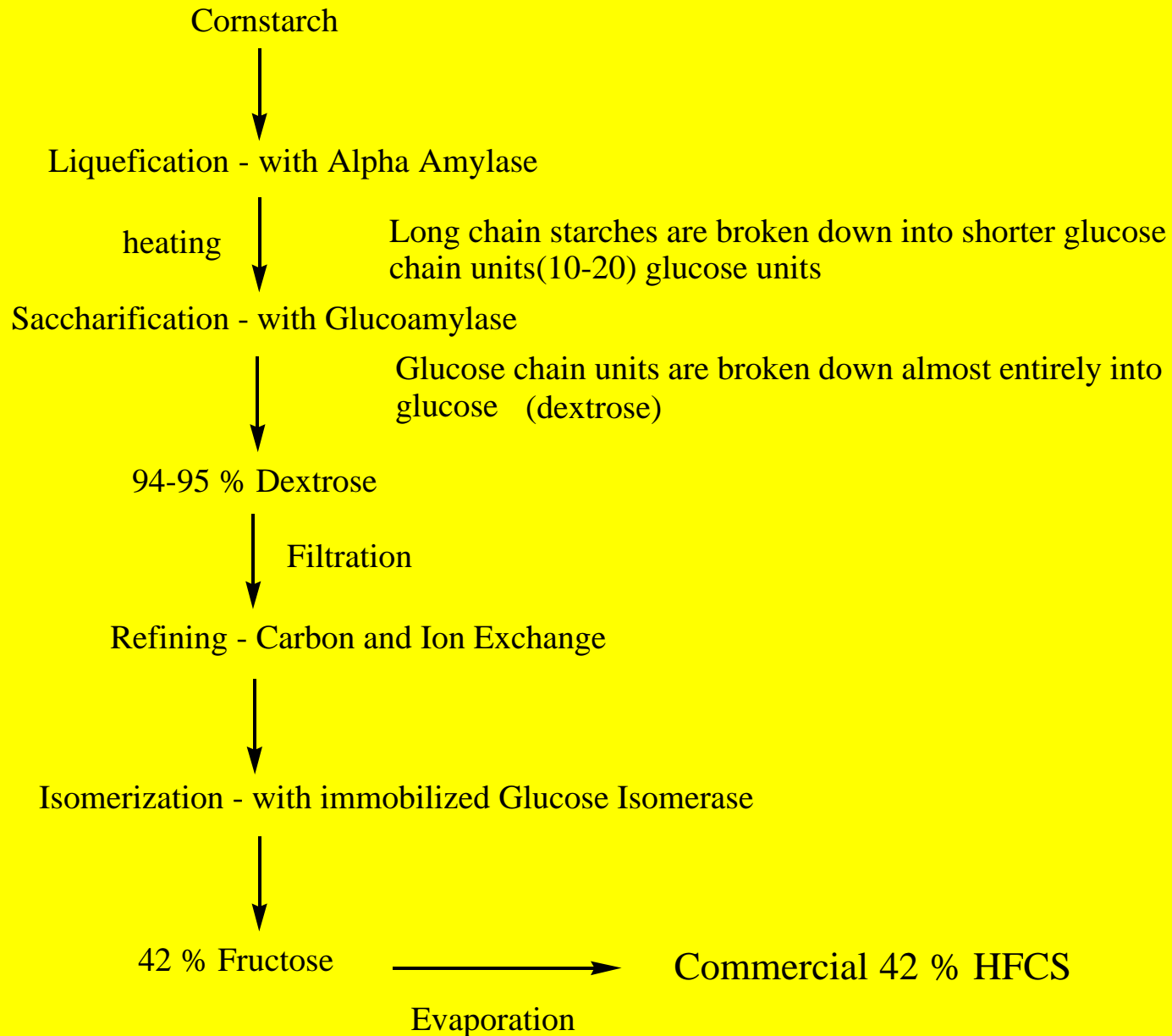
Industrial Manufacture of HFCS

- α -amylases: produced from genetically and protein engineered bacteria: *Bacillus licheniformis* and *Bacillus stearothermophilus*
- R & D performed by large enzyme companies that engineered these enzymes specifically for HFCS and detergent industries

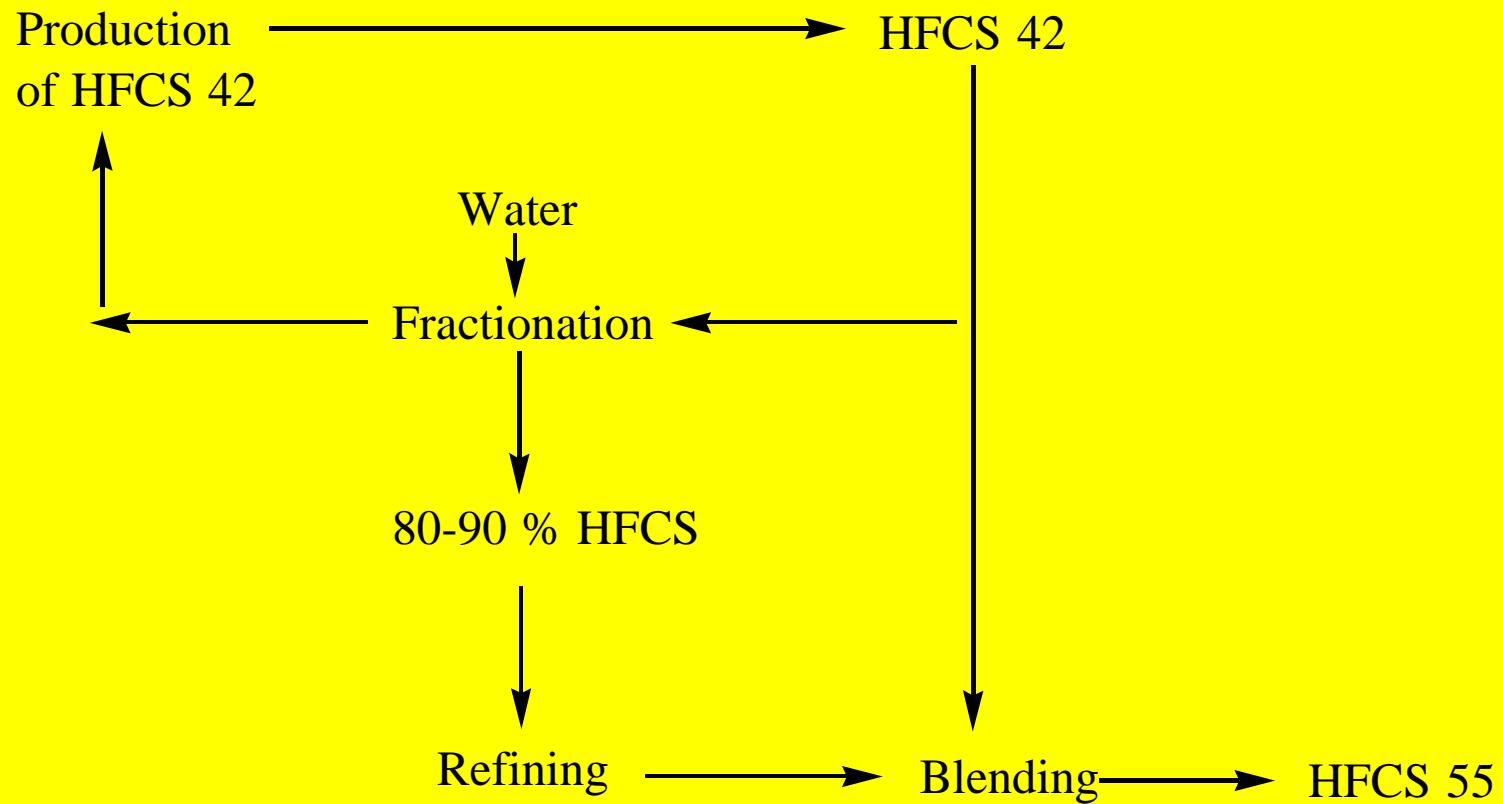


From: Mikoyo and Bisgaard-Frantzen, 2000

HFCS 42 Manufacturing



HFCS 55 Manufacturing

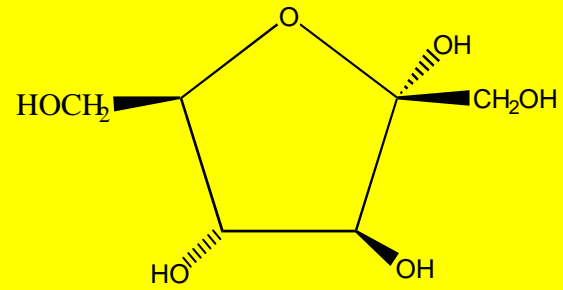


Long, John, E. Food Science and Technology 1991, 48, 247-258

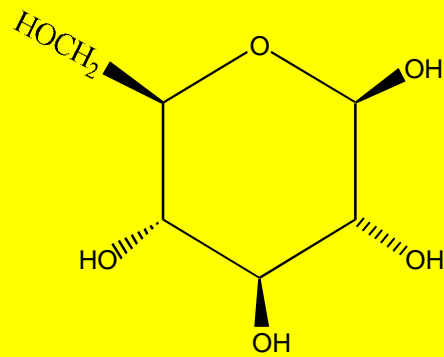
Physical properties of HFCS

Sample	Density	BRIX	Thermal Conductivity	% Dry Weight Solids	% Moisture
	g/mL	@ 20 C	$\text{wm}^{-1} \text{ } ^\circ\text{C}^{-1}$		
A-42	1.34±0.004	68.69±0.012	0.372±0.004	70.446	29.56
A-55	1.38±0.008	75.54±0.006	0.350±0.000	77.470	22.53
B-42	1.33±0.012	69.76±0.006	0.363±0.005	71.051	28.99
B-55	1.38±0.002	75.47±0.006	0.348±0.004	77.970	22.03
C-42	1.32±0.005	69.52±0.006	0.360±0.000	71.516	28.48
C-55	1.32±0.005	75.50±0.006	0.344±0.005	78.253	21.75
D-Blend	1.37±0.008	75.93±0.006	0.350±0.000	77.953	22.15
D-55	1.37±0.006	75.46±0.006	0.340±0.004	76.892	23.11

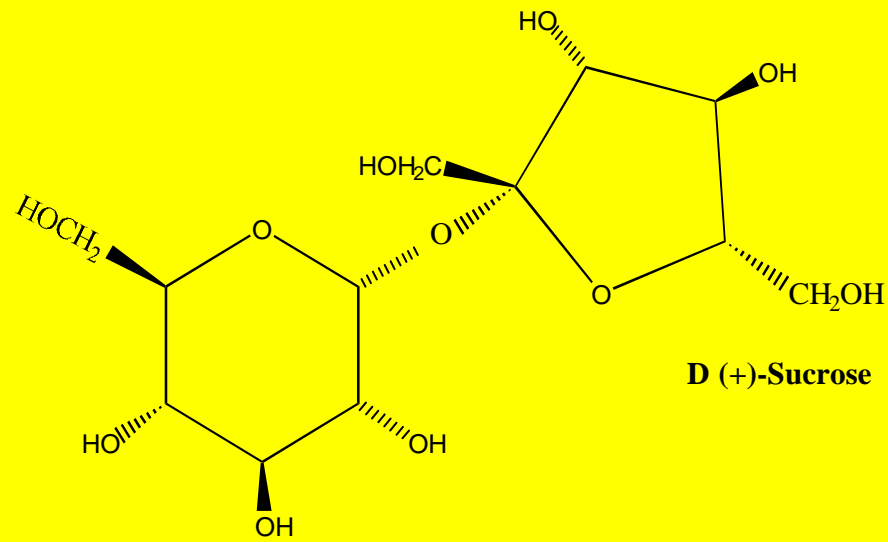
Chemical Structures of Sweeteners



D(-)-Fructose



D (+)-Glucose

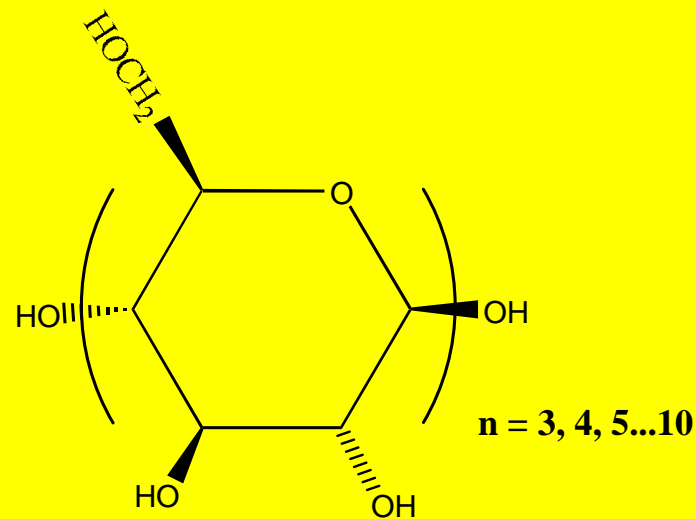


D (+)-Sucrose

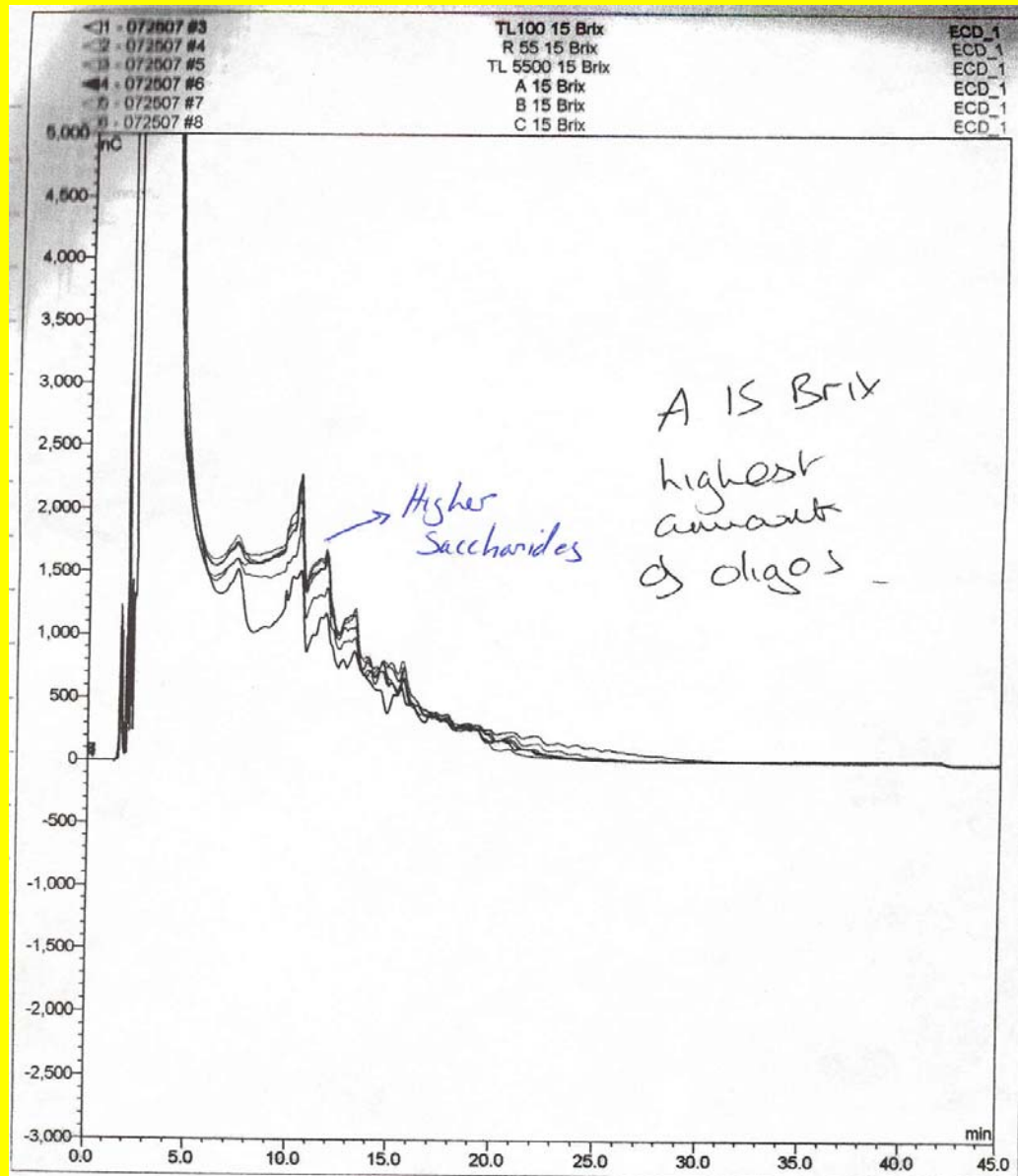
Impurities as a result of the method of manufacture

Confirmed: Maltooligosaccharides (long chain polymers of glucose) resulting from incomplete hydrolysis of starches are present in HFCS as a result of incomplete breakdown into glucose

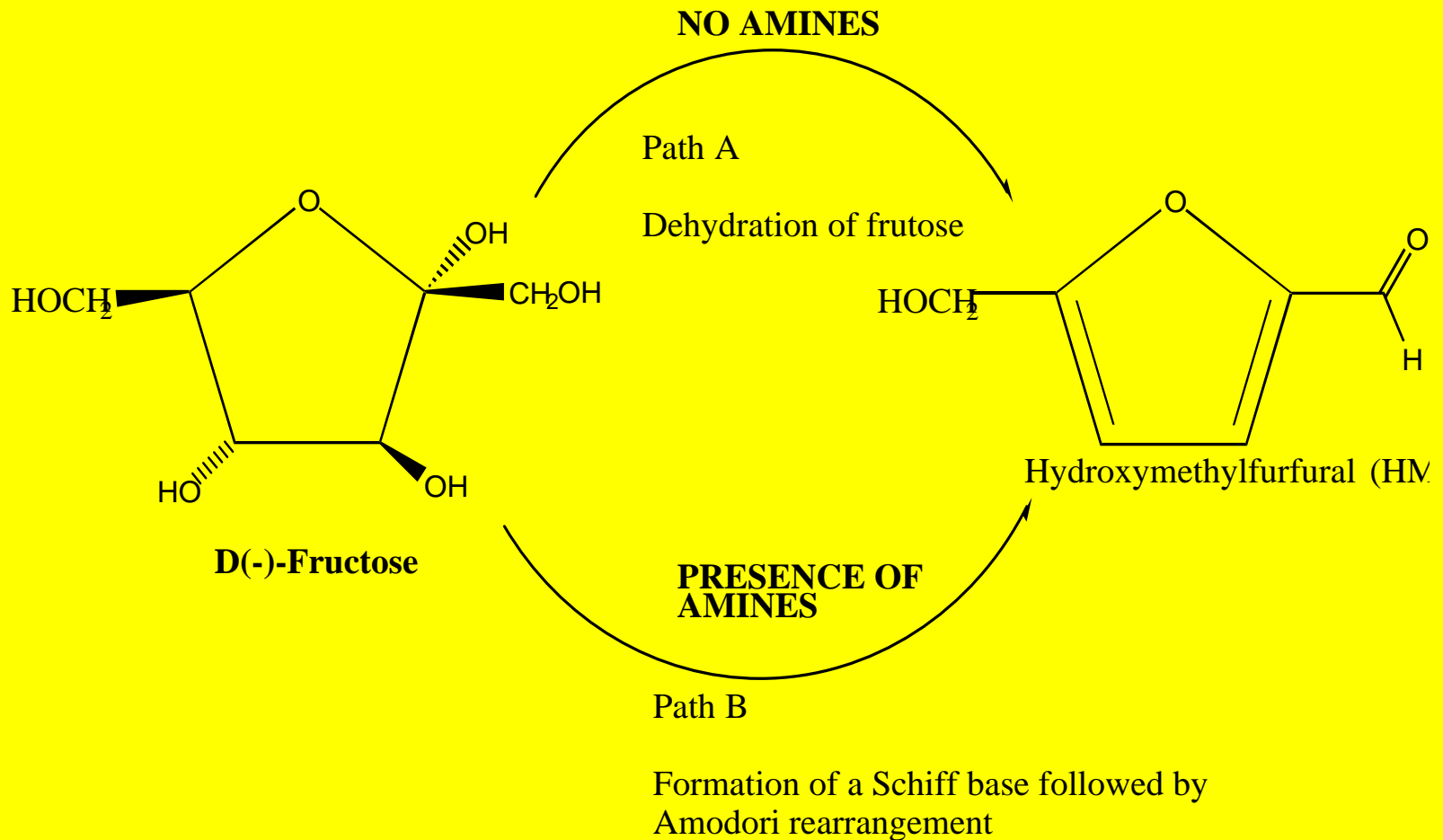
→ These are in HFCS samples and are going to be tested on bees for toxicity. We suspect that the bees cannot digest these large molecules because stachyose and raffinose are known to be toxic to bees



Liquid Chromatography Chromatogram of an HFCS sample diluted by a factor of 5

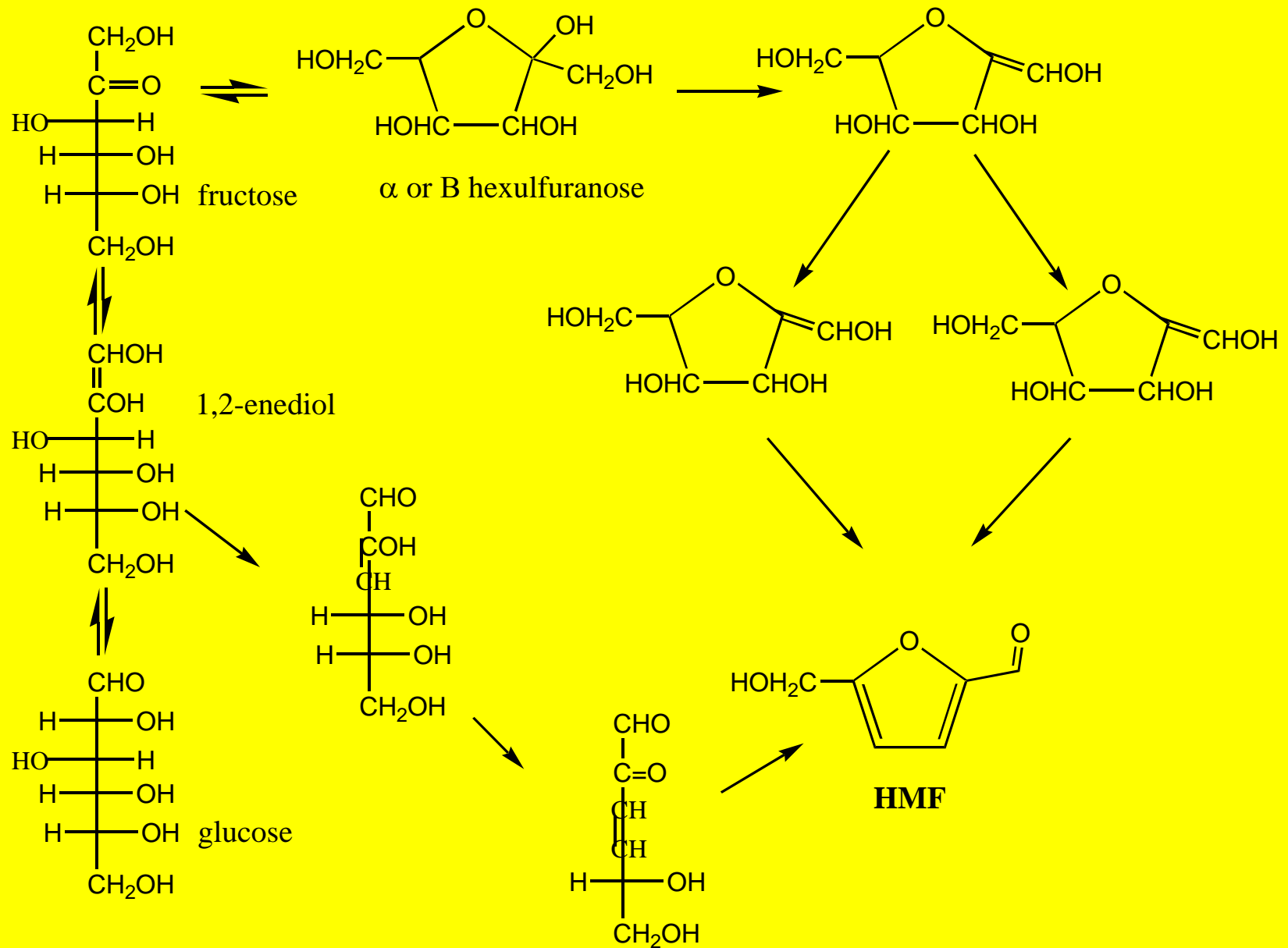


Formation of Hydroxymethylfurfural (HMF)



HMF is a dehydration product in this study (NO NITROGEN).
If there are nitrogen or amine compounds present then HMF would be classified as a Maillard product.

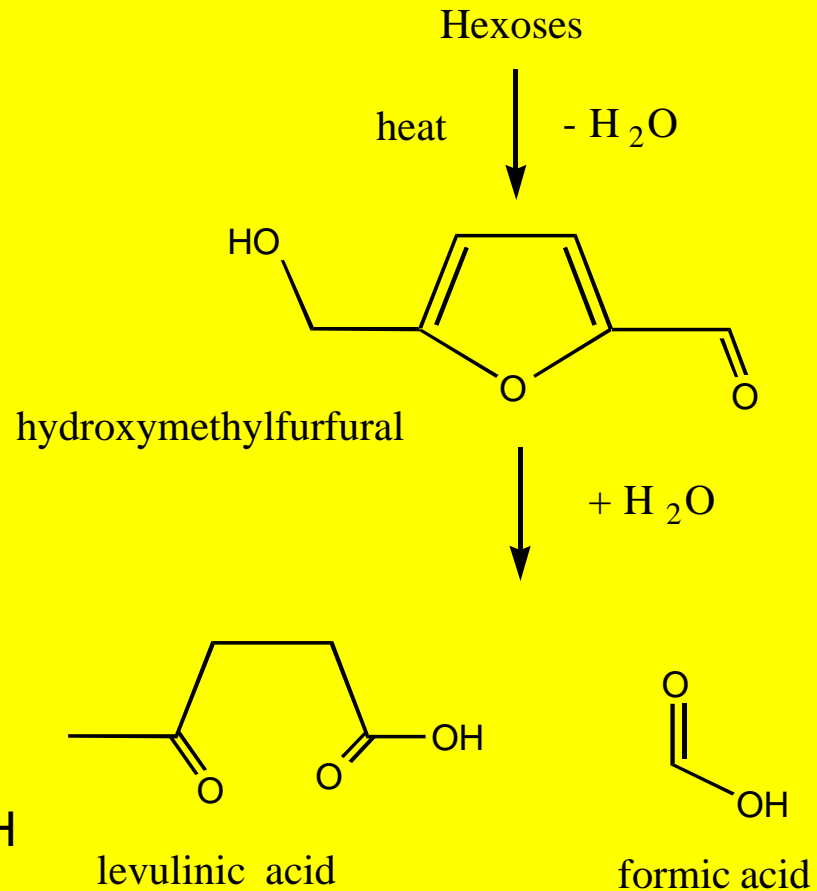
Proposed mechanism for HMF formation



Hydroxymethylfurfural (HMF) in HFCS

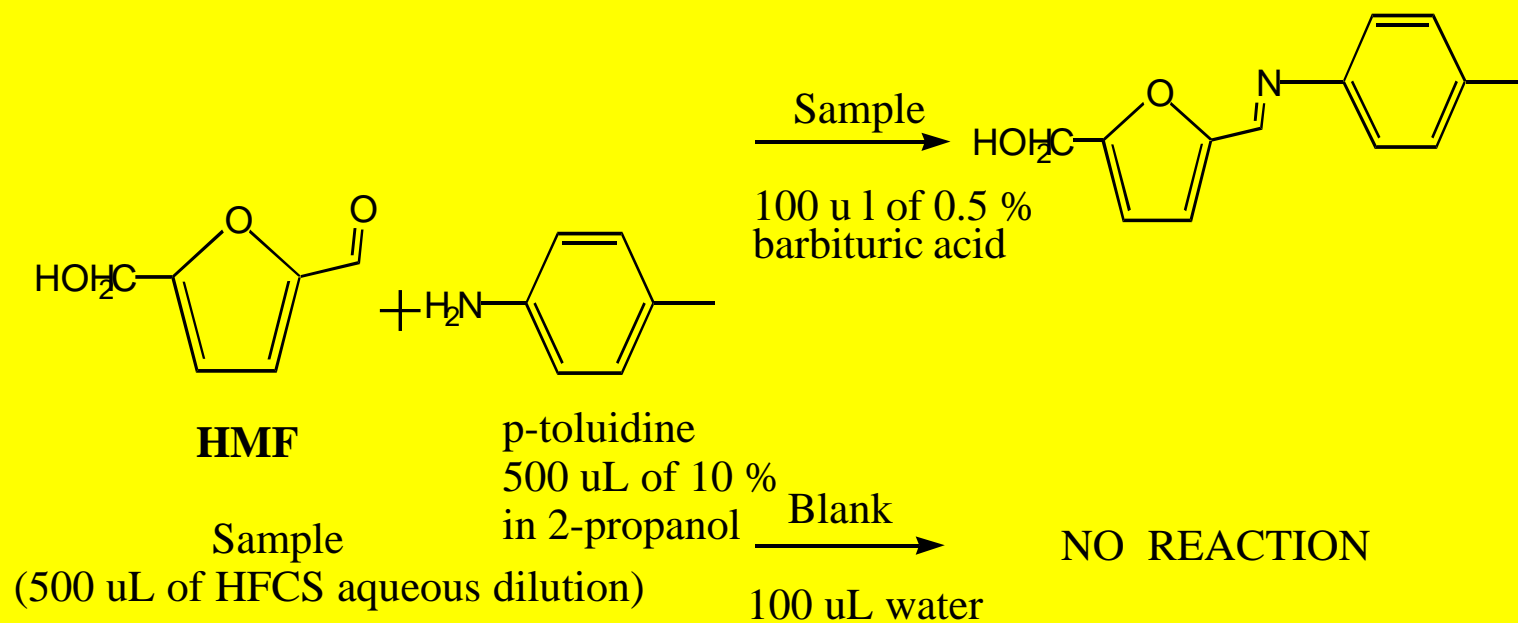
Favor HMF formation

- Fructose (starting material)
- Heat
- Time
- Low pH (Acidic conditions)
- Metal Ions (stabilize HMF)
- Dehydrating agents
- Breakdown of HMF to levulinic acid and formic acid lowers the pH
- This further catalyzes HMF formation



Spectrophotometric method to detect HMF

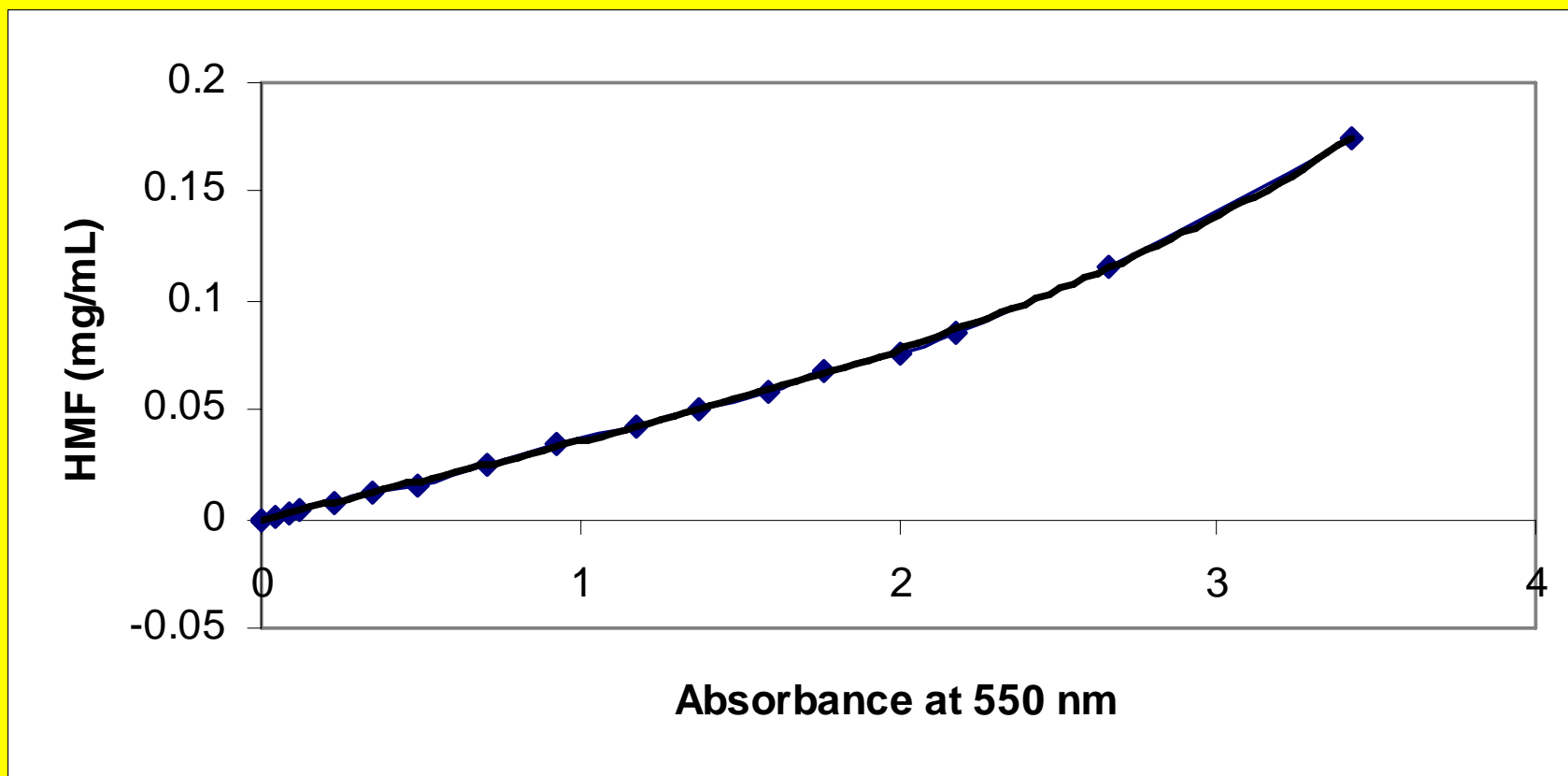
AOAC method, initially reported by Winkler, scaled to microliter levels to accommodate 96 well micro-plates



Measure absorbance at 550 nm of sample and blank. Determine Concentration by running against standard curve. Plot concentration in mg/mL versus absorbance.

O. Winkler and Z. Lebensm, Unters. Forsch. 102 (1955), p. 160.

Calibration curve for determination of Hydroxymethylfurfural (HMF) in samples



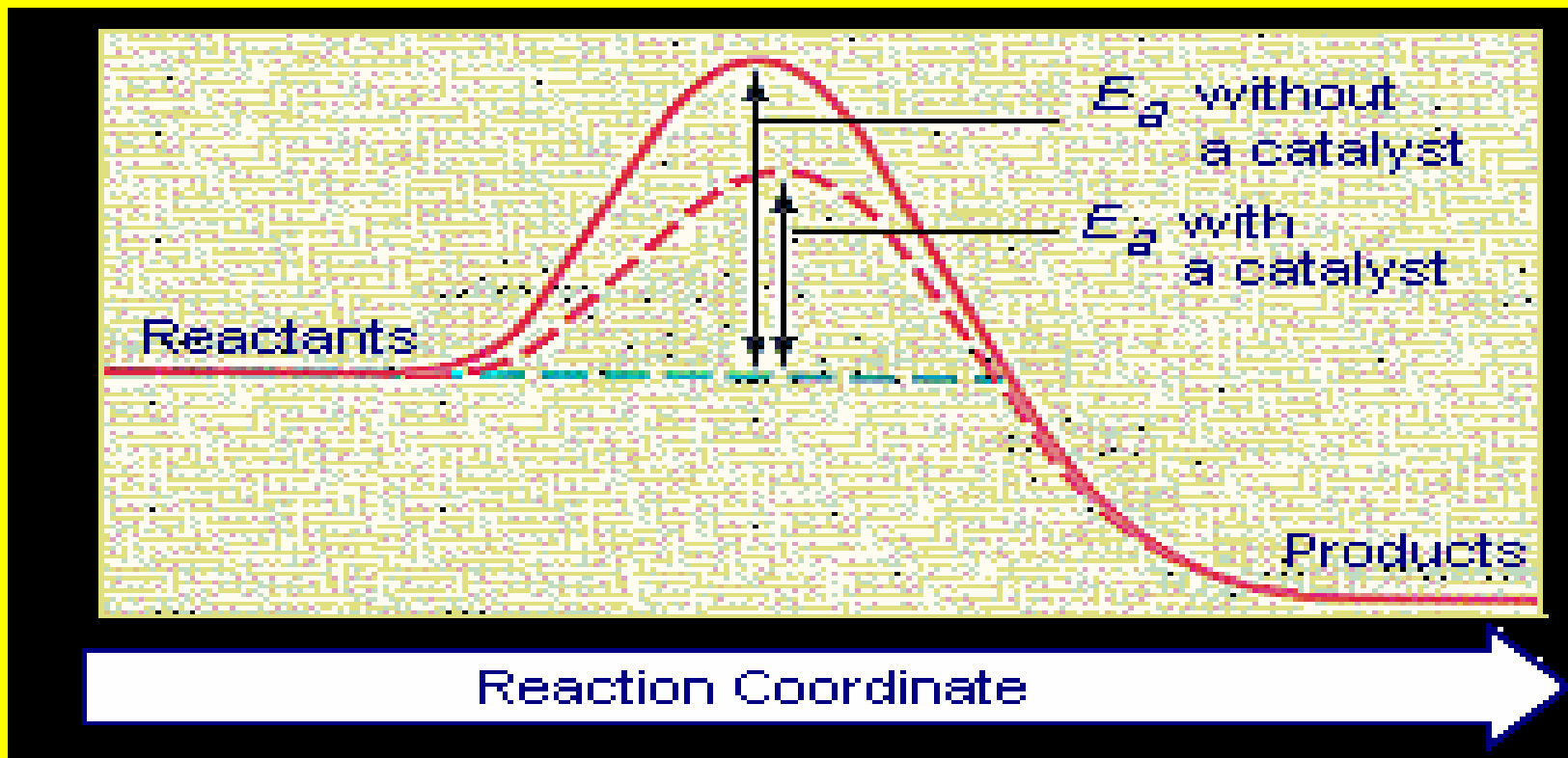
$$\text{HMF (mg/mL)} = 0.0001 \times \text{Abs}^4 + 0.0013 \times \text{Abs}^3 - 0.0019 \times \text{Abs}^2 + 0.0362 \times \text{Abs} - 0.0003$$

$R^2 = 0.9997$

Chemical Properties of Domestic HMF Samples

HFCS	pH	% HCl equivalents	HMF _o (ug/g) ppm	% Fructose	% C, H, N, S
A-42	4.15	0.014	20.75	42	29.30, 5.55, 0, 0
A-55	4.86	0.078	28.65	55	31.72, 7.39, 0, 0
B-42	3.86	0.011	3.07	42	29.50, 7.65, 0, 0
B-55	4.16	0.009	20.77	56	31.56, 7.42, 0, 0
C-42	4.18	0.0092	8.13	42	29.53, 7.55, 0, 0
C-55	5.02	0.0074	7.89	56	31.69, 7.43, 0, 0
D-Blend	6.08	0.0062	4.05	50	32.79, 7.19, 0, 0
D-55	4.34	8.47	27.47	55	31.70, 7.41, 0, 0

Reaction coordinate diagram



If the Energy of Activation (E_a) can be determined then the rate of formation of product can be estimated for any temperature. The rate of formation of product must be studied at least for two temperatures for the E_a to be estimated.

How to determine the energy of activation

In 1889 the chemist Svante Arrhenius showed that the relation between temperature (T), rate constants (k) and energy of activation (E_a) obeys an equation of the form:

$$k = Z e^{-E_a/RT}$$

k = rate constant in moles / second

Z = proportionality constant and is reaction specific

E_a = the energy of activation in Joules / mole or kCal / mole

R = gas constant 8.314 Joules / mole / Kelvin

T = temperature in Kelvin

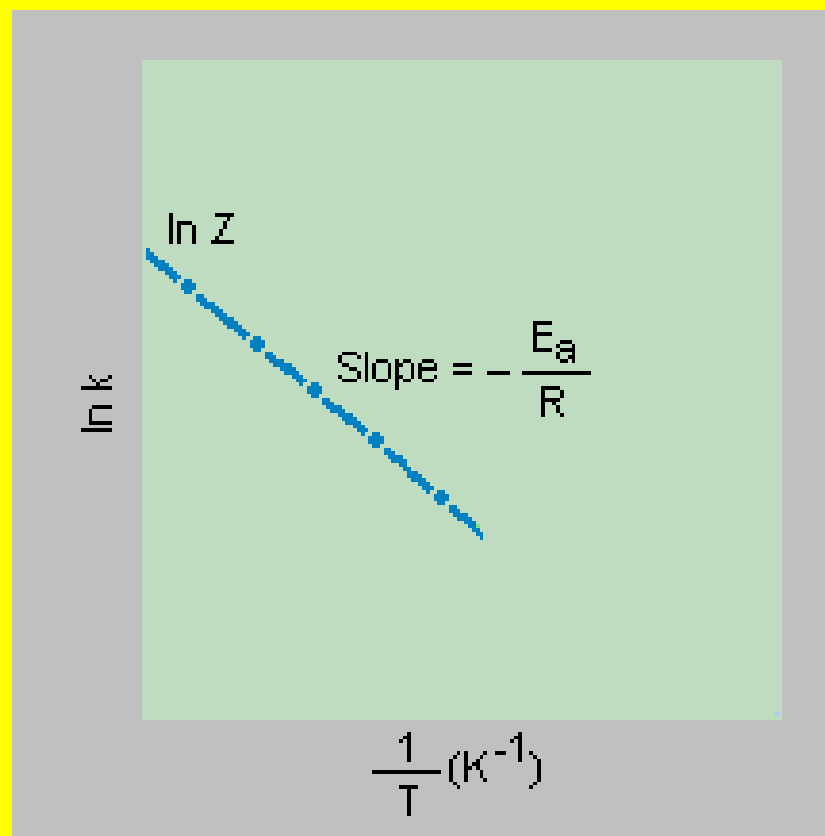
$$\ln k = \ln (Z e^{-E_a/RT})$$

$$\ln k = \ln Z - E_a/RT$$

$$\ln k = - E_a/RT + \ln Z$$

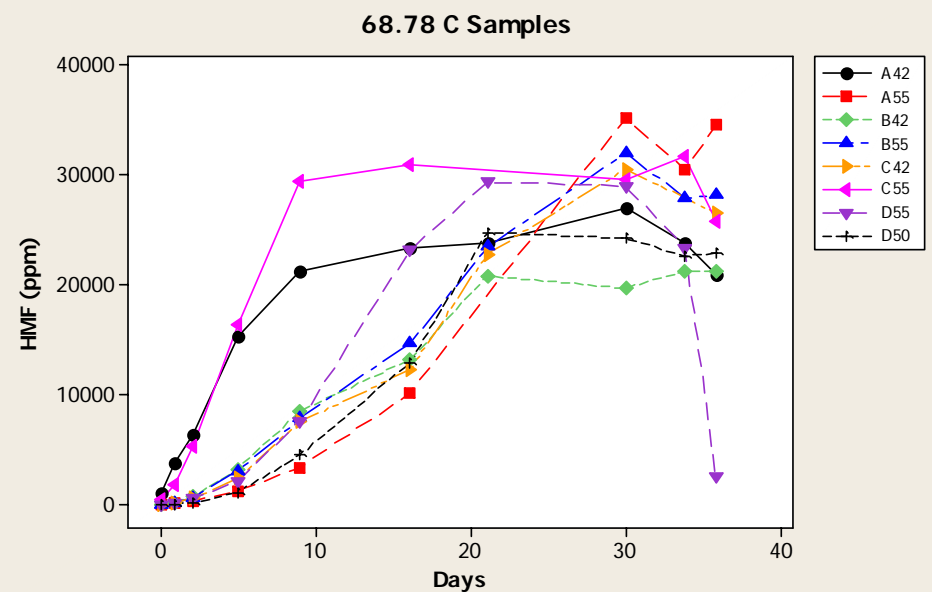
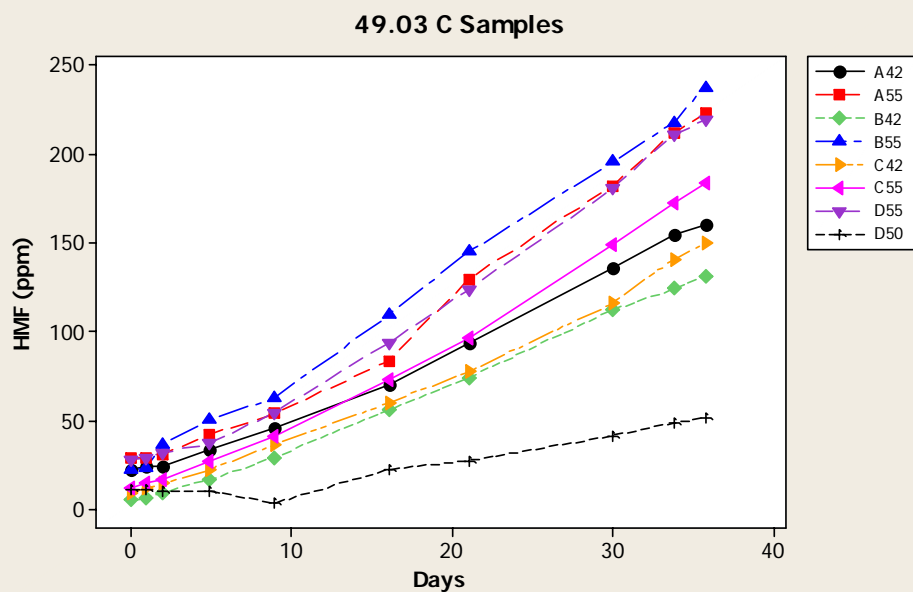
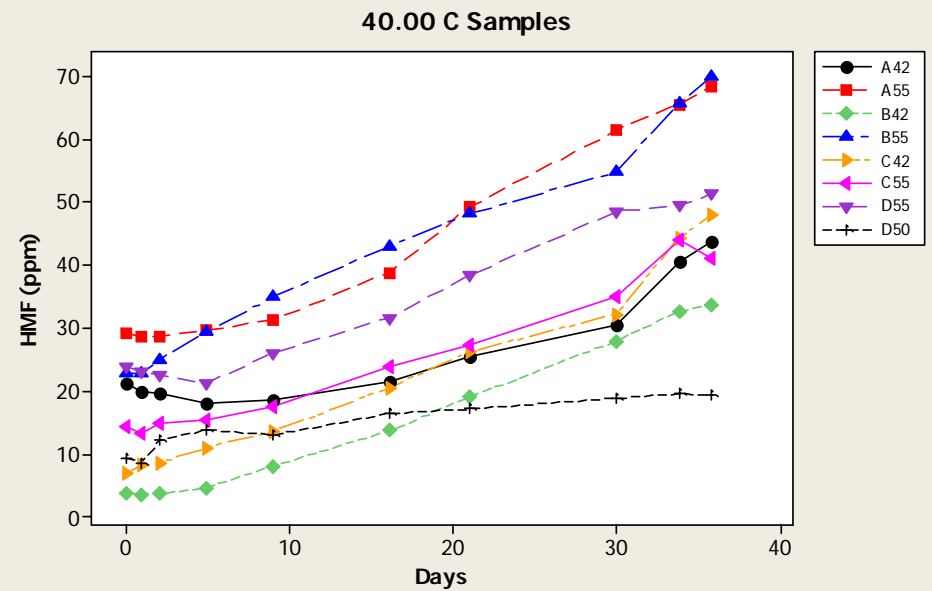
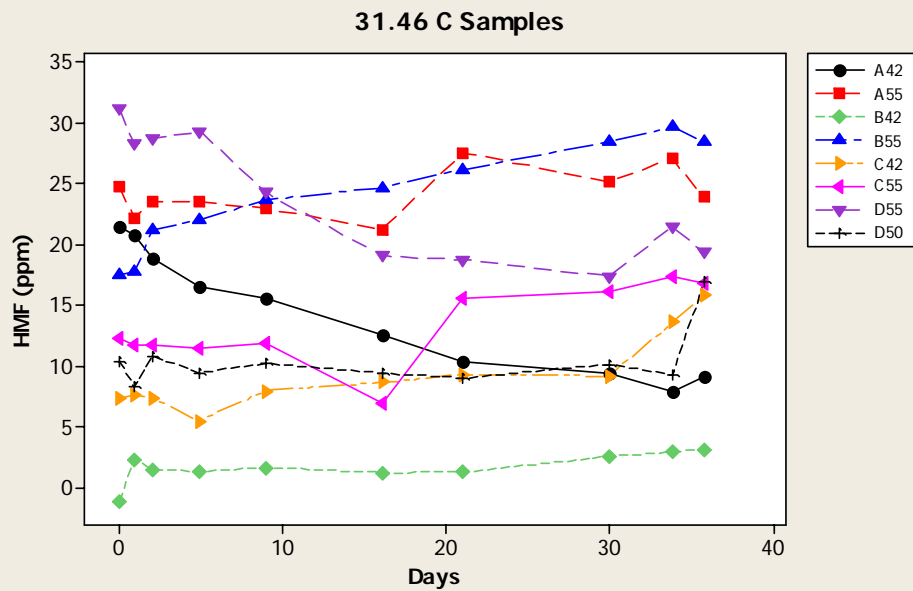
$$y = m x + b$$

plot $\ln k$ versus $1/T$, slope = $-E_a/R$

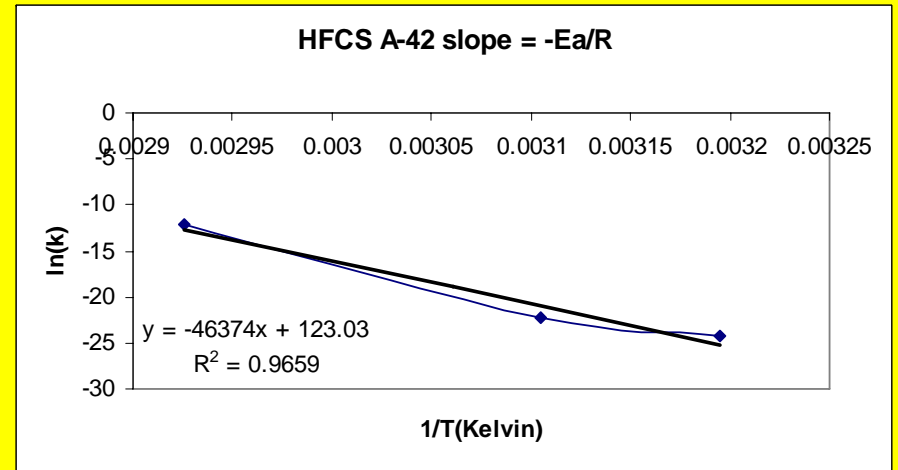
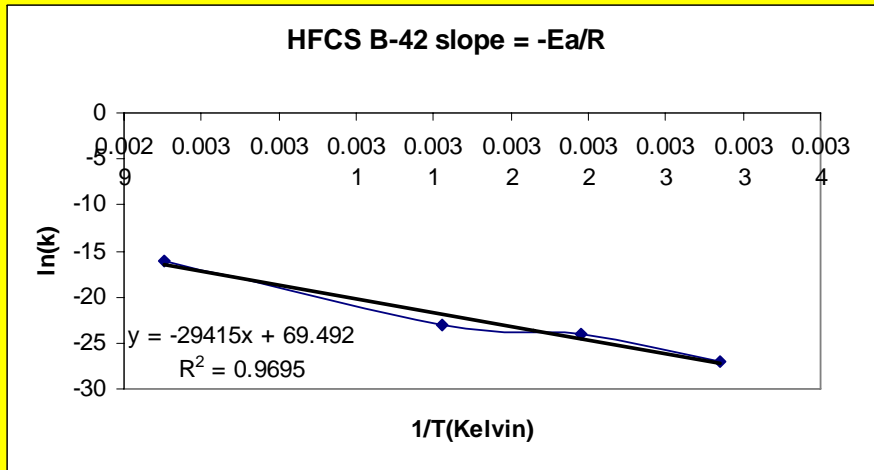


Study rates of formation of HMF at different temperatures

The temperatures are time weighted averages



Correlation of E_a with pH



Brand	E_a (kCal/mol)	pH
A-42	94.26	4.15
B-42	56.64	3.86

$$\ln(k_1/k_2) = E_a/R(1/T_2 - 1/T_1)$$

If I know the rate at one temperature and the energy of activation, then I can estimate the rate at formation at a selected temperature.

Estimate of HMF consumed annually

2007 estimated per capita consumption of HFCS at 44.2 lb/year

$44.2 \text{ lb} \times 2.2 \text{ kg/lb} = 97.24 \text{ kg HFCS}$

$97.24 \text{ kg} \times 3.07 \text{ mg/kg} = 298.52 \text{ mg annually (lowest sample measured)}$

$97.24 \text{ kg} \times 28.65 \text{ mg/kg} = 2,785.93 \text{ mg annually (highest sample measured)}$

<http://www.hfcsfacts.com/PerCapitaConsumption.html>

<http://www.pubmed.org>

<http://www.sciencedirect.com>

Conclusion & Current & Future Projects with HFCS

- HFCS syrups are provided acidic from the manufacturers to prevent fermentation. We are experimenting with neutralizing the syrups and have found that natural terpenoids prevent fermentation.
- A slight difference in pH will enhance HMF formation
- By increasing the pH (more basic), HMF formation may be minimized
- By minimizing HMF concentrations, Levulinic Acid and Formic Acid will be prevented
- Current and long-term projects will involve studying HMF formation in buffered HMF samples and in different containers (stainless steel, steel, plastic, glass)
- We will also study the rate of formation of Levulinic acid and Formic Acid from heat damaged syrups and surrogate samples that are spiked with HMF
- Conduct an Ames mutagenicity study on HMF
- Ultimately develop a field test for HMF

Acknowledgements (in alphabetical order of last name)

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