A New Acid Stable Thermostable Maltogenic Alpha Amylase for High Maltose Syrup

58 Detmold Starch Convention, Germany

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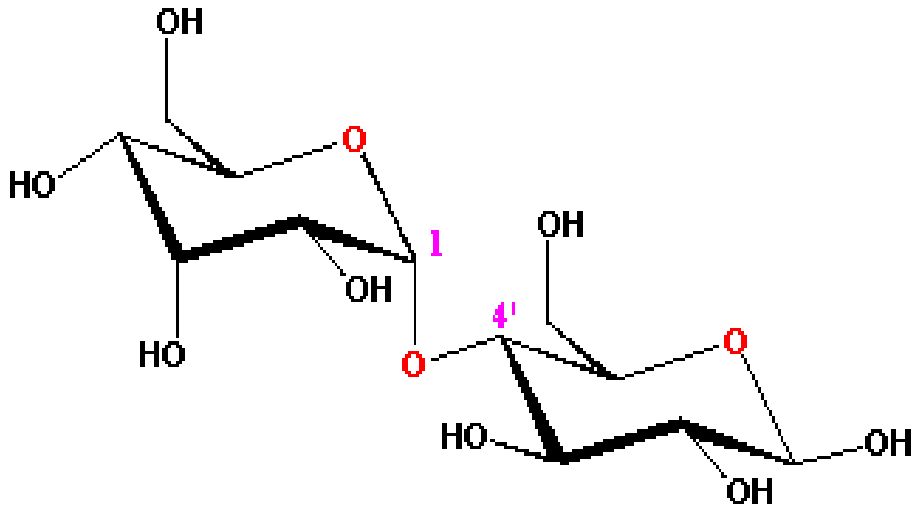

Agenda

- Current maltose production and application
- The issue
- What is the new enzyme?
- Application data
- Summary

Maltose Application

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Applications	Functions
Baking	•Good heat stability •Reduced browning
Brewing	•High fermentability
Candy	•Low hygroscopicity •Resists crystallization
Canning	•Low sweetness
Soft drinks	•Body/flavor characteristics •Pleasant mouth feel
Food formulations	•Good heat stability •Low hygroscopicity •Low viscosity
Hydrogenated maltose (maltitol)	•Low calorie sweetener
Nutrient (Japan)	•Intravenous feeding
Health (bifidobacteria) and functional food ingredients	•Substrate for IMO sugars

Typical Process of Maltose Production

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Starch (Maize or Tapioca or other)
(15-32% dsb)

↓

Liquefaction
(DE: 4-11)

← **Alpha Amylase**
(SPEZYME ® FRED or SPEZYME ® AA)

↓

Alpha Amylase Inactivation
(pH 4.2, 30 min. at 95 ºC, or second jet at 120+ °C)

↓

Saccharification
(pH 5.2-6.0, 55-60 ºC)

← **Fungal AA (CLARASE(R) L)**
β - Amylase (OPTIMALT ® BBA)
Pullulanase (OPTIMAX ® -L 1000)

↓

Refining and finish
Depending on original DE some residual starch may remain that can be removed with alpha amylase.

REACTION MECHANISM *of* BETA AMYLASE, PULLULANASE *and* FUNGAL ALPHA AMYLASE

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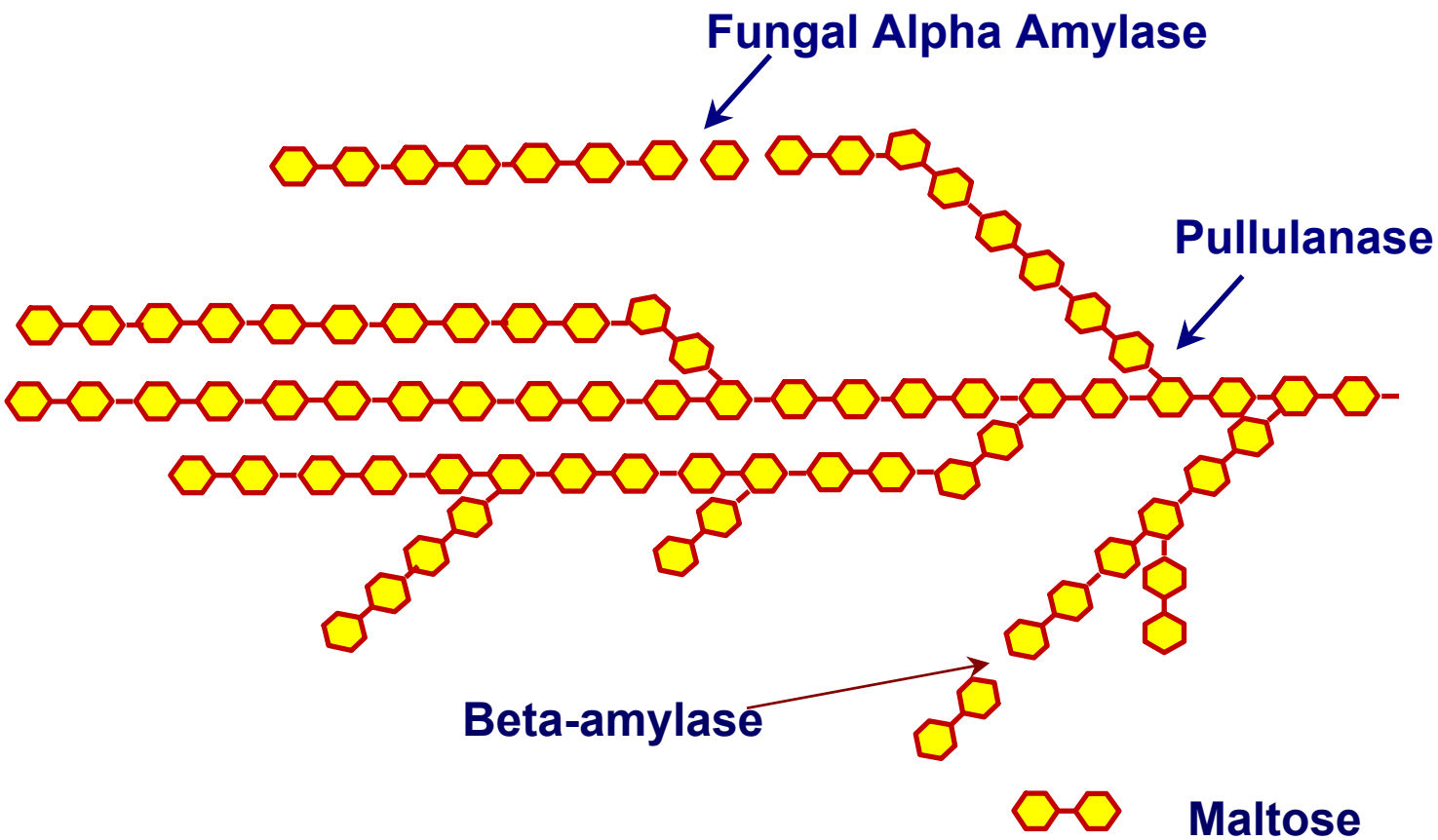

Factors Affecting the Production of Very High Maltose Syrup

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- Maltogenic Enzymes
- Sugar profiles
- Impact of DE
- Impact of pH and Temperature
- Impact of residual thermal stable and pH stable liquefaction enzyme
- Trouble shooting

Analytic Method

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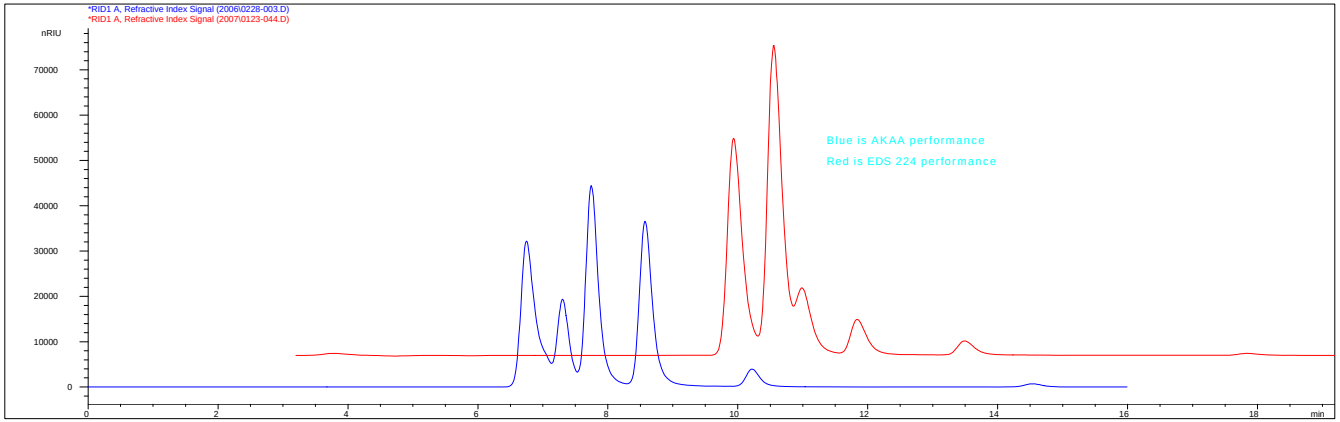
Analytical Methods for Carbohydrate Analysis

Recommended Conditions	
HPLC column	HPX 87-H (Bio-Rad)
Column temperature	60° C
Mobile phase	0.01 N H_2SO_4
Detection	RI
Injection volume	20µl, 1.0% solution



Sugar profile

Syrup Type	Sugar Composition (%)		Recommended Enzyme(s)
	Maltose	Glucose	
Maltose Syrup	40 - 45	1 - 10	Fungal alpha-amylase CLARASE® (R)-L
High Maltose Syrup	55 - 60	1 - 5	Beta-amylase OPTIMALT® BBA
Very High Maltose Syrup	> 80	< 1	Beta-amylase + pullulanase OPTIMALT® BBA + OPTIMAX®

The issue of the current process -pH Drop

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- DAMAGING MICROBE LACTOBACILLUS (fermentum)
- VERY THERMOSTABLE UP TO 60 ^{o}C
- POPULATION GROWTH 52-56 ^{o}C
- FAST ACID FORMATION CAPABILITY
- FACULTATIVE ANAEROBIC GROWTH

What the industry do to deal with the issue

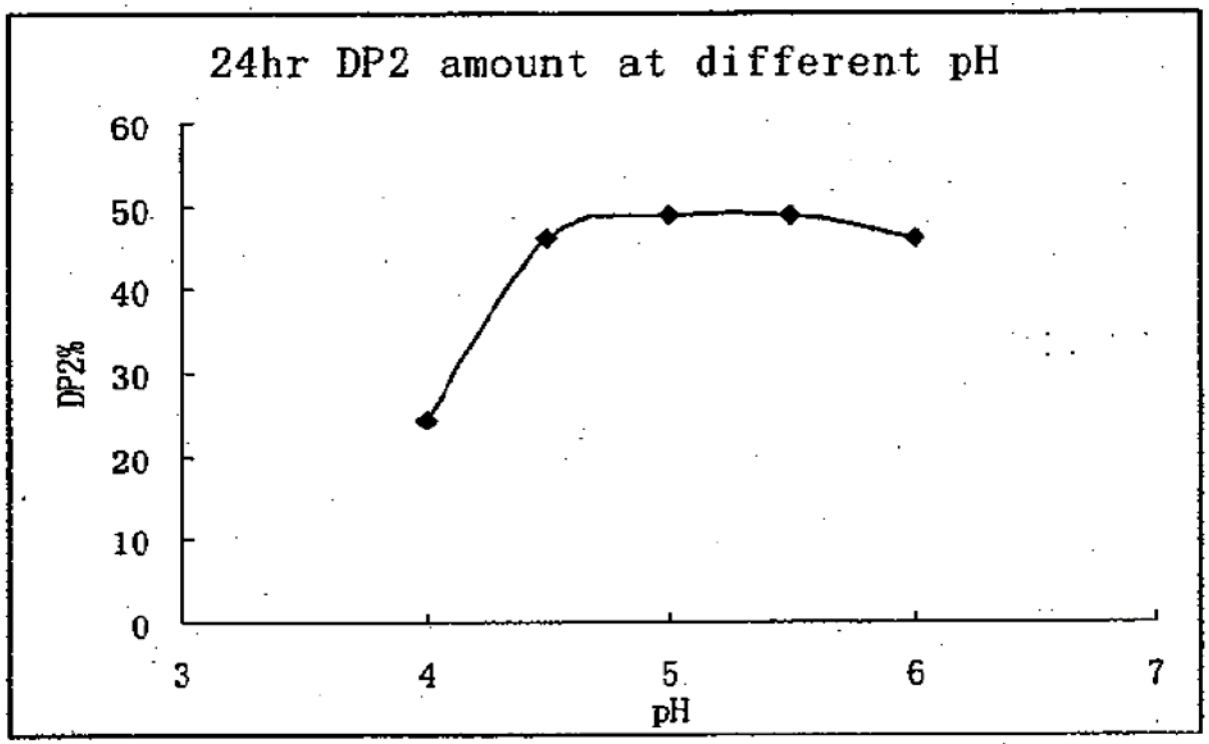
- **Cleaning with/without chemical from time to time**
  - **WASHING PROCESS EQUIPMENTS WITH HOT app. 60 °C, 1% NaOH, rinsing**
  - **WASHING AGAIN WITH HOT appr. 60 °C, HNO_3 (Note: Materials of tanks etc.), rinsing**
  - **FINALLY RINSING WITH COOL H_2O_2**
  - **REGULAR CLEANING (Own schedule must be created.)**
- **Short reaction time**
- **Add lysozyme**

New Fungal AA from *Trichoderma reesei*

Sample:	**ALPHA AMYLASE [SCAFFOLD 3]**
Conversion:	Endohydrolysis of 1,4-α-D-glucosidic linkages in polysaccharides containing three or more 1,4-α-linked D-glucose units
E.C. number:	3.2.1.1
Application:	Starch degradation in bread baking, brewing, dishwashing and sweeteners and bioethanol applications
Description:	It is an overexpressed enzyme produced by *Trichoderma reesei.*

What's new, TrAA, a low pH FAA

- pH profile, TRAA,



Enzyme used in this study

- **Clarase (R)-L :**
  - Fungal Alpha amylase
  - standard (#107-04330-001) 40690 SKBU/g
- **Optimax L-1000**
  - Acid stable pullulanase
  - standard (#107-04224-001) 1036 ASPU/g
- **SPEZYME Fred** Liquefact
  - Spezyme Fred: Low pH thermostable AA
  - DE -10
- **TrAA-sample**(150906): 17865 SKBU/g

Dose response of TrAA for maltose

Enzyme 1 (dose)	Enzyme 2 (dose)	T (°C)	pH	Time (hr)	% DP1	% DP2	% DP3	% HS
TrAA (10 SKBU/g)	NA	60	4.5	24	4.5	37.8	23.7	34.0
				48	6.4	46.2	21.7	25.7
TrAA (15 SKBU/g)	NA	60	4.5	24	6.7	47.4	21.3	24.6
				48	8.9	52.8	17.3	21.1
TrAA (20 SKBU/g)	NA	60	4.5	24	8.7	52.6	17.6	21.1
				48	10.5	55.1	14.5	19.9
TrAA (20 SKBU/g)	PU (0.25 kg/mt)	60	4.5	24	8.9	54.5	19.4	17.2
				48	11.3	58.9	16.5	13.4

Comparison of TrAA with FAA and BBA at low pH

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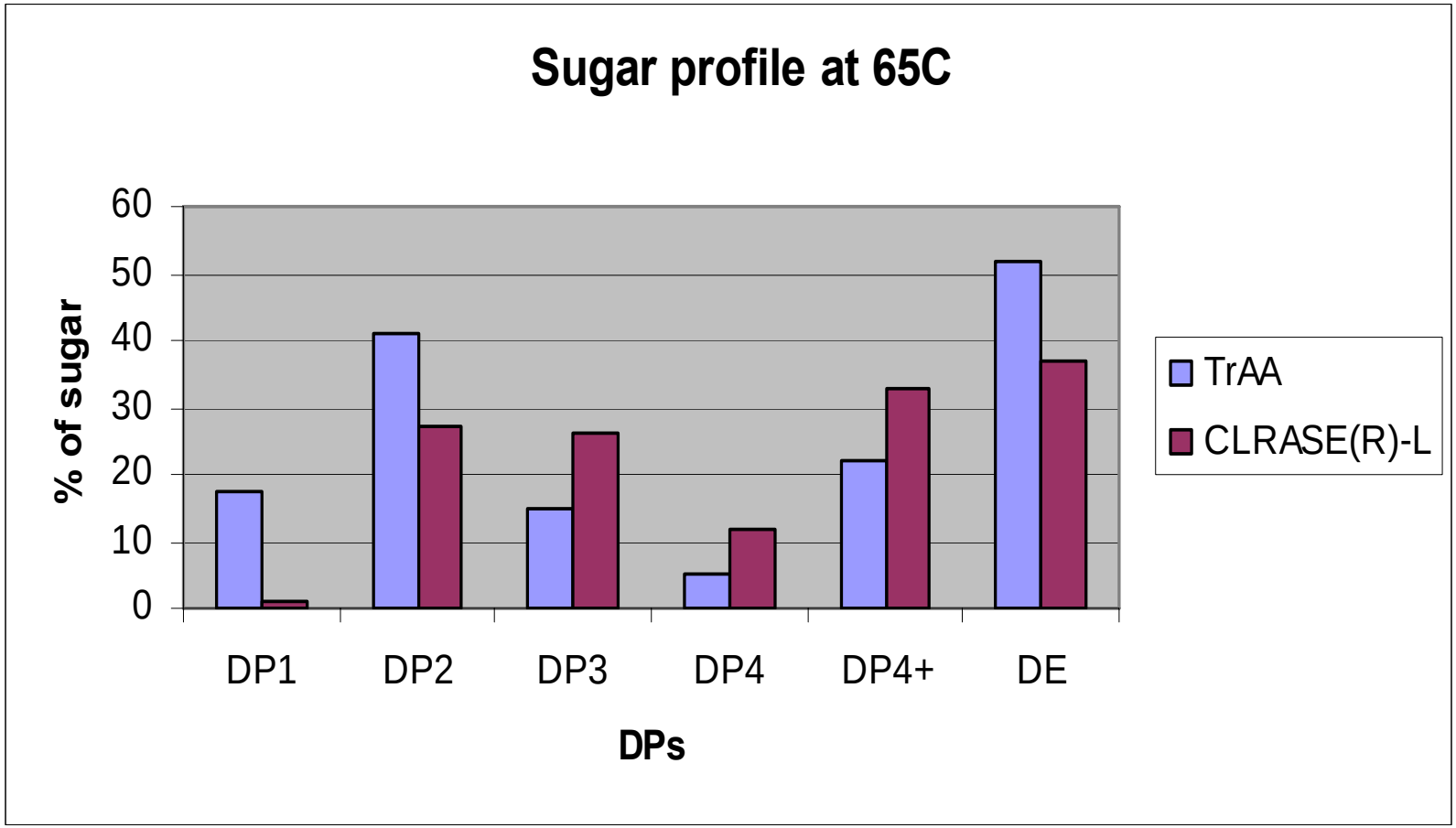
Enzyme (dose)	T (°C)	pH	Time (hr)	% DP1	% DP2	% DP 3	% HS	DE
BBA (0.2 kg/mt ds)	58	4.6	24	0.6	12.5	3.2	83.6	25
			48	0.3	12.2	3.1	84.4	24
Clarase® L (0.22 kg/mt ds)	58	4.6	24	0.6	9.9	17.9	71.6	28
			48	10.0	18.0	14.2	71.3	28
TrAA (0.5 kg/mt ds)	58	4.6	24	6.0	44.4	20.8	28.7	46
			48	8.1	51.2	17.8	22.9	49

Comparison of CLARASE and TrAA on maltose formation at higher temperature



Temperature: 65 C; pH: 5.4

Doses of enzymes: TrAA: 0.5kg/tds; CLARASE(R)-L: 0.22kg/tds

Reaction time: 24 hrs

Effect of added Pullulanase on Maltose level

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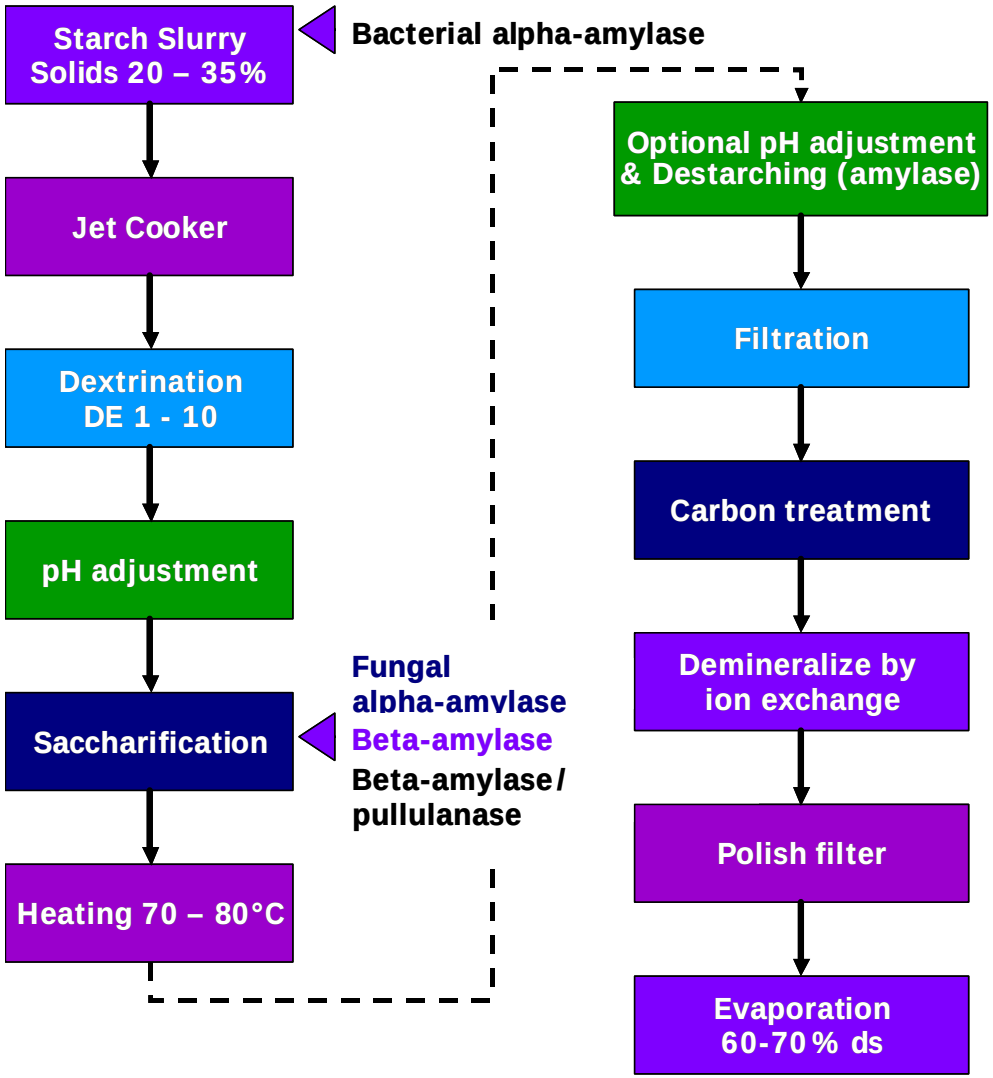
TrAA	PU	T (°C)	pH	Time (hr)	% DP1	% DP2	% DP3	% HS	DE
0.5 kg/mt ds	None	58	4.6	24	7.2	44.8	20.1	27.9	47
				48	9.2	51.6	16.8	22.3	50
0.5 kg/mt ds	0.1 kg/mt ds	58	4.6	24	6.7	44.9	21.5	26.9	47
				48	10.4	61.8	22.4	5.3	57
0.5 kg/mt ds	0.25 kg/mt ds	58	4.6	24	7.8	49.4	23.6	19.2	50
				48	10.8	61.7	22.0	5.5	57
0.5 kg/mt ds	0.5 kg/mt ds	58	4.6	24	7.5	50.3	25.0	17.2	51
				48	10.3	60.9	22.9	5.9	56

Flow chart of the maltose process



Benefits of using the new enzyme

- No pH drop during Saccharification
- Less contamination, less color substance
- No need for pH adjustment after AA deactivation

Summary

- With the new TrAA, saccharification of liquefied starch for maltose production could be done at low pH 4.6, or higher temperature, under which the microbial contamination issue will be suppressed. The will offer operational benefits as no pH adjustments after deactivation of alpha-amylase and during saccharification, less cleaning of the reaction tank…

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New Facilities in Wuxi, China