UNIVERSITÄT HOHENHEIM

Agrartechnik Hohenheim

Stevia opportunities, concerns and challenges for the EU market

---

UNIVERSITÄT HOHENHEIM

Agrartechnik Hohenheim

UNIVERSITÄT HOHENHEIM

Agrartechnik Hohenheim

Universität Hohenheim

Contents

Agrartechnik Hohenheim

The human love to sweet taste

Agrartechnik Hohenheim

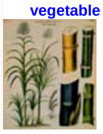
Meat, Fat, Beeries, Mushrooms

Cereals, Vegetables, Pulses Milk, Chesse, Yoghurt, Wine, Beer, vegetable Fat and Bread

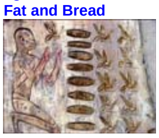
Sugar as mass product

Date palmtrees in oasis

Cave paintings Honey collector Spain



Sugar cane in Papua-Neuguinea



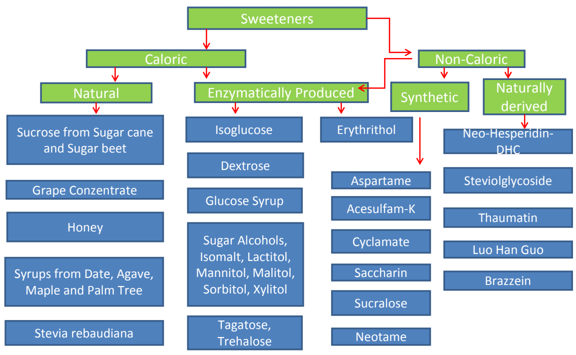
Honey in Egypt

Figs in Egypt

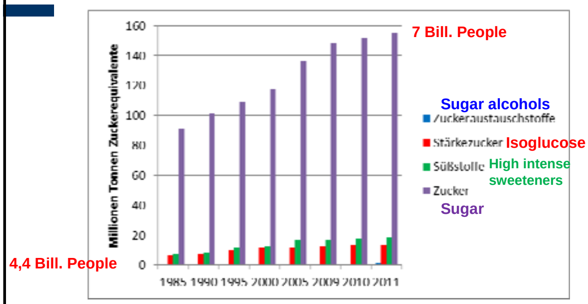
Sugar Beet

50.000 v. Chr. — 20.000 v. Chr. — 10.000 v. Chr. — 3.000 v. Chr. — 2.000 v. Chr. — 1870

Human inventions for the sweet taste

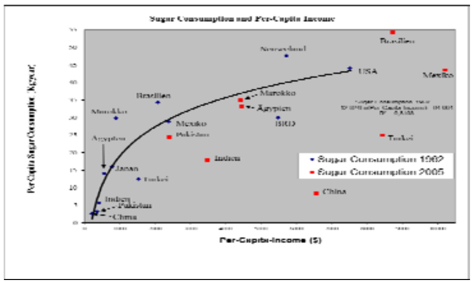


Development of world sweeteners consumption



Quelle: Internationale Zuckerorganisation, London, 2012

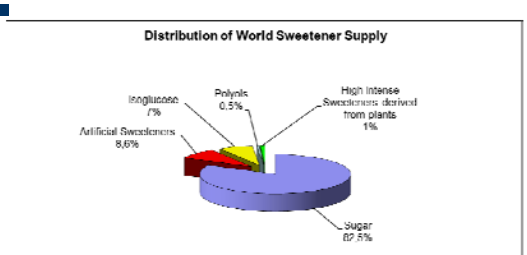
Sugar Consumtion and Per-Capita-Income



Opportunities

Markt Dynamics in the world an in the EU

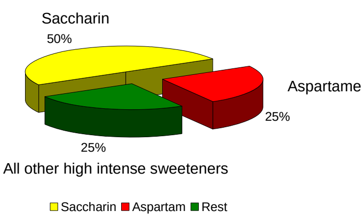
Distribution of World Sweetener Supply



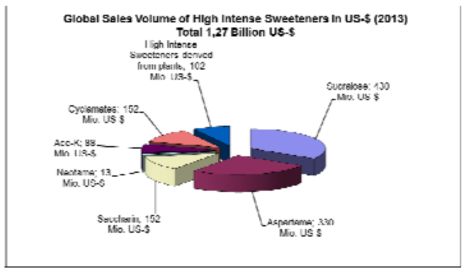
Quelle: ISO, 2013

Distribution of World Market Shares of High Intense Sweeteners

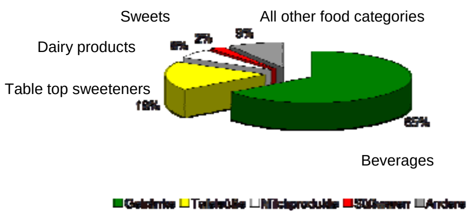
Total Volume: 18 Mio. TSE (tonnes sucrose equivalents)



Sales Volume of Low Calorie Sweeteners



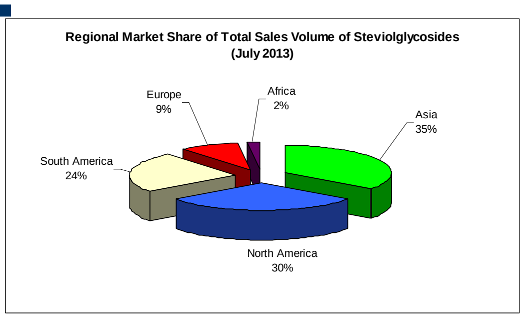
Use of high intense sweeteners in food



Share of sugar-reduced poducts in certain food categoris

- Chewing gum 95%
- Beverages 20%
- Table top Sweeteners 15%

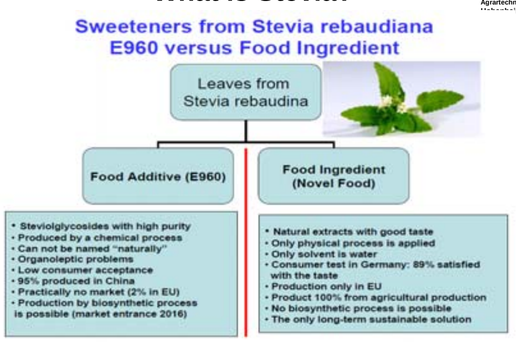
Regional Market Shares of Steviolglycosides



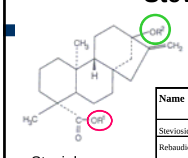
Concerns

What are the problems of Steviolglycosides?

What is Stevia?



Sweeteners from Stevia rebaudiana Bertoni

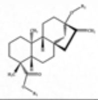


Steviol

Name	R 1	R 2	Süßkraft	Natürliche Zusammensetzung
Stevioside	ß-D-Glucose	ß-D-Glucose-ß-D-Glucose (2-1)	110 - 270	60 - 70%
Rebaudioside A	ß-D-Glucose	ß-D-Glucose-ß-D-Glucose (2-1) ß-D-Glucose (3-1)	150 - 320	20 - 30%
Rebaudioside C	ß-D-Glucose	ß-D-Glucose-α–Rhamnose (2-1) ß-D-Glucose (3-1)	40 - 120	5 - 10%
Rebaudioside D	ß-D-Glucose-ß-D-Glucose	ß-D-Glucose-ß-D-Glucose (2-1)-ß-D-Glucose (3-1)	200 - 250	Spuren
Rebaudioside E	ß-D-Glucose-ß-D-Glucose	ß-D-Glucose-ß-D-Glucose (2-1)	150 - 200	Spuren
Dulcoside A	ß-D-Glucose	ß-D-Glucose-α–Rhamnose (2-1)	40 - 70	5 - 10%

Allowed Steviolglycosides according to approval Status 2011

Will be produced by genetically modified yeast



Between natural occuring, chemcial synthesis, enzymatic and genetic engineering

Typ	Reste an Steviol - Gerüst R1	Reste an Steviol - Gerüst R2	Formel	Molekular-gewicht (g/mol)	Süßkraft*
Rebaudioside A	β-glc-	(β-glc)$_2$-β-glc-	$C_{44}H_{70}O_{23}$	967.01	200-300
Rebaudioside B	H	(β-glc)$_2$-β-glc-	$C_{38}H_{60}O_{18}$	804.88	150
Rebaudioside C	β-glc-	(β-glc, α-rha-)-β-glc-	$C_{44}H_{70}O_{22}$	951.01	30
Rebaudioside D	β-glc-β-glc-	(β-glc)$_2$-β-glc-	$C_{50}H_{80}O_{28}$	1129.15	221
Rebaudioside E	β-glc-β-glc-	β-glc-β-glc-	$C_{44}H_{70}O_{23}$	967.01	174
Rebaudioside F	β-glc-	(β-glc, β-xyl)-β-glc-	$C_{43}H_{68}O_{22}$	936.99	200
Stevioside	β-glc-	β-glc-β-glc-	$C_{38}H_{60}O_{18}$	804.88	210
Steviolbioside	H	β-glc-β-glc-	$C_{32}H_{50}O_{13}$	642.73	90
Rubusoside	β-glc-	β-glc-	$C_{32}H_{50}O_{13}$	642.73	114
Dulcoside A	β-glc-	α-rha-β-glc-	$C_{38}H_{60}O_{17}$	788.87	30

glc = glucose rha = rhamnose xyl = xylose
*aus Kinghorn et al 1999, pg 403

Metabolite

Breeding

New Steviolglycosides from Stevia rebaudiana MORITA 2010

Will be produced by genetically modified yeast

No. (Steviol Glycoside)	R1	R2
I (Dulcoside B)	β-glc-α-rha(2→1) β-glc(3→1)	H
II (Rebaudioside G)	β-glc-β-glc(3→1)	β-glc
III (Rebaudioside I)	β-glc-β-glc(2→1) β-glc(3→1)	β-glc-β-glc(3→1)
IV (Rebaudioside H)	β-glc-α-rha(2→1)-β-glc(3-1) β-glc(3-1)	β-glc
V (Rebaudioside L)	β-glc-β-glc(2-1) β-glc(3-1) }-β-glc(6-1)	β-glc
VI (Rebaudioside K)	β-glc-α-rha(2→1) β-glc(3→1)	β-glc-β-glc(2→1)
VII (Rebaudioside J)	β-glc-β-glc(2→1) β-glc(3→1)	β-glc-α-rha(2→1)
VIII (Rebaudioside M)	β-glc-β-glc(2→1) β-glc(3→1)	β-glc-β-glc(2→1) β-glc(3→1)
IX (Rebaudioside N)	β-glc-β-glc(2→1) β-glc(3→1)	β-glc-α-rha(2→1) β-glc(3→1)
X (Rebaudioside O)	β-glc-β-glc(2→1) β-glc(3→1)	β-glc-α-rha(2→1)-β-glc(3→1) β-glc(3→1)

Quelle:
Morita WO201/038911

2.2 Actual Application for Food Use of Steviolglycosides according to EU directive 1131/2011

About 30% of sugar is replaceable in foods and beverages

Kategorie	Max. Steviol-äquivalente mg/l oder mg/kg	Max. Steviol-glycoside (Stevia) mg/l oder mg/kg
Getränke (incl. Milchhaltige und Getränkepulver	80	240
Alkoholhaltige Getränke mit weniger als 15 % Alkohol	150	450
Joghurt	100	300
Speiseis	200	600
Konfitüren und Fruchtaufstriche	200	600
Soßen inkl. Ketchup	120	360
Schokolade Zuckerfreie Süßwaren Zuckerfreier Kaugummi	270 350 3300	810 1050 9900
Table-top Süßungsmittel	Quantum satis	

Japanese Cancer Study on Stevioside 1997

Carcinogenicity studies of stevioside 599

Table 1. Body weights, daily intakes of food and stevioside, final survival rates and survival times for F344 rats given stevioside in the diet for 104 wk

Group Sex	Group Treatment	Body weight at wk 104 (g)	Daily intake of Food (g/rat)	Daily intake of Stevioside (mg/kg body weight)	Final survival rate (%)	Survival time (wk)
Male	Control	455 ± 34	15.2 ± 0.6	0	78	105.8 ± 5.1
	2.5%	445 ± 54	15.4 ± 0.7	969 ± 308	72	104.3 ± 9.2
	5%	431 ± 31*	15.5 ± 0.7	1997 ± 617	60‡	102.8 ± 9.5
Female	Control	305 ± 38	10.0 ± 0.8	0	78	105.4 ± 6.3
	2.5%	294 ± 30	10.1 ± 0.9	1120 ± 285	70	103.2 ± 10.4
	5%	263 ± 29†	10.0 ± 0.7	2387 ± 508	78	105.5 ± 7.5

Values are means ± SD.
*Significant difference from controls ($P < 0.05$; Scheffe's test).
†Significant difference from controls ($P < 0.01$; Scheffe's test).
‡Significant difference from controls ($P < 0.05$; Fisher's exact probability test).

Legal Production Proecedure

18.000 Liter Water per one ton of leaves → **Extraction**

Zur Extraktion Zugabe von Formaldehyd

↓

Percipitation ← z.B. 86 kg Al-Salts per one ton of Stevia leaves

↓

Ion Exchange Resins

↓

Decouloring with Resins ← 2500 Liter Resins, 7.500 Liter Ethanol

↓

100 kg Steviolglycoside davon z.B. 20-30 kg Rebaudioside A → **Cyristallisation with Alcohols**

90% of all plant constituents are destroyed

Challenges

Do we need really high intense sweeteners?

Do we need Stevia?

General Situation

Agrartechnik Hohenheim

Sweetness and Sweeteners is a Hot Topic in Socienty and Science

World Nutrition Situation

Agrartechnik Hohenheim

- 870 Million People (12% of world population) suffer of under-nutrition.
- Worldwide obesity has more than doubled since 1980.
- In 2014, more than 1.9 billion adults, 18 years and older, were overweight. Of these over 600 million were obese. Double as much as under-nutrition!
- 39% of adults aged 18 years and over were over-weight in 2014 (38% of men and 40% of women), and 13% (11% of men and 15% of women) were obese.
- 42 million children under the age of 5 are overweight

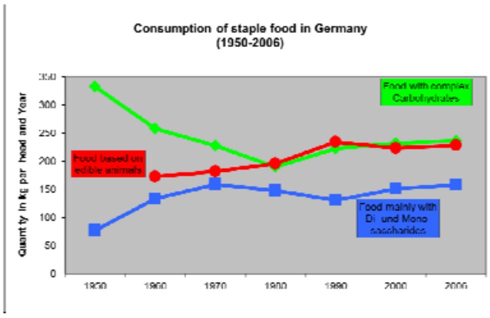
Worldwide Epidemic of Diabetes

Agrartechnik Hohenheim

Share of diabetic people of total population in 2030.

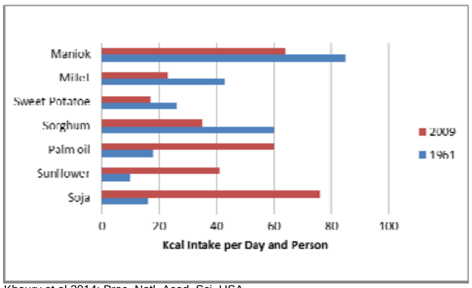
Change in Nutrition Habits in Germany

Agrartechnik Hohenheim



Change in per Person and per day intake of calorie sources

Agrartechnik Hohenheim



Source: Khoury et al 2014; Proc. Natl. Acad. Sci, USA

Three Hypthesis: How Intense Sweetener may work in human body

Agrartechnik Hohenheim

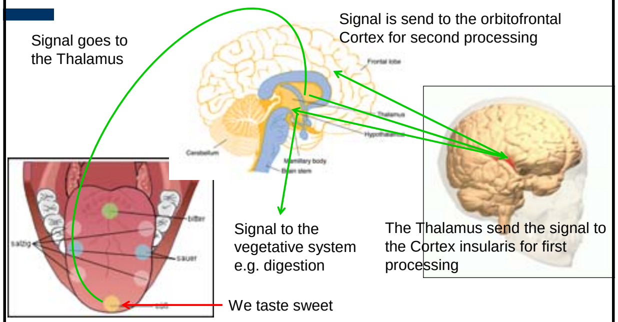
- The sweet taste of Intense Sweeteners lead to an increased appetite because of lack of energy together with the sweet taste
- Intense Sweeteners change the carbohydrate metabolism, sugars utilization is increased = more fat
- Intense Sweeteners change intestine microflora leading to obesity and diabetes

Processing of Sweet Taste in the Brain

Agrartechnik Hohenheim

Signal goes to the Thalamus

Signal is send to the orbitofrontal Cortex for second processing



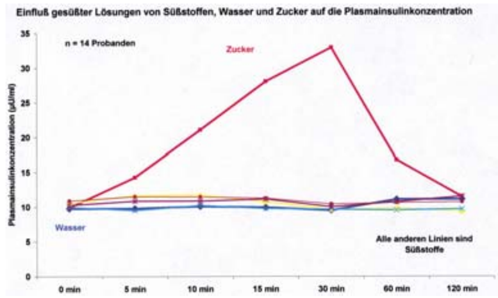
Signal to the vegetative system e.g. digestion

The Thalamus send the signal to the Cortex insularis for first processing

We taste sweet

Cephalic Effect of sugar and high intense sweeteners on insulin segregation

Agrartechnik Hohenheim



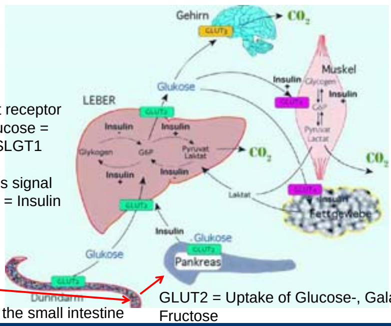
Carbohydrate Metabolism in Human Body

Agrartechnik Hohenheim

Source: Uni Graz

T1R3 sweet receptor = sensor glucose = increasing SLGT1

SLGT1 gives signal to pancreas = Insulin segregation



T1R3

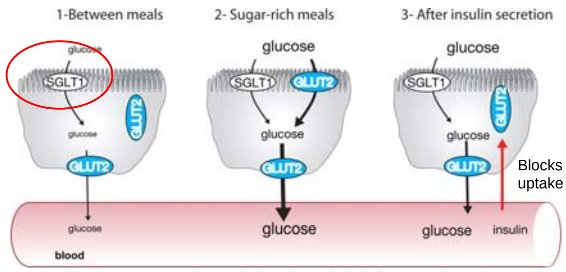
Entrance of the small intestine

GLUT2 = Uptake of Glucose-, Galactose- and Fructose

Have high intense sweeteners influence on glucose up-take?

Agrartechnik Hohenheim

SGLT1= Natrium/Glucose-Cotransporter 1

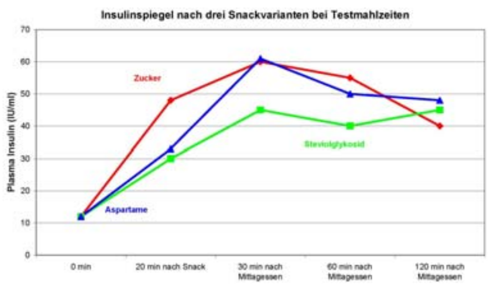


Blocks uptake

Armelle Leturque et al: **Source: GLUT2 mutations, translocation, and receptor function in diet sugar managing;** American Journal of Physiology - Endocrinology and Metabolism 5 (2009) E985-E992

Influence of Steviolglykosides on the insulin level after a testmeal

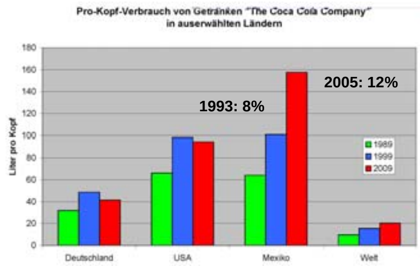
Agrartechnik Hohenheim



Consumption of Softdrinks and Diabetes

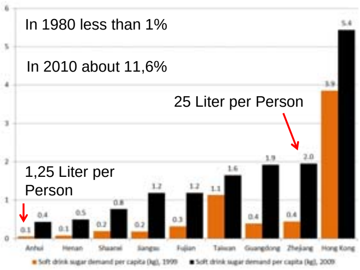
Agrartechnik Hohenheim

Diabetes Prevelance in Mexico



Softdrink and Diabetes Prevelance in China

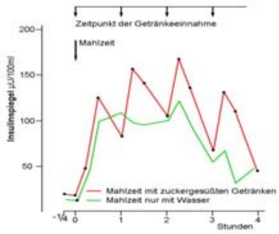
Agrartechnik Hohenheim



67 Liter per Person

Influence of sugar sweetended beverages on insulin level

Agrartechnik Hohenheim



Drink (red line -) with 19 Gram of sugar each drink

Eat (green line -)

76 Gram sugar in the cake

Difference in Insulin segregation

Additional problem: Sugared Softdrinks do not influence satiety

Conclusions

Agrartechnik Hohenheim

- In modern nutrition we will need low calorie sweeteners because we need to unlock sweet taste from calories
- Low calorie must not only be safe in toxicology terms
- Low calorie must also be safe in terms of human physiology
- **This is the research gap: Here we contribute with the Go4Stevia-Project to match the needs of society!**

Agrartechnik Hohenheim

- Thanks for your attention!
- udo.kienle@uni-hohenheim.de
- www.go4Stevia.eu