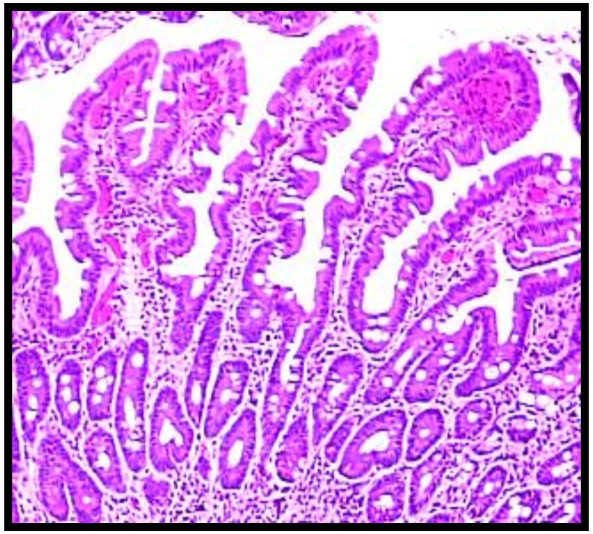
Gluten-free beers: new aspects for reflection

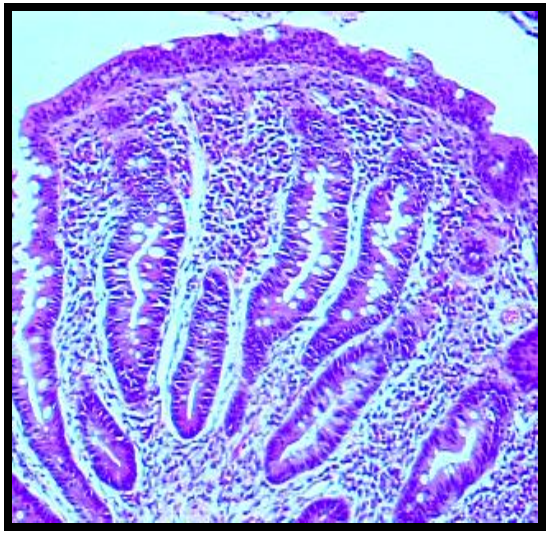
Martin Zarnkow

Celiac disease

- ✓ autoimmune enteropathy triggered by the ingestion of gluten in genetically susceptible individuals
- ✓ intestinal mucosa becomes damaged and its functionality severely impaired
- ✓ estimated prevalence is ~ 1% population



normal

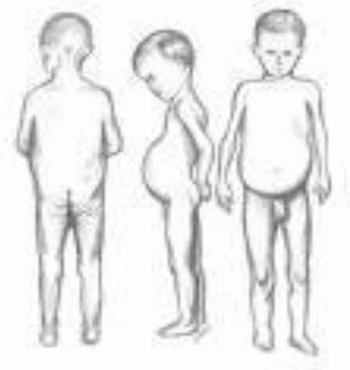


total atrophy

source: Catassi Epidemiology and natural history of celiac disease

Symptoms

- ✓ can present at any age
- ✓ typical malabsorption syndrome
  - Chronic diarrhea
  - Weight loss
  - Abdominal distension
- ✓ atypical presentation
  - Secondary to malabsorption
  - *Dermatitis herpetiformes*
  - Hypo/hyperthyroidism
  - Dental enamel
- ✓ asymptomatic form
  - Presence of histological changes
  - Apparent absence of symptoms



Strict lifelong elimination of gluten from the diet (gluten-free diet)

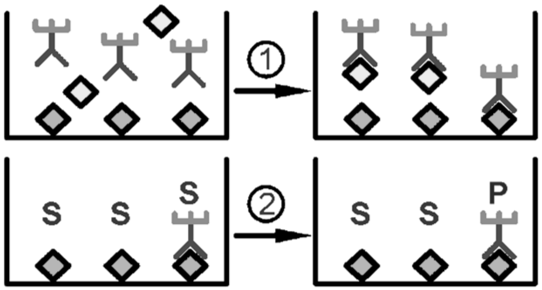
Measurement of gluten

– International regulations* are based on CODEX STAN 118 – 1979
– CODEX standardizes food that contains:
  a) less or equal 20 ppm to be *gluten-free*
  b) >20–100 ppm to be labeled as *very-low gluten*
– Certified measurement methods:

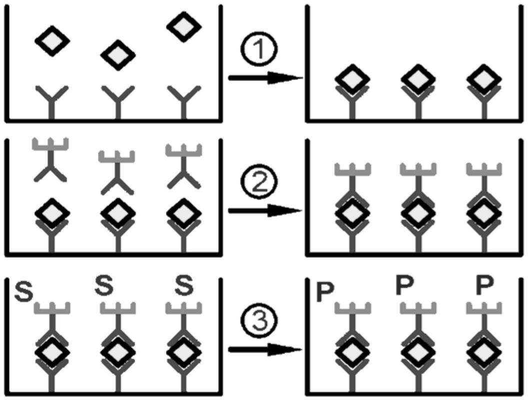
Type	Ridascreen Gliadin competitive R7021	Ridascreen Gliadin R7001
Test Format	Microtiter plate with 96 wells (12 strips with 8 removable wells each)	
Standard Range	0 ng/ml (zero standard), 10 ng/ml, 30 ng/ml, 90 ng/ml, 270 ng/ml gliadin. The RIDASCREEN® standard material is a hydrolysate (mixture of wheat, rye and barley).	0 ppb (zero standard), 5 ppb, 10 ppb, 20 ppb, 40 ppb, 80 ppb gliadin
Sample Preparation	homogenization and extraction	
Incubation Time	40 min	1 h 30 min
Detection Limit	Limit of detection (LOD): 1.36 mg/kg (ppm) Gliadin (2.72 ppm gluten)	Detection limit (LOD): 1.5 ppm gliadin, corresponding to 3 ppm gluten
	Limit of quantification (LOQ): 5 mg/kg (ppm) Gliadin (10 ppm gluten)	**Limit of quantification (LOQ): 2.5 ppm gliadin, corresponding to 5 ppm gluten**
Cross Reactivity	The monoclonal antibody R5 reacts with the gliadin fractions from wheat and corresponding prolamins from rye and barley. No cross reaction with soy, oats, corn (maize), rice, millet, teff, buckwheat, quinoa and amaranth.	

*= Some countries e.g. Australia and New Zealand have stricter rules (<LOQ)

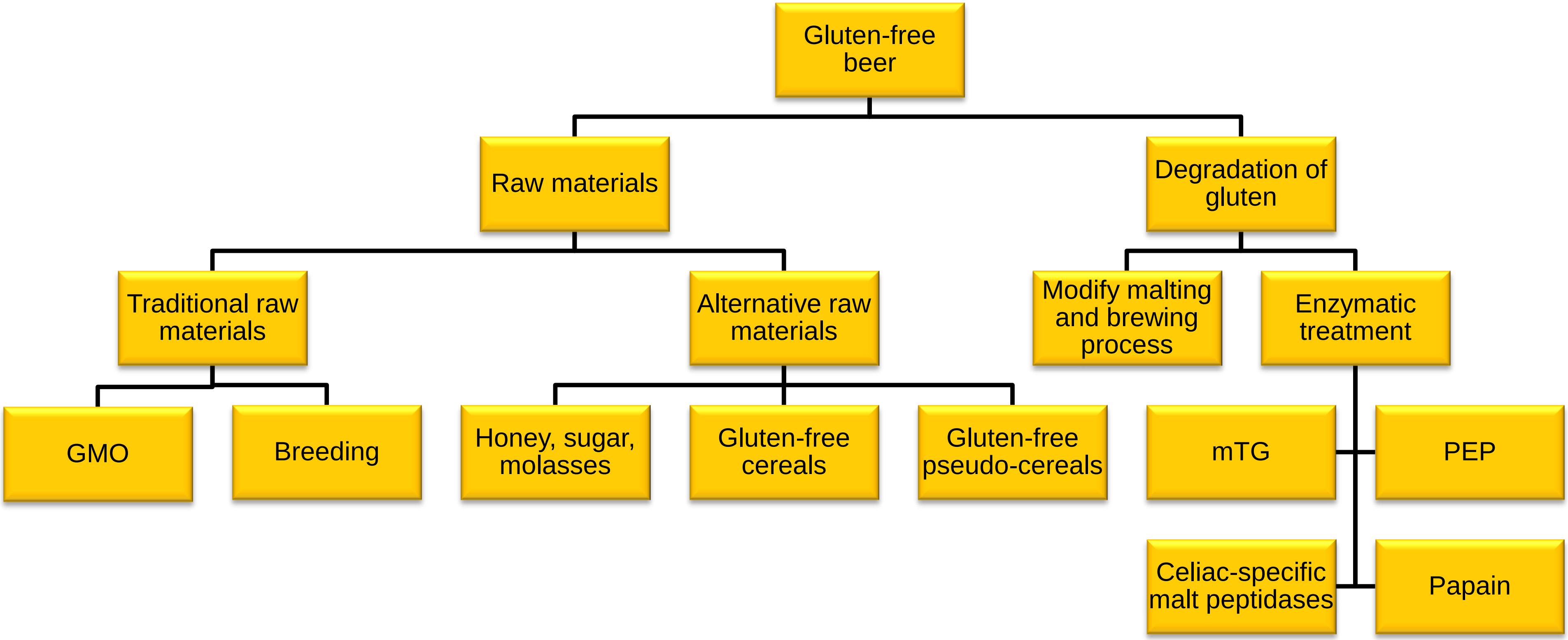
<u>Competitive ELISA</u>



<u>Sandwich ELISA</u>



Possibilities to produce gluten-free beer



Source: Bamforth, C.W., 2009: In Arendt, E.K., Dal Bello, F. (Eds.), The Science of Gluten-Free Foods and Beverages. AACC, pp.113–117.
Zarnkow, M.; Kerpes, R.; Knorr, V.; Köhler, P.; Becker, T. (2013): Eintrag vermeiden – Strategien zur Herstellung glutenfreier Biere. In: Brauindustrie. Pp.20–23

Cereals and pseudo cereals

gluten-containing cereals:

barley (*Hordeum vulgare*)
wheat (*Triticum astivum*)
oat (*Avena sativa*)
rye (*Secale cereale*)

emmer (*Triticum dicoccum*)
einkorn (*Triticum monococcum*)
kamut (*Triticum polonicum?*)
spelt (*Triticum aestivum* ssp. *spelta*)
triticale (*xTriticosecale)*
tritordeum (hexaploid)

guten-free cereals and pseudo cereals:

millet (e. g. *Panicum miliaceum*)
sorghum (*Sorghum bicolor*)
rice (*Oryzae sativa*)
maize (*Zea maize*)

amaranth (*Amaranthus hypochondriacus*)
buckwheat (*Fagopyrum esculentum*)
quinoa

Introduction

- ✓grains must be subjected to degradation processes (physical and enzymatic) as their primary constituents are not water soluble
- ✓the water-soluble molecular fragments allow further treatment of the grain making them accessible to fermentation microorganisms
- ✓these processes correspond to malting and mashing in the production of beer and whiskey

Introduction...cont'd

- ✓ this presentation focuses on: sorghum and small -seeded millets
- ✓ sorghum and the numerous small-seeded millet varieties have been widely exploited in Africa and Asia
- ✓ corn replaced both of these grains in the wetter climates in Africa
- ✓ high resistance to drought in harsh climates allows them to retain their position as the ideal grains for many regions of Africa and Asia

Source: http://inco-fonio-en.cirad.fr/

Fermented alcoholic and non-alcoholic beverages from sorghum, millets and other starch sources

Beverage	Starch Source	Microorganism	Country
amgba	sorghum	*S. cerevisiae, …*	Cameroon
affouk	sorghum	*S. cerevisiae, …*	Cameroon
omulamb	sorghum, maize, banana	*S. cerevisiae, …*	Uganda
Bantu beer, chibuku	sorghum, millet, maize	*S. cerevisiae, …*	South Africa, Zimbabwe
biere de mil	millet	*S. cerevisiae, …*	Senegal
bili bili	sorghum	*S. cerevisiae, …*	Chad, Central African Republic
bojalwa	sorghum	*S. cerevisiae, …*	Botswana
boza	wheat, millet, maize	*S. cerevisiae, Lactobacillus, Leuconostoc*	Turkey, Bulgaria, Romania, Albania
burukutu, pito	sorghum, maize, cassava, guinea corn	*S. cerevisiae, S. chavelieri, Leuconostoc mesenteroides, Candida, Acetobacter*	Nigeria, Benin, Ghana, Ethiopia
busaa	maize, sorghum, finger millet	*S. cerevisiae, Lactobacillus*	Kenya
bushera	sorghum, millet	*Weissella confusa, Lactobacillus*	Uganda

Main source: Dendy, A. V. D. (1995) Sorghum and Millets – Chemistry and Technology, St. Paul, Minnesota, AACC

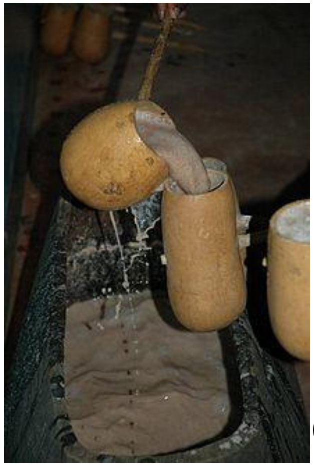
Fermented beverages … cont'd

Product	Starch source	Microorganism	Country
cochate	millet	*S. cerevisiae, …*	Chad
chapalo	sorghum	*S. cerevisiae, …*	Niger
dam	Millet	*S. cerevisiae, …*	Togo
doro	finger millet	yeast, bacteria	Zimbabwe
dolo	Sorghum	*S. cerevisiae, …*	Burkina Faso, Mali
Hirsebier (millet beer), Krieger Bräu, Riedenburg	proso millet, agave	*S. pasteurianus, …*	Germany
kaffir beer	maize, kaffir corn	yeast, *Lactobacillus*	South Africa
kasi kasi	sorghum, banana	*S. cerevisiae, …*	Zaire
katata	finger millet, maize	*S. cerevisiae, …*	Zambia
kibuku	sorghum	*S. cerevisiae, …*	Zaire
kwete	millet, maize	*S. cerevisiae, …*	Uganda
mahewu	maize, sorghum, millet	*L. delbrueckii, L. bulgaricus*	South Africa
mbege	millet, banana	*S. cerevisiae, …*	Tanzania
merissa	sorghum	*S. cerevisiae, Lactobacillus,* acetic acid bacteria	Sudan

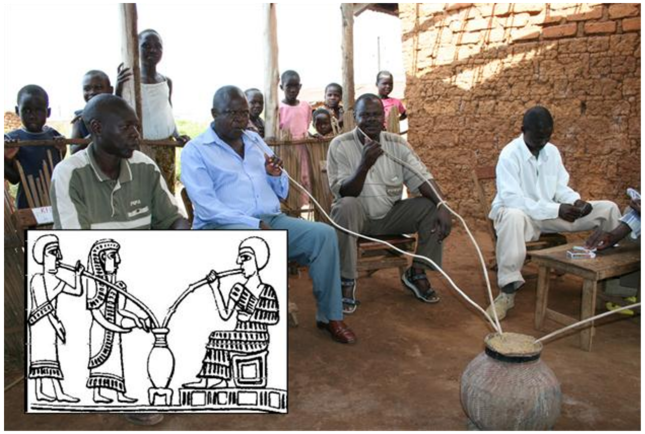
Main source: Dendy, A. V. D. (1995) Sorghum and Millets – Chemistry and Technology, St. Paul, Minnesota, AACC

pombe	sorghum	*S. cerevisiae, …*	Tanzania
sibamu	sorghum, millet	*S. cerevisiae, …*	Zambia
Red bridge	sorghum	*S. cerevisiae, …*	USA
togowa	millet, sorghum	*Lactobacillus plantarum, L. brevis, L. fermentum, L. cellobiosus, Pediococcus pentosaceus, Weissella confusa, Issatchenkia orientalis, S. cerevisiae, Candida pelliculosa, C. tropicalis*	Tanzania
talla	sorghum, finger millet	*S. cerevisiae, …*	Ethiopia
uji	maize, sorghum, millet	*Lactobacillus*	East Africa

Main source: Dendy, A. V. D. (1995) Sorghum and Millets – Chemistry and Technology, St. Paul, Minnesota, AACC



Mbege; source: dmvafricans.com



Men using straws to drink locally brewed beer in Uganda (Michael Eberhard©). Inset: Mesopotamian beer drinkers using straws (Berlin, Vorderasiatisches Museum, Inv.-Nr. VA 522; Drawing: D. Hinz)

Raw materials...cont'd

Storage

- ✓ particular significance for the storage of sorghum and millet are problems caused by mold infestations
- ✓ impact of mold infestation can vary:
  - ✓ mold consumes a certain quantity of the valuable substances in the grain and, in less favorable circumstances, causes problems like gushing
  - ✓ mold can also release mycotoxins

Storage jars in Ethiopia

Teff (*Eragrostis tef* (Zucc.) Trotter)

- ✓ very small kernel with thousand kernel weight of 0.3–0.4 g
- ✓ originated in Ethiopia, and its name is derived from the Amharic word *teffa* which means “lost” due to small size of the grain and how easily it is lost if dropped
- ✓ unmalted teff and non-leavened teff bread are used in the production of opaque beers

Stages of development over four days of germination (Zarnkow/Reichenwallner©)

Source: wikipedia

- ✓ malting: steeping and germination at 22 ° C, 48 % moisture, germination for 4 days

Kinetics in *L. amylolyticus* fermentation in teff malt wort

Objectives

✓to study the kinetics of lactic acid formation

✓to determine the lactic acid to sugar ratio in the course of the fermentation process with balanced sensory quality

Materials and Methods

✓*L. amylolyticus* at the concentration of 10^7 cells per ml was inoculated to 1 L teff malt wort

✓the samples (triplicate) with initial pH 5.4 were incubated at 48 ° C for 72 h with sampling in 4 h interval for the first 16 h, and 8 h interval for the remaining period

✓samples centrifuged and kept in a deep freeze until analysis but pH and cell concentration were measured immediately after sampling

Teff – materials and methods

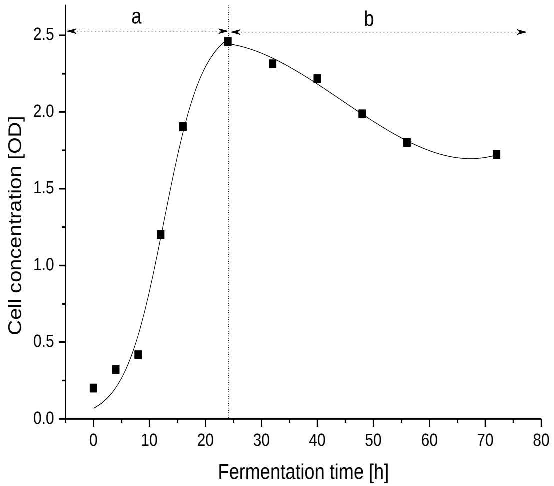
✓sensory qualities of teff malt beverages with different lactic acid to sugar ratio (0.21, 0.62, and 1.45) were assessed using a 5 point hedonic scale

Kinetics of lactic acid fermentation

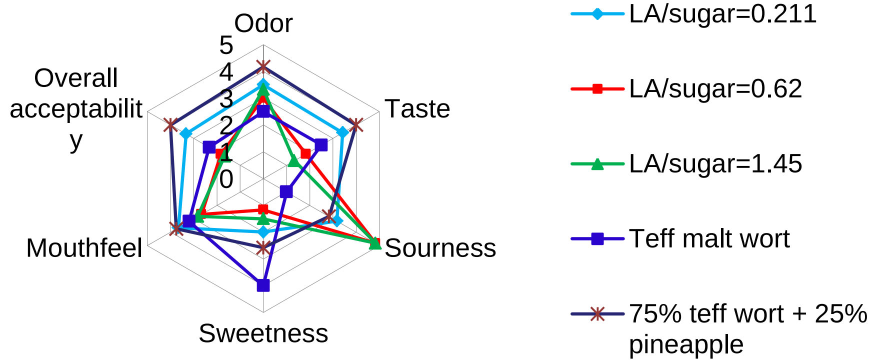
- ✓ the fermentation process was considered as an irreversible biological reaction in which the bacteria (x_o) consume substrates/sugar (S) to produce more bacteria (x) and lactic acid (P)
- ✓ OriginLab Data Analysis and Graphing Software, OriginPro Version 8.6, was used for data fitting

Teff – results and discussion

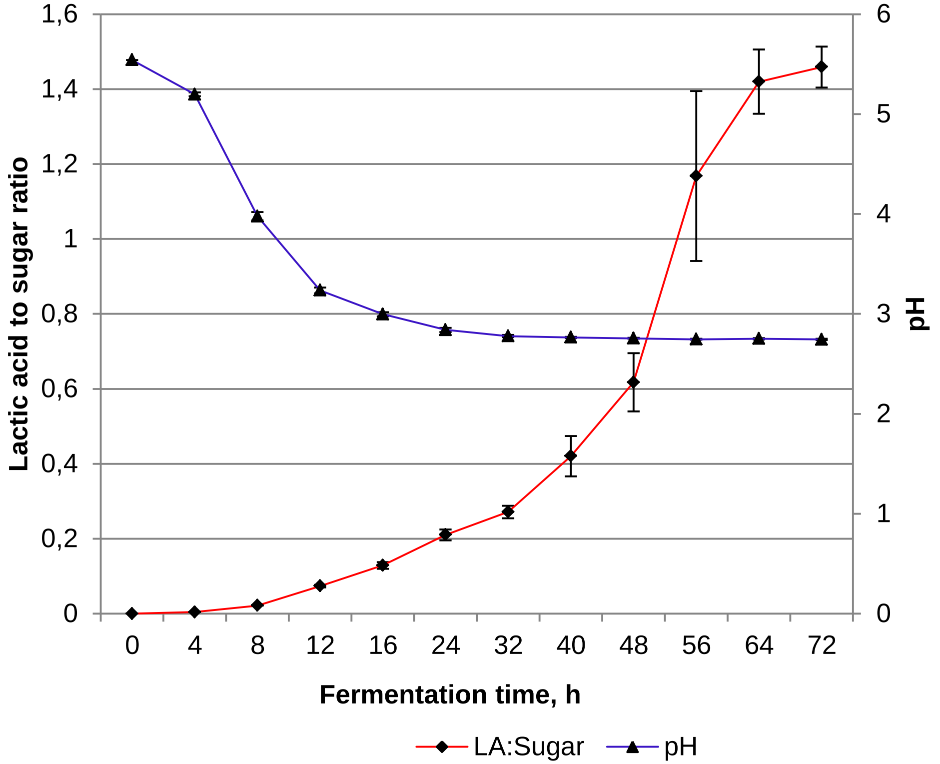
✓the growth and death phases of *L. amylolyticus* were treated independently. Portion ‘a’ fitted to sigmoidal logistic function , type I (R^2 = 0.998), and portion ‘b’ fitted to exponential decay I (R^2=0.98)



Cell concentration profile during the fermentation process



Sensory qualities and overall acceptability of teff malt beverages with different lactic acid to sugar ratio



Profiles of lactic acid to sugar ratio, and pH

Teff – conclusion

- ✓ the kinetics of lactic acid formation was successfully described by the logistic expression adapted from logistic growth model
- ✓ lower lactic acid to sugar ration resulted in a beverage with better acceptability in relation to its sensory qualities
- ✓ the beverage with 25% pineapple had also good acceptability
- ✓ adjustment of the lactic acid to sugar ration by adding commercial lactic acid leads to an inferior quality
- ✓ pH of 2.7 or higher for the fermented beverage was considered as a good pH for better acceptability

Teff – problem!

There exists an European patent and an agreement with the Ethiopian government

EP 1 646 287 B1

PROCESSING OF TEFF FLOUR

Proprietor: Health & Performance Food

International B.V.

9407 TG Assen (NL)

Inventor: ROOSJEN, Jans

NL-9414 AB Hooghalen (NL)

But not for malted teff!

Proso millet (*Panicum miliaceum* L.)

- ✓ the origin of proso millet is thought to be in China
- ✓ chemical composition: carbohydrate 69.8 %, protein, 6–16 % (N × 6.25), 4.1–9.0 % and minerals 1.5–4.2 %
- ✓ has small seeds and therefore the endosperm is quickly depleted during germination
- ✓ relatively thick husks on millet cause water to be absorbed more slowly
- ✓ moisture content of more than 44 %, temperature of 22 °C, and five days of germination have been shown to produce good results

(Zarnkow/Reichenwallner©)

Source: wikipedia

Proso millet

- ✓ wild brown variety of proso millet appears to be best suited for malting among many others
- ✓ kilning at 80 ° C preserves sufficient amylolytic enzyme activity
- ✓ starch degradation is less dependent on α-amylase and β-amylase, rather on content of limit dextrinase and most likely amyloglucosidase
- ✓ mashing program: rests between 40 and 55 ° C, and the mash needs to be acidified to a pH of 5.2
- ✓ proso millet wort fermented with *Lactobacillus* strains do not exhibit any deficiencies
- ✓ both homo-fermentative and hetero-fermentative *Lactobacillus* strains can be utilized

Sorghum (*Sorghum bicolor* (L.) Moench)

Source: geo.de

- ✓ probably originated in Ethiopia
- ✓ now a days it is cultivated in most African countries, India, southeastern Asia, Australia and USA
- ✓ extremely drought-tolerant and is capable of growing in regions too dry for corn
- ✓ globally, 65 million tons are harvested each year
- ✓ the seeds vary in color ranging from chalky white to yellowish, reddish and dark brown
- ✓ occupies third place in the world next to corn and rice as a malt substitute

Sorghum

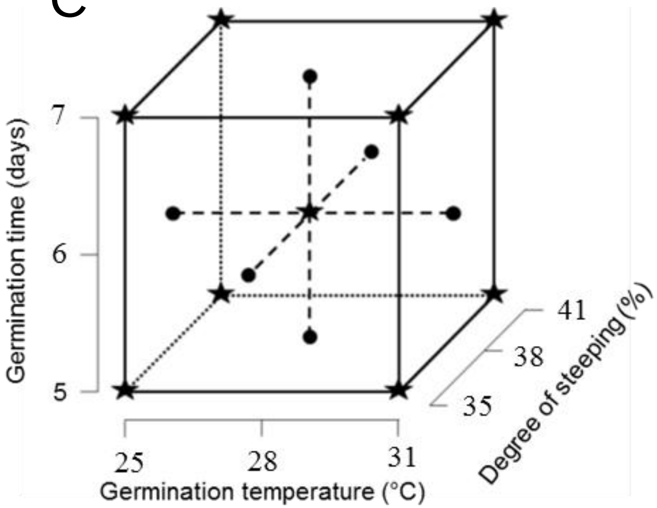
- primarily utilized in the production of traditional beverages
- beer brewed with sorghum can be divided into:
  - sweet beer, which is relatively clear with little particulate matter and lacking sourness, e.g., *dolo* from West Africa, and
  - sour, pinkish brown, opaque (cloudy) beer, e.g. *Bantu beer*
- major difference: the latter has undergone some means of acidification, generally achieved through the presence of microorganisms such as *Lactobacillus* or *Acetobacter*

Sorghum – malting properties

- ✓ determining malting and mashing conditions which yield the best activities of the amylolytic enzymes and the optimal quality attributes, for producing a high quality of sorghum wort
- ✓ determining the impacts of malting processes on the bioactive functional ingredients
- ✓ studying the possibility of sorghum wort to be a potential carrier of probiotic *Lactobacillus* bacteria (LAB)

Sorghum malting conditions – experimental design

- ✓ malting parameters:
  - ✓ degree of steeping: 35, 38 and 41%
  - ✓ germination temperature: 25, 28 and 31° C
  - ✓ germination time: 5, 6 and 7 days



- ✓ Response Surface Methodology (RSM)
  face-centered design

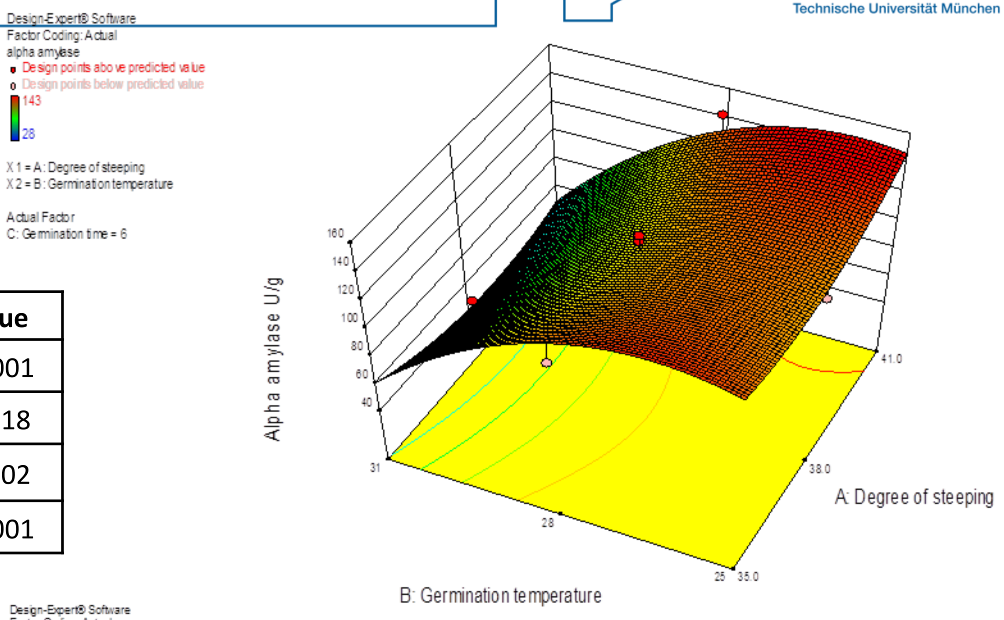
- ✓ responses:
  - ✓ amylolytic specifications: α- and β-amylase activity, extract proteolytic specifications: Free amino nitrogen (FAN), Kolbach-index
  - ✓ bioactive components: water-extractable arabinoxylan (WEAX) and B2 vitamin

Sorghum malting conditions – results

✓ amylolytic specifications

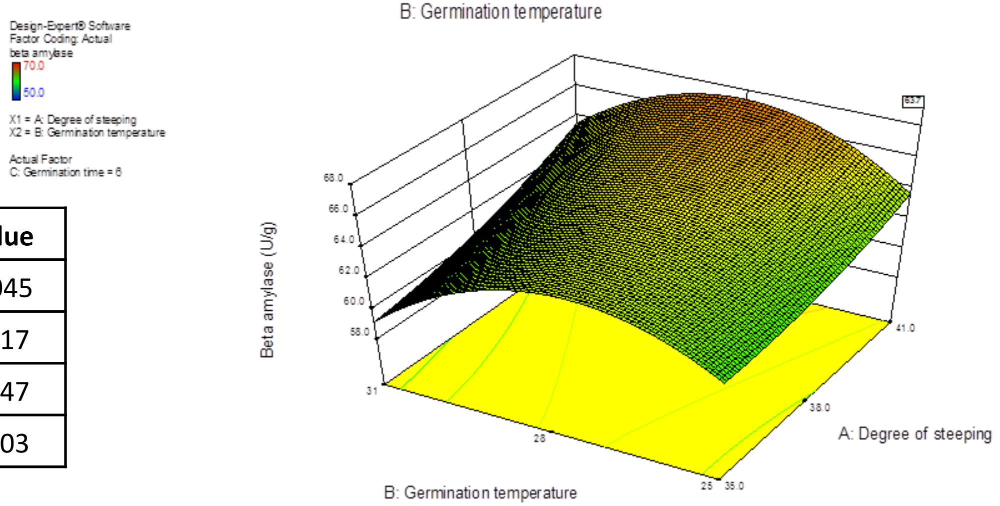
α-amylase: 28–143 U/g
model: quadratic, R^2 = 0.98

Significant terms	F Value	P-value
Germination temperature	586.4	< 0.0001
Germination time	14.32	0.0018
Degree of steeping*Germination temperature	23.43	0.0002
(Germination temperature)^2	64.59	< 0.0001



β-amylase: 50–70 U/g
model: quadratic, R^2 = 0.81

Significant terms	F Value	P-value
Germination time	11.38	0.0045
Degree of steeping*Germination temperature	14.89	0.0017
Degree of steeping*Germination time	11.28	0.0047
Germination temperature*Germination time	22.64	0.0003



Sorghum malting conditions

Zarnkow/Reichenwallner©

- ✓ optimum conditions:
  - ✓ degree of steeping: 41%
  - ✓ germination temperature: 27° C
  - ✓ germination time: 7 days

- ✓ attribut values at the optimum conditions

α –amylase U/g	β –amylase U/g	Extract %	AAL %	FAN mg/100 g	Kolbach-index %	WEAX g/L	B2 µg/L
139	60	83.8	83	117.8	26.6	0.3	114.9

- ✓ low α- and β-amylase but satisfactory levels of extract yield, AAL and FAN (compared to barley)

Sorghum malting conditions – conclusions

- ✓ RSM methodology was used to investigate the optimum conditions of malting with sorghum
- ✓ among the tested parameters, the germination time had the highest effect on malting attributes
- ✓ optimum conditions are: 41% degree of steeping, 27° C germination temperature after 7 days of germination
- ✓ despite of the low activity of α- and β-amylase (amylolytic enzymes), the levels of extract was comparable to barley
- ✓ sorghum malt is a promising raw material in beverages industry

Over all conclusion

- ✓ comprehensive trials have shown that different beverage styles can be produced from gluten-free grain without a problem
- ✓ many different beverages have been made on base of sorghum and millets and fermenting microorganisms
- ✓ in many cases malt is the main source of these beverages to enhance the amylolytic enzymes for starch degradation
- ✓ RSM is a powerful tool to prove the malting abilities
- ✓ biodiversity!
- ✓ drough resistant – climate change
- ✓ these grains are all gluten-free
- ✓ gluten contamination still could cause problems!

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