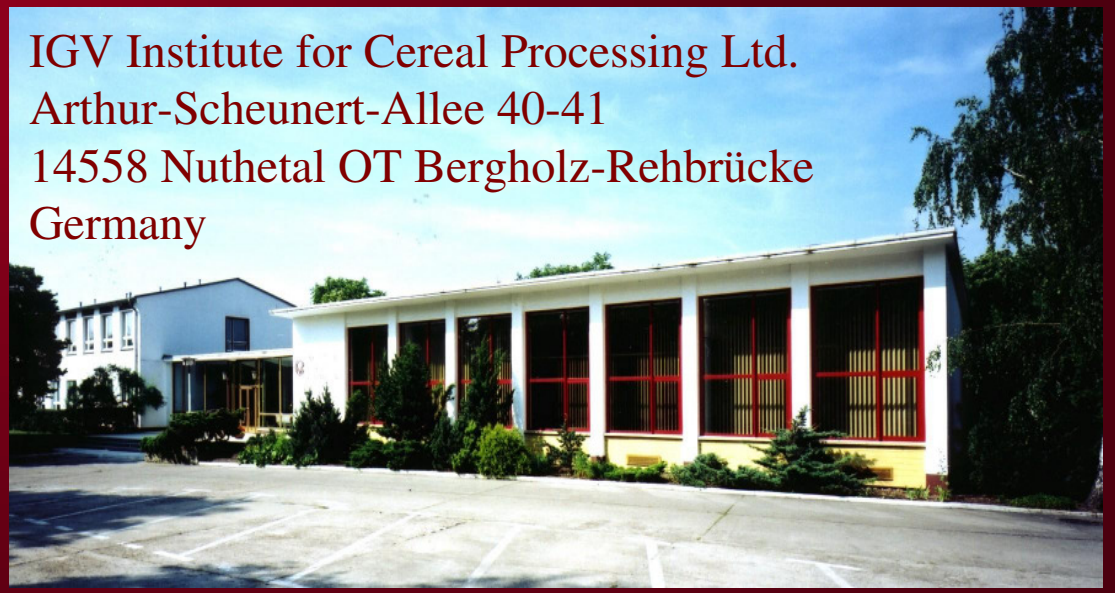
MAP Milling Project - Measure and control of mycotoxins, pesticides and acrylamide in grain milling sector

Training material about

* Mycotoxins
* Acrylamide



department
analytics/quality management
technical part
Dr. Monika Springer, Ute Meister

department
technology transfer
financial part
Ines Gromes

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MAP Milling - Part mycotoxins

Assessment of rapid methods for mycotoxins

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Mycotoxins
Maximum levels for the *Fusarium* toxins DON and zearalenone

Mycotoxin	Product	Maximum level (µg/kg)
DON	Unprocessed cereals	1250 (durum, oats, maize*): 1750)
	Cereal flour, bran, germ	750
	Bread, pastries, biscuits, cereal snacks, breakfast cereals	500
	Pasta (dry)	750
	Processed cereal-based baby food	200
zearalenone	Unprocessed cereals, maize flours/germs	100 (maize*): 200)
	Cereal flour, bran, germ (without maize)	75
	Bread, pastries, biscuits, cereal snacks, breakfast cereals	50
	Processed cereal-based baby food	20

Source: commission regulation (EC) No 1881/2006 of 19 December 2006; *) max. levels for maize / processed maize products: from 1.7.2007

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Mycotoxins
Maximum levels for other mycotoxins in cereals / cereal products

Mycotoxin	Product	Max. level (µg/kg)
OTA	Unprocessed cereals Cereal products Processed cereal-based baby food	5 3 0.5
Fumonisin B_1+B_2	Unprocessed maize*) Maize flour, germ, oil *) Processed maize products *) Processed cereal-based baby food *)	2000 1000 400 200
Aflatoxin B_1 / Aflatoxins (sum)	Cereals and cereal products Maize for sorting or other treatment Processed cereal-based baby food	2 / 4 5 / 10 0.1 / -

Source: commission regulation (EC) No 1881/2006 of 19 December 2006;
*) max. levels for fumonisins in maize / processed maize products: from 1.10.2007

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Mycotoxins
Maximum levels for other mycotoxins in feed

Mycotoxin	Product	Max. level (mg/kg)
Aflatoxin B_1	feedingstuffs	0.005 - 0.02
DON*)	Cereals and cereal products Other complementary / complete feedingstuffs	8 (maize by-products: 12) 0.9 - 5
Zearalenone*)	Cereals and cereal products Other complementary / complete feedingstuffs	2 (maize by-products: 3) 0.1 - 0.5
Fumonisin*) B_1+B_2	Maize and maize products Other complementary / complete feedingstuffs	60 5 - 50
OTA*)	Cereals and cereal products Other complementary / complete feedingstuffs	0.25 0.05 - 0.1

*) guidance values

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Mycotoxins
Accuracy and precision of analytical methods for mycotoxins

Performance criteria according to commission regulation (EC) No 40172006

for DON:

Level µg/kg	RSD_r %	RSD_R %	Recovery %
> 100 - ≤ 500	≤ 20	≤ 40	60 to 110
> 500	≤ 20	≤ 40	70 to 120

for zearalenone:

Level µg/kg	RSD_r %	RSD_R %	Recovery %
≤ 50	≤ 40	≤ 50	60 to 120
> 50	≤ 25	≤ 40	70 to 120

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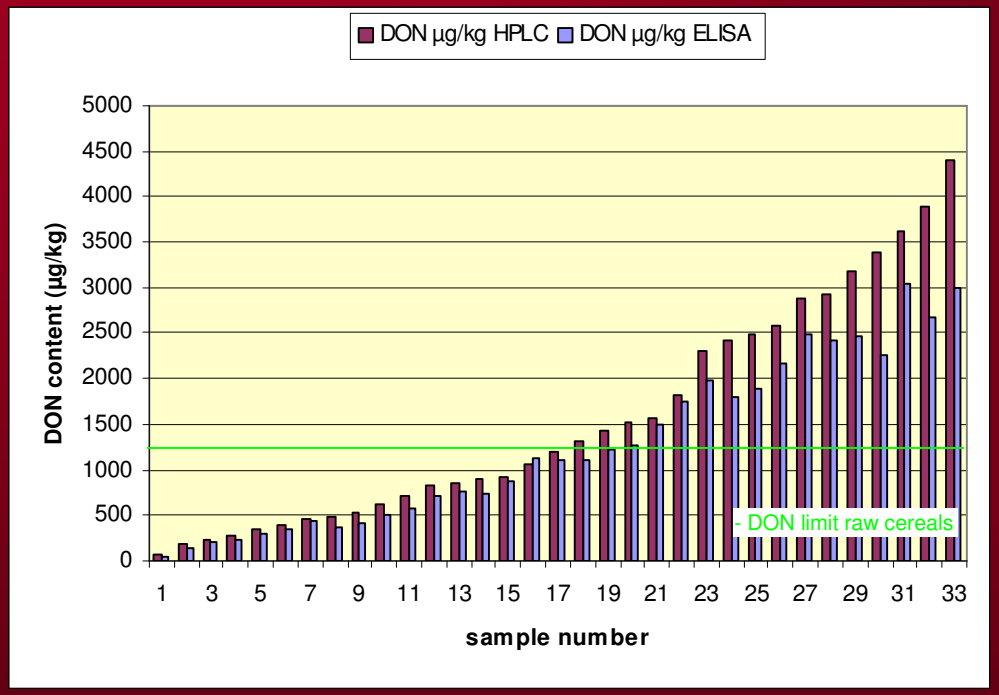
Mycotoxins

Results of DON ELISA test – comparison with HPLC



Anthos reader

DON contents of wheat samples, analyzed with HPLC and ELISA (NEOGEN Veratox® DON test)

Result:

➡ DON contents, analyzed by ELISA were with one exclusion lower than the values measured by HPLC (HPLC values by 4-33 % higher than ELISA)

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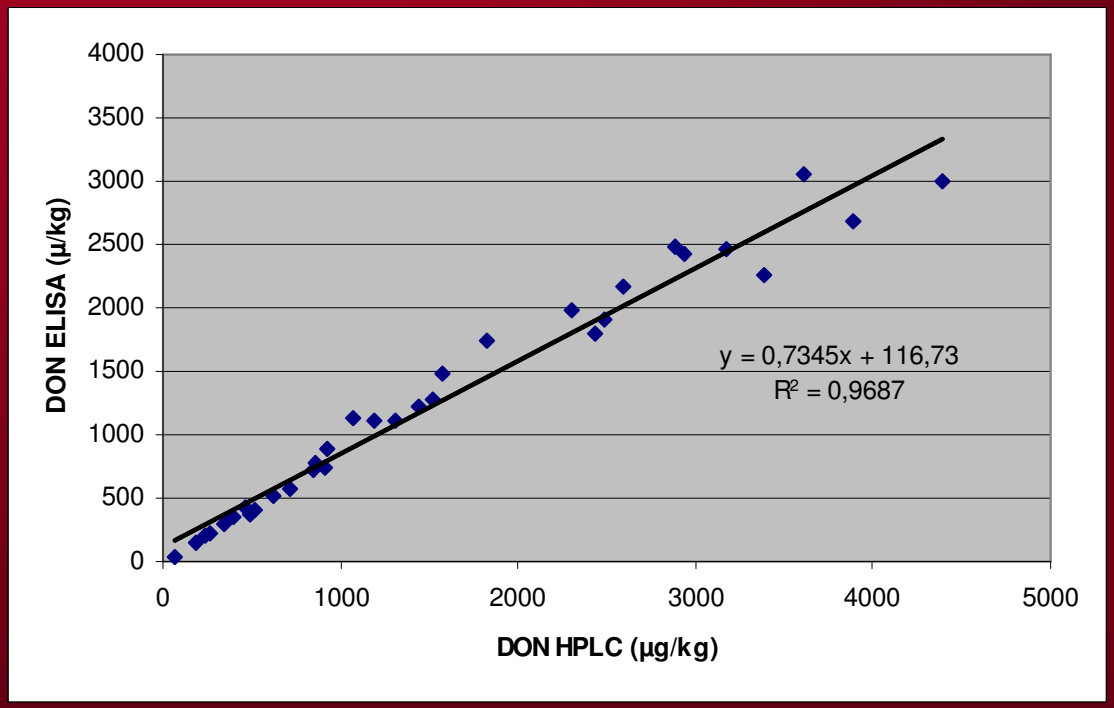
Mycotoxins

Results of DON ELISA test – correlation with HPLC



correlation coefficients:

- 0.984 for all values
- 0.991 for the values up to 2000 µg/kg

→ Correlation between HPLC and ELISA was very good

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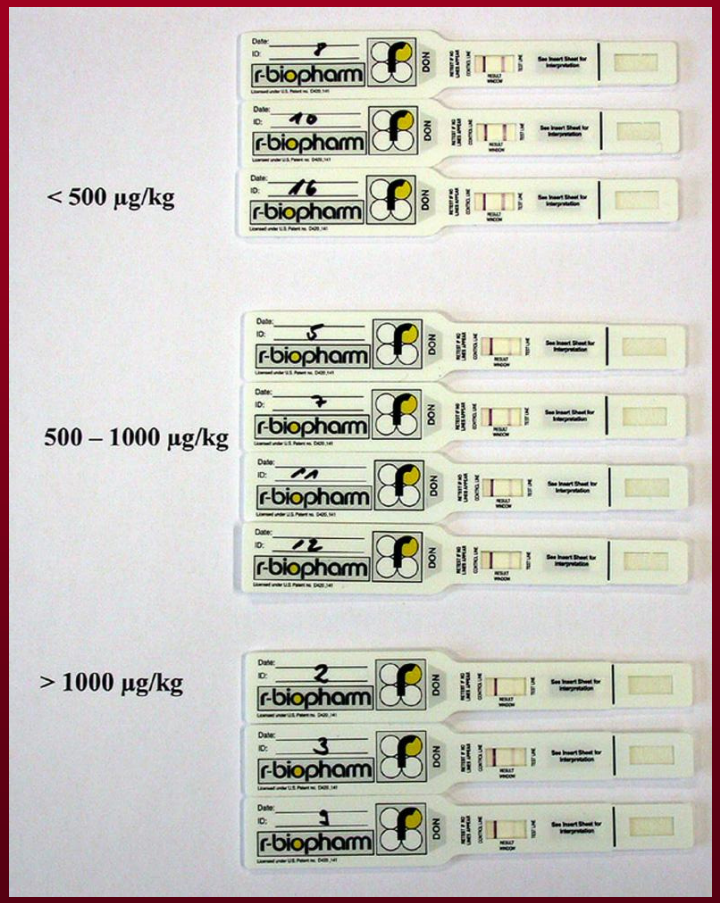
Mycotoxins
Results of DON strip test of r-biopharm – comparison with HPLC



RIDA® QUICK DON strip test of r-biopharm:

- Deviations from a limit value of 1000 or 2000 µg/kg are checkable (depending on dilution and sample weight)

- Interpretation of the measuring results is difficult because fading of colour intensity of a visible red-brown colour band on the strip has to be evaluated visually

- Evaluation is not a simple Yes/No decision because the changes from maximum to weak colour intensity are flowing

- In case of DON contents from 500 µg/kg onwards the colour intensity of the bands was so low that measuring results were evaluated as positive (i.e. > 1000 µg/kg)

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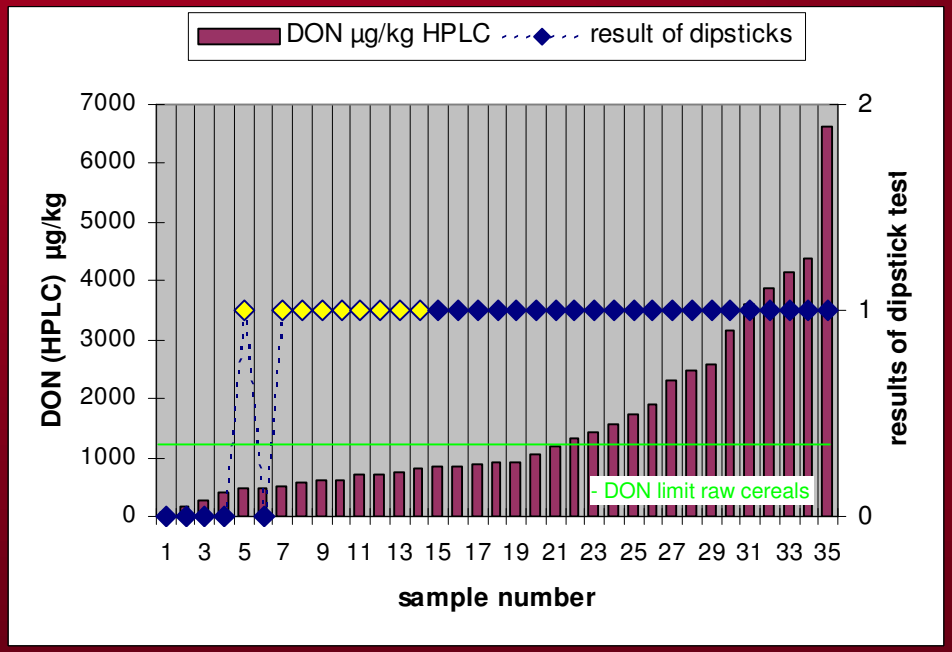
Mycotoxins
Results of DON strip test of r-biopharm – comparison with HPLC



Comparison of RIDA® QUICK DON strip test (r-biopharm) with HPLC

Result:

➡ Of the total of 35 wheat samples, 9 samples had an incorrectly positive evaluation
-> Correlation between HPLC and RIDA® QUICK DON strip test was low

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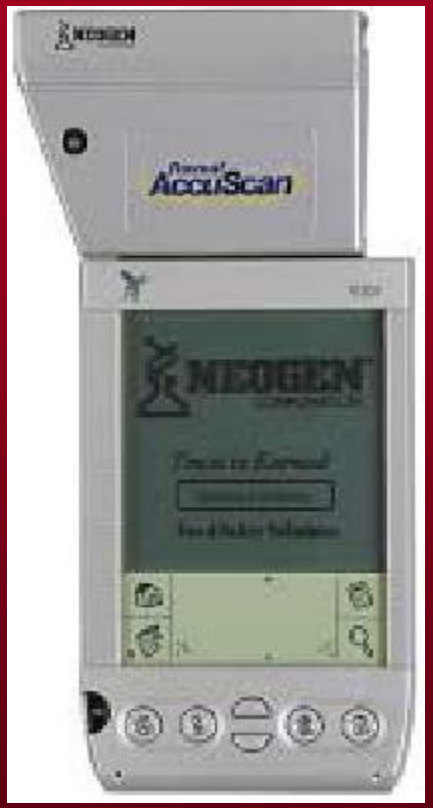
Mycotoxins

DON strip test of neogen – comparison with HPLC



AccuScan reader

REVEAL®DON strip test of neogen

- in this strip test , too, differently coloured lines are obtained, but the intensity of the lines can be measured by AccuScan reader

- Algorithms programmed into the AccuScan reader convert the measured line density into a semi-quantitative result:

0	=	not detectable	DON content < 0.5 mg/kg
0.5	=	positive result	DON content between 0.5 – 1 mg/kg
1.0	=	positive result	DON content between 1 – 2 mg/kg
2.0	=	positive result	DON content > 2 mg/kg

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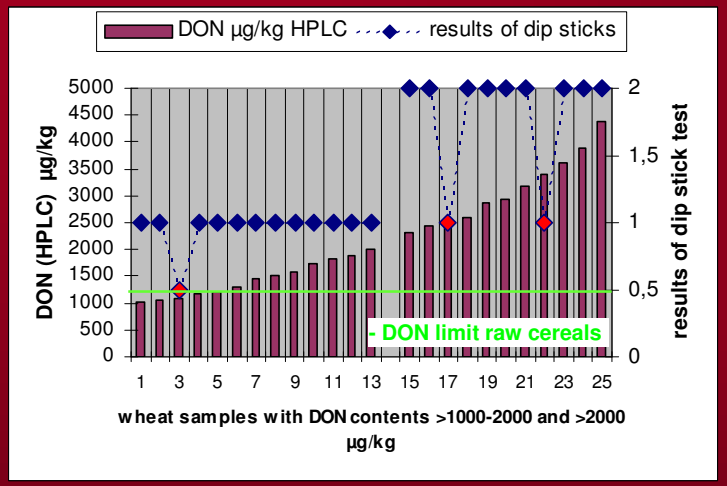
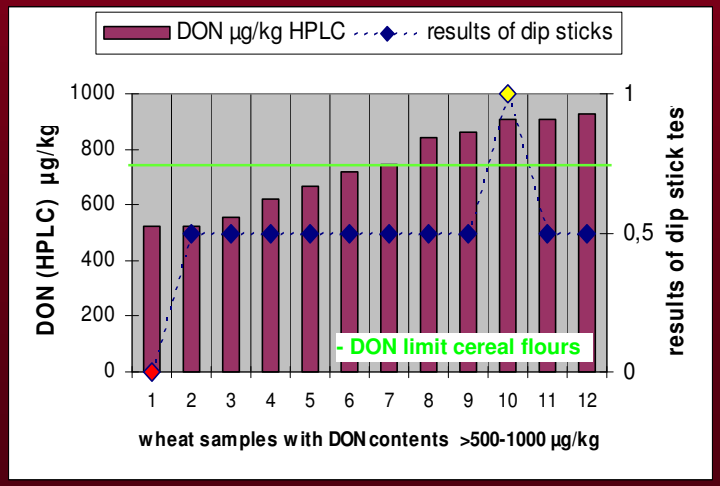
Mycotoxins
Results of DON strip test of neogen – comparison with HPLC





Comparison of neogen REVEAL®DON strip test with HPLC

- Of 56 measured samples, only 2 of 12 samples in the range of concentrations >500 - <1000 had a too low/too high semiquantitative result (but both samples had DON contents in a borderland of decision: 520 respectively 909 µg/kg)

- In the range of DON-concentrations >1000 µg/kg 3 samples had a lower result than expected (2 samples had DON contents widely above the legal limit of raw grain and 1 sample had a DON content of just above 1000 µg/kg)

Result:

➡ good correlation between HPLC and Reveal DON strip test

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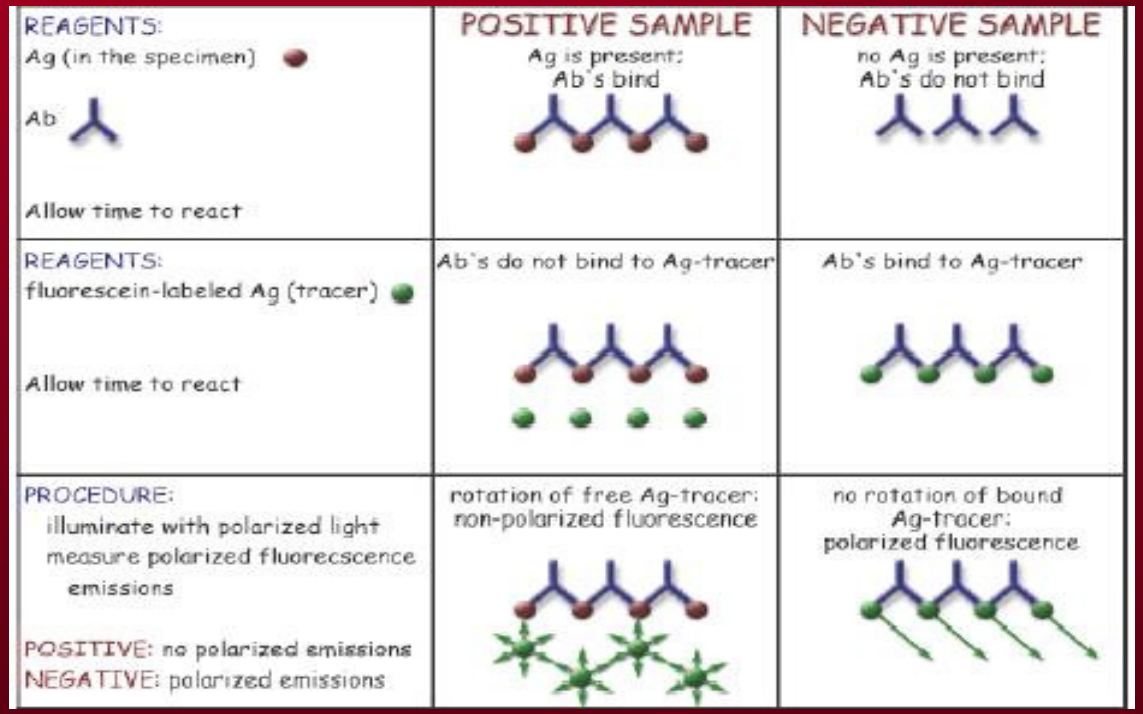
Mycotoxins
Principle of Fluorescence polarisation immunoassay (FPI)



Source: http://www.lfl.bayern.de/labor_aktuell/artikel/12074/linkurl_0_0_0_1.pdf

Competition of mycotoxin (red Ag) with fluorescein labelled mycotoxin (green Ag) for binding to antibody (Ab)

At illumination with polarized light:

- negative samples with bound fluorescein labelled mycotoxin (green Ag) show a polarized fluorescence emission (= 100 %)

- samples with mycotoxin content show reduced or no polarized fluorescence emission (= 100 % - x)

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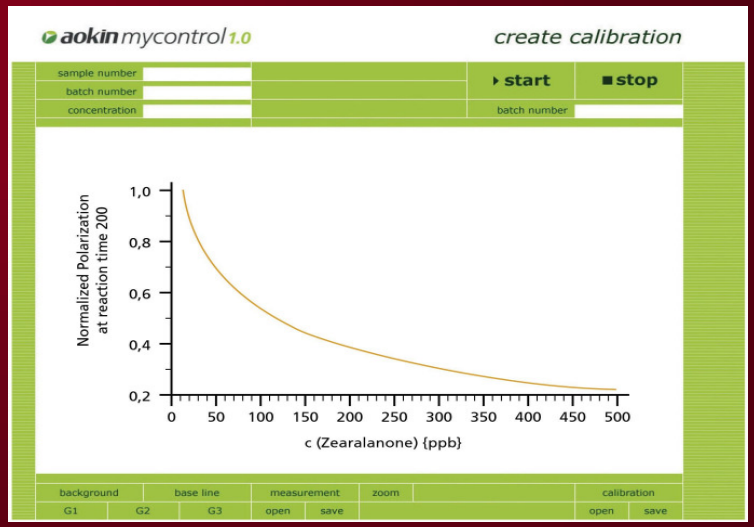
Mycotoxins
Fluorescence polarisation immunoassay (FPI) for zearalenone

Prototype of the FP 470 of AOKIN AG



Calibration curve of zearalenone

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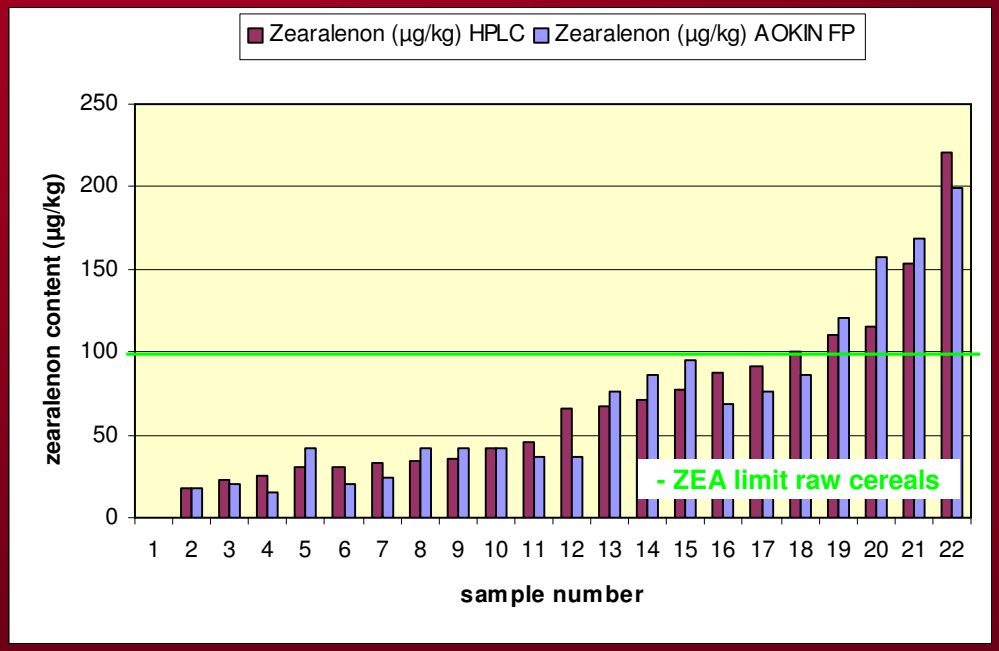
Mycotoxins
Results of FPI for ZEA – comparison with HPLC



AOKIN FP 470

Zearalenone contents of wheat samples, analyzed with HPLC and fluorescence polarization

Result:

➡ ZEA contents, analyzed by HPLC, were confirmed by FPI with deviations of ± 40 % (positive deviations are due to the fact that zearalenone derivatives are co-detected by antibody-based FP)

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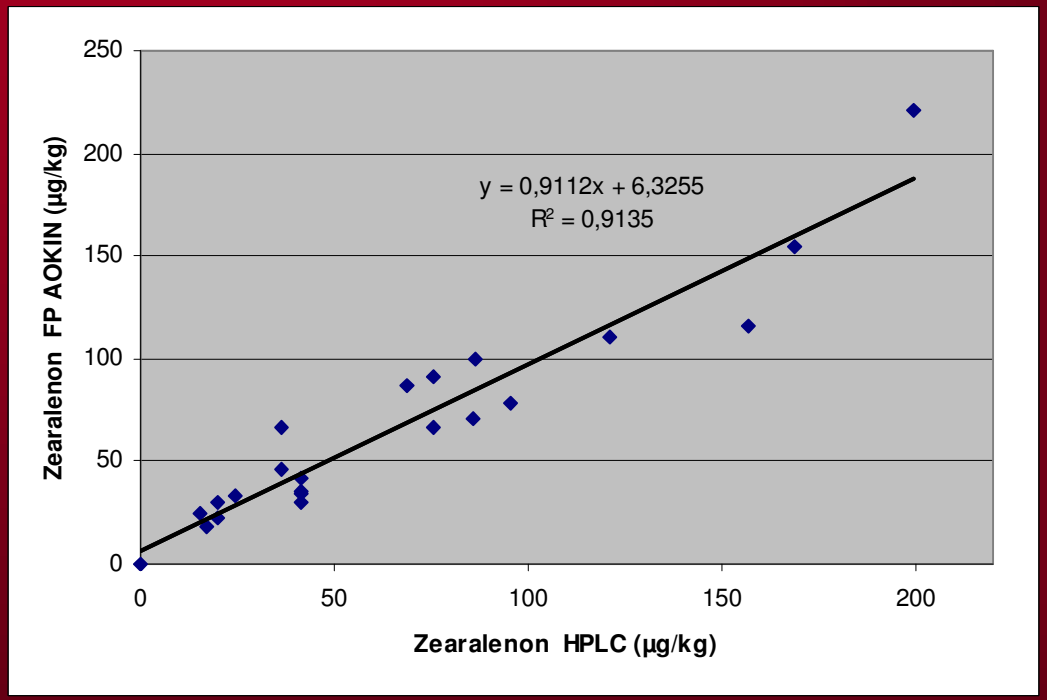
Mycotoxins

Results of FPI for zearalenone – correlation with HPLC



Correlation between HPLC and fluorescence polarization

Result:

➡ Correlation between HPLC and fluorescence polarization was adequate to a rapid method (correlation coefficient: 0.956)

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Mycotoxins
Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were performed with a FTIR spectrometer 8400S from Shimadzu, fitted with a single reflection diamond ATR element (Golden Gate)

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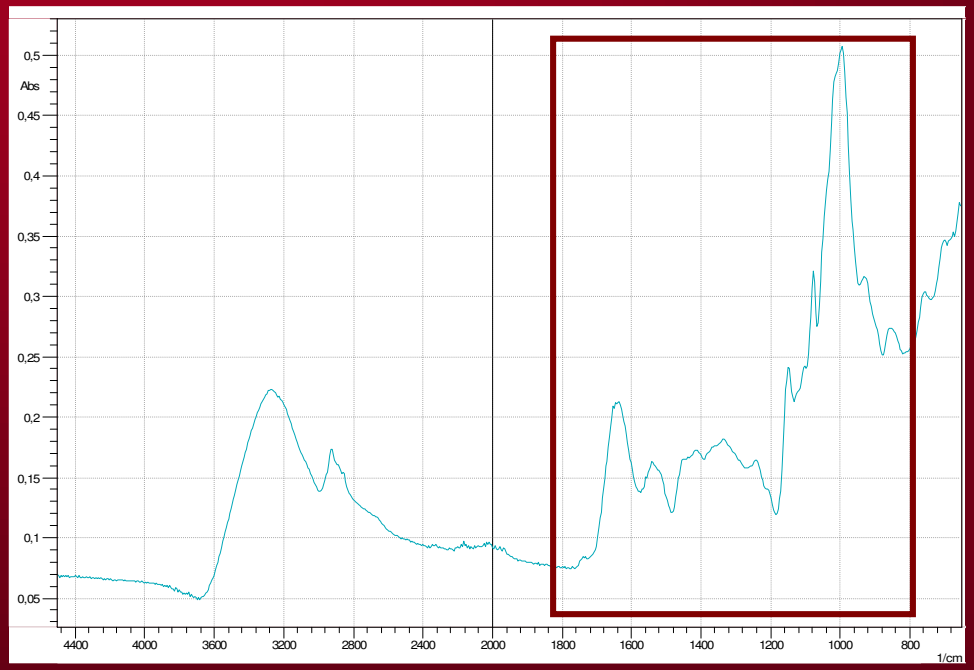
Mycotoxins
Fourier transform infrared spectroscopy (FTIR)



Mid-infrared spectrum of wheat

The rectangle marks the range chosen for data analysis according to Kos et al. (2003) Anal Chem 75: 1211-1217

-> they observed the greatest changes caused by mould in the spectral range of 1800-800 cm^{-1} (bands of carbohydrates, proteins, lipids).

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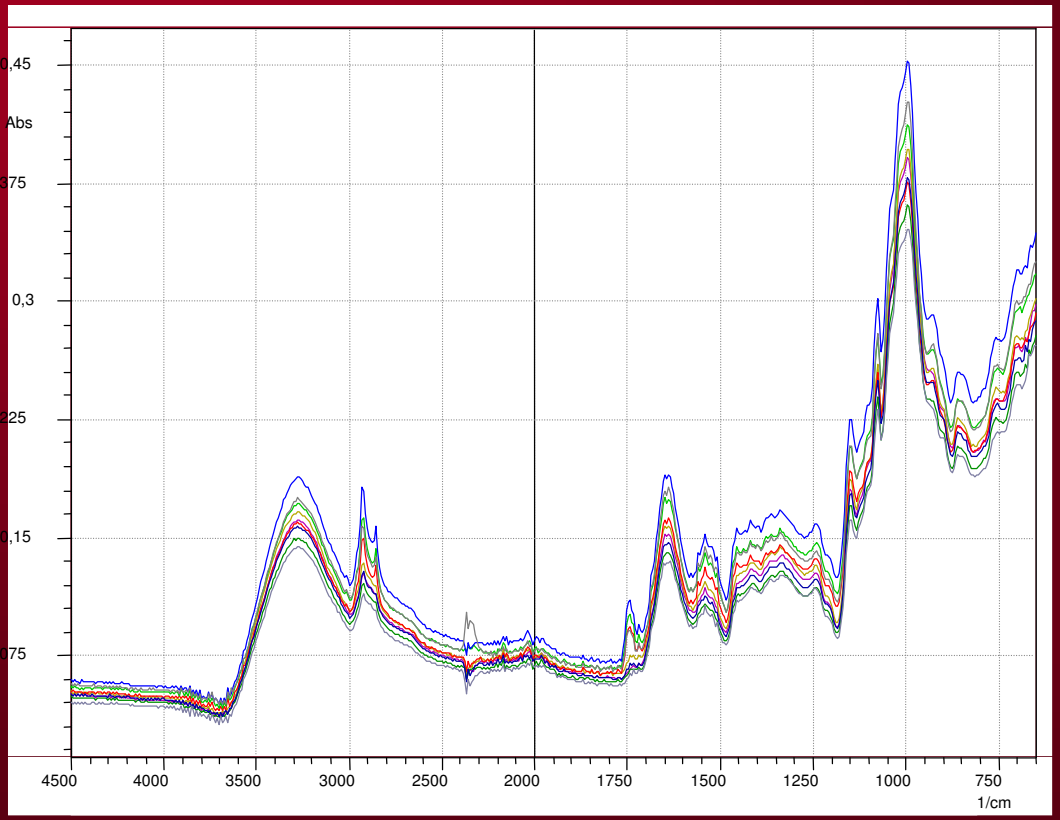
Mycotoxins
Fourier transform infrared spectroscopy (FTIR)



Mid-infrared spectrum of wheat - unsieved sample
-> 10 measurements: 10 relatively different spectra

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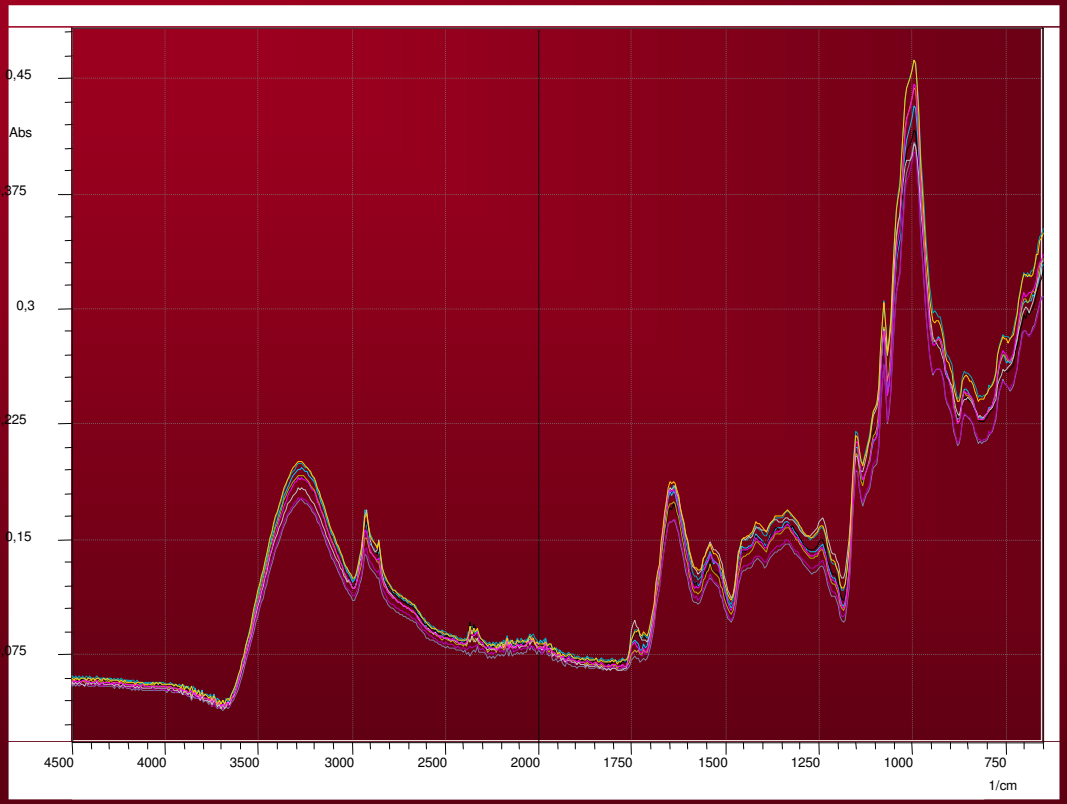
Mycotoxins
Fourier transform infrared spectroscopy (FTIR)



Mid-infrared spectrum of wheat - sieved sample (112-250 µm)
-> better agreement between 10 measured spectra

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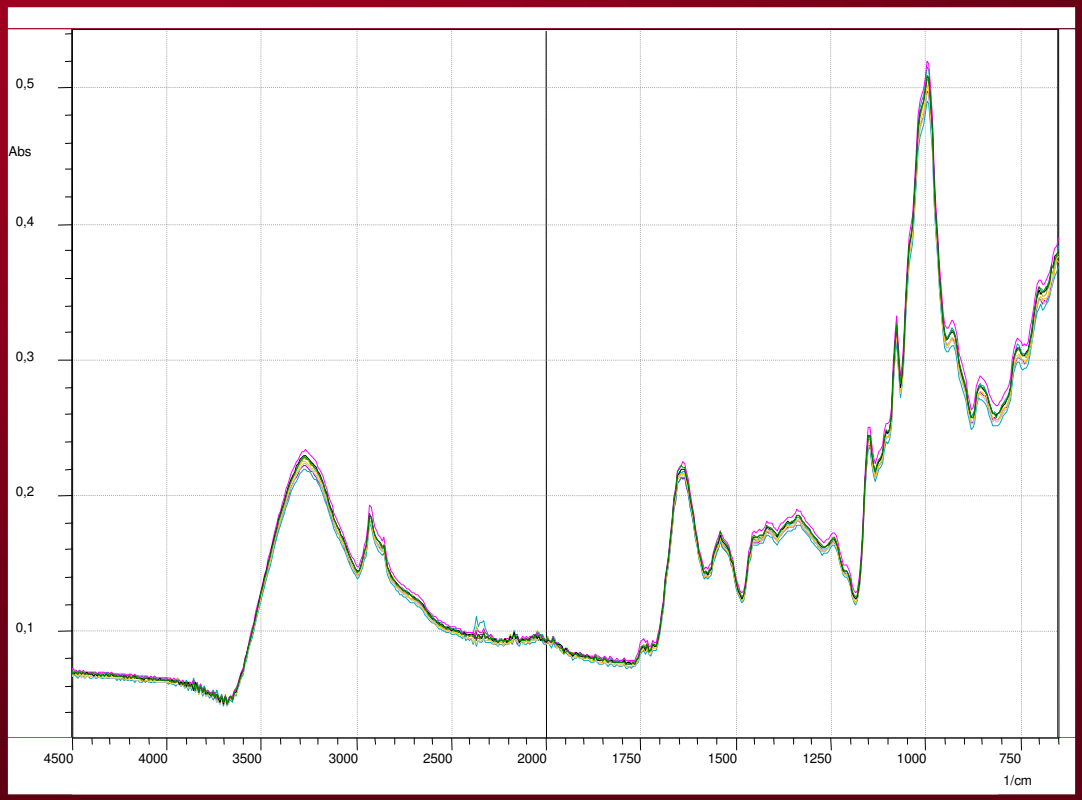
Mycotoxins
Fourier transform infrared spectroscopy (FTIR)



Mid-infrared spectrum of wheat - sieved sample (< 112 µm)
-> good agreement between 10 measured spectra

For calibration, validation and measurement of unknown samples only the sieving fractions <112 µm of the ground samples were used

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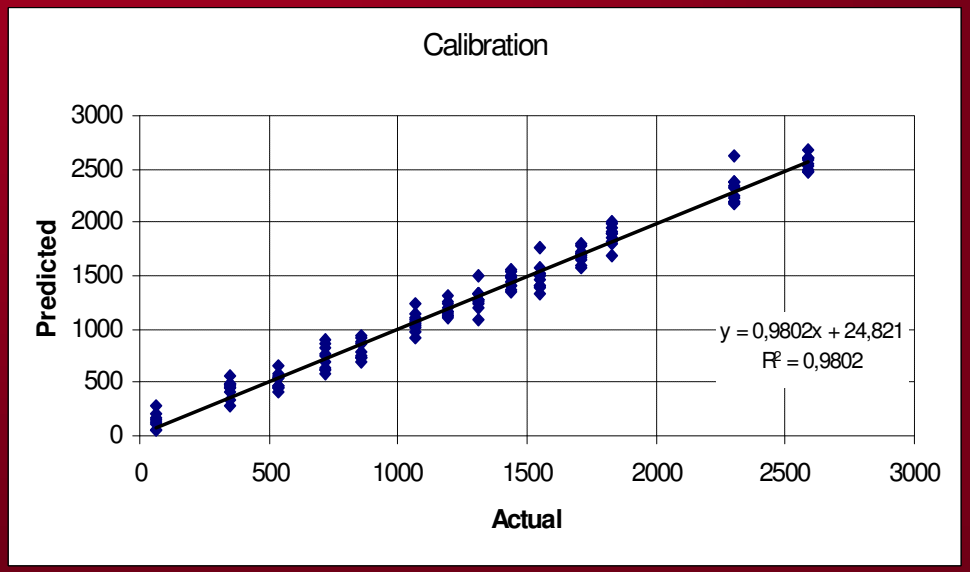
Mycotoxins
DON: Correlation of HPLC / FTIR



Calibration of FTIR 8400S
with 14 wheat samples
(DON contents: 60-2590 µg/kg)

Result:

➡ A calibration with a good correlation was achieved (correlation coefficient: 0.9901)

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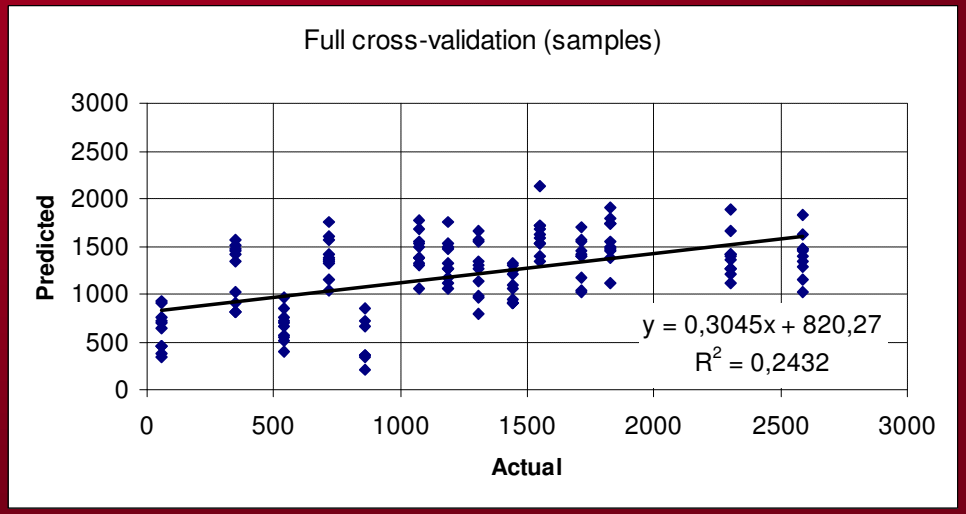
Mycotoxins
DON analysis possible with FTIR?



Full cross-validation
with 14 wheat samples
(DON contents: 60-2590 µg/kg)

Result:

- Correlation significantly worsened at full cross-validation (correlation coefficient only 0.4932)
- Correct predictions of the DON content of unknown samples were not possible
- Obviously there is no direct connection between changes of the components of the grain kernels (carbohydrates/proteins/fats) by moulding and the DON content (other fungi which do not produce DON could have caused the measured changes)

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Mycotoxins
Correlation DON HPLC – *Fusarium* damaged kernels ?

healthy kernels, normal size

white to reddish colour, strongly shrunken fusarium damaged kernels

kernels of normal appearance with red tip (seldom)

dented kernels, normal size, mycel in the ventral fold

Source: http://www.lfl.bayern.de/labor_aktuell/artikel/12074/linkurl_0_0_0_1.pdf

Due to frequent request of farmers, grain-chandlers and millers, the suitability of the visual assessment (analysis of Fusarium damaged kernels) also was compared with HPLC.

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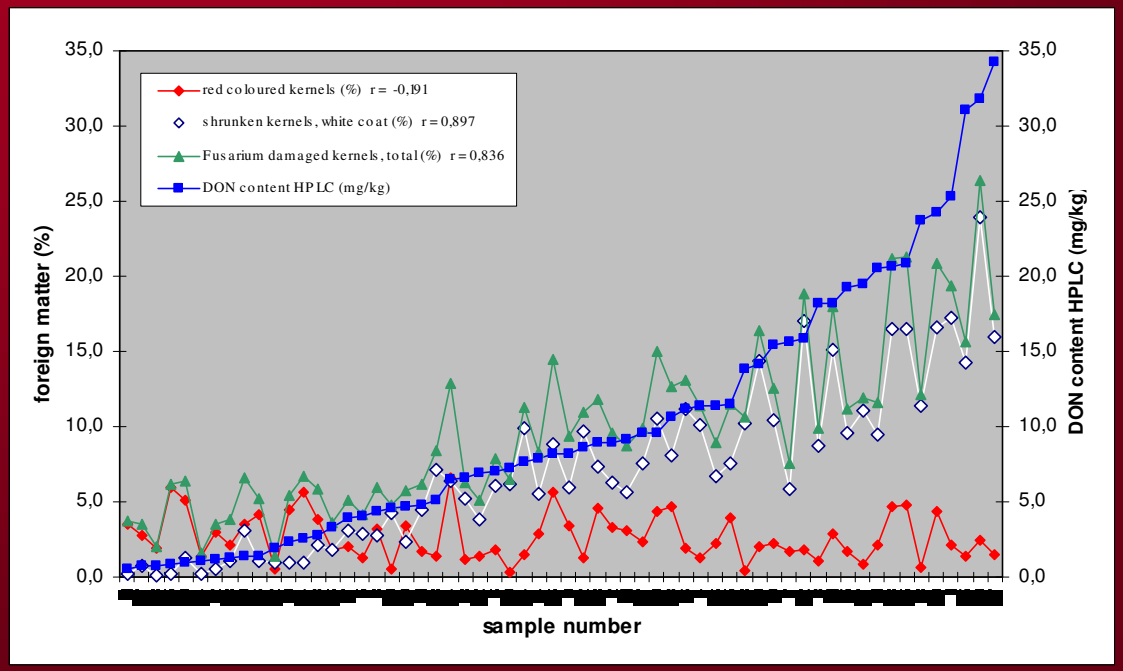
Mycotoxins
Correlation DON HPLC / *Fusarium* damaged kernels ?



Comparison of the DON contents of all 60 wheat samples (0.5 - 34 mg/kg) with the analysis of *Fusarium* damaged kernels

Result:

Correlation between HPLC and
- white, shrunken kernels with whitish coat
- Fusarium damaged kernels (total)

No correlation between HPLC and
- red coloured kernels

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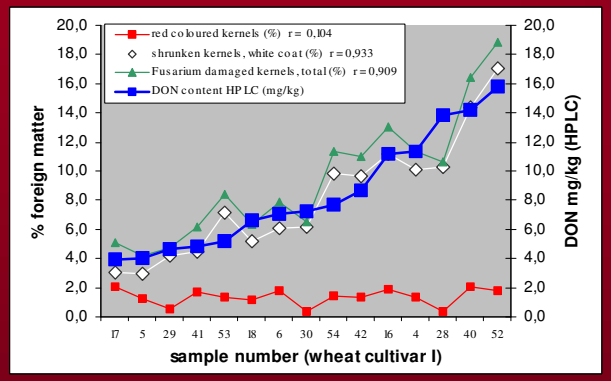
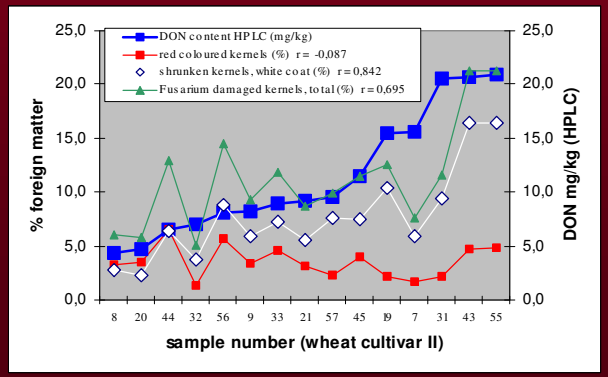
Mycotoxins
Correlation DON HPLC / *Fusarium* damaged kernels ?

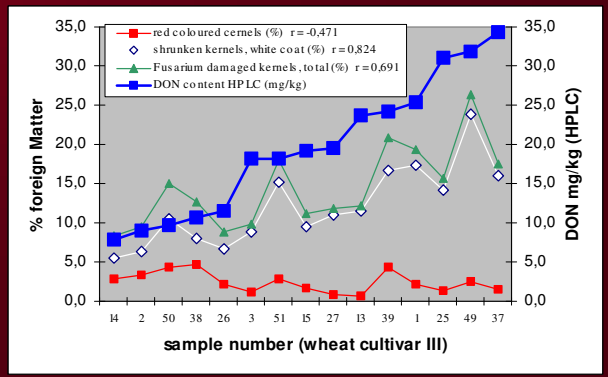
Correlation at high DON contents (4 – 34 mg/kg):



good correlation
in wheat samples
of cultivar I

lower correlation in wheat samples of cultivar II and III :





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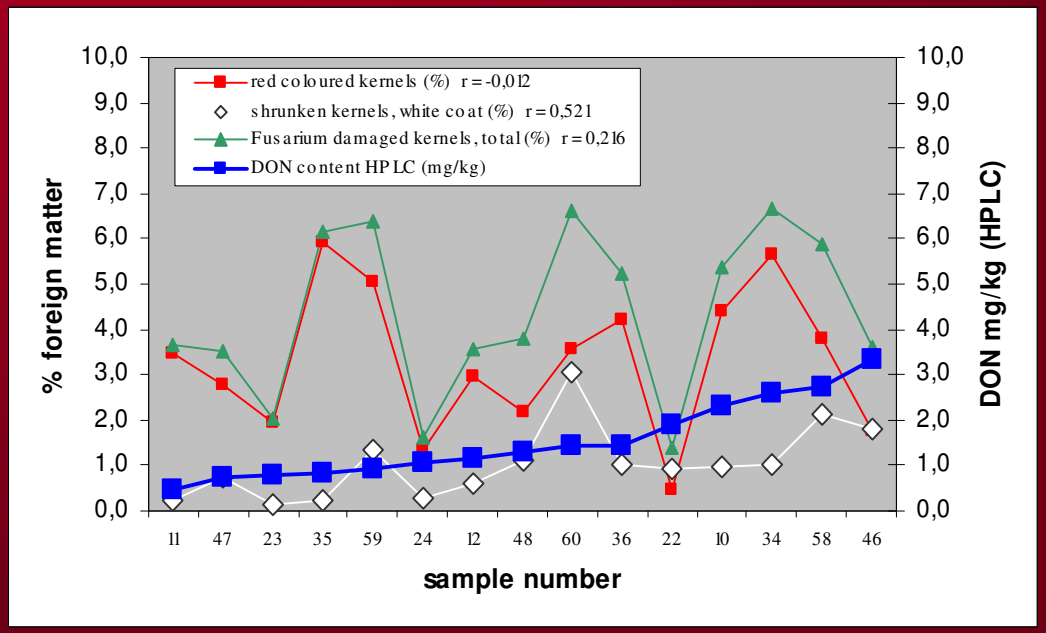
Mycotoxins
Correlation DON HPLC / *Fusarium* damaged kernels ?



Comparison of the DON contents of wheat samples with low DON content (0.5 - 3 mg/kg) with the analysis of *Fusarium* damaged kernels

Result:

- No or very low correlation at DON concentration range of interest to the milling industry (0.5 – 3 mg/kg) !
- Visual assessment – althogh frequently used in practice – is not suited as rapid method !

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Mycotoxins
Assessment of methods for mycotoxins (DON, zearalenone)

Method	Exact quantific.	Control of legal limit	Rapidity	Required expe-rience /qualific.	Invest-ment	Material costs per sample	Summary
HPLC	**(10)**	**(10)**	**(2)**	**(0)**	**0**	**(2)**	reference
ELISA	**8**	**8**	**8**	**6**	**6**	**4**	**40**
Dipstick r-biopharm	**0**	**4**	**10**	**8**	**10**	**8**	**40**
Dipstick neogen	**4**	**7**	**10**	**10**	**8**	**8**	**47**
Fluor. polarization	**8**	**8**	**8**	**6**	**(6)?****	**(4-6) ?****	**41 ?**
FTIR	**0**	**0**	**(0*/ 9)**	**4**	**2**	**10**	**15-25**
Fus. damag. kernels	**0**	**0**	**6**	**6**	**10**	**10**	**32**
Scale:							
10	r = 1	possible	≤ 20 min	low	0	0	
8	r ≥ 0,95		≤ 30 min		≤ 2 T€	< 10 €	
6	r ≥ 0,90		≤ 1 h		>2 - 10 T€	10 - 20 €	
4	r ≥ 0,85		≤ 2 h		>10 - 30 T€	20 - 30 €	
2	r ≥ 0,80		≤ 4 h		>30 - 50 T€	30 - 40 €	
0	r < 0,80	impossible	≥ 1 day	high (chemist)	> 50 T€	> 40 €	

* time-consuming calibration with HPLC required for rapid measurement ** costs unknown yet

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Mycotoxins
Assessment of methods for mycotoxins - conclusion

Conclusion:

The rapid methods were evaluated with respect to the possibilities to control the legal limits for mycotoxins and also to their rapidity, the required experience / personnel qualification and the costs for investment / analysis.

Bearing in mind these criteria the Neogen Reveal® DON strip was evaluated best. The strip test can be used for the detection of a transgression or falling-below a limit value.

Exact quantification of mycotoxin contents according to this test is not possible. For this purpose only HPLC or another chromatographic method can be used.

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MAP Milling - Part acrylamide

Study about acrylamide formation and minimization

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Acrylamide
Signal values for acrylamide

cereals	product	signal value (µg/kg)					
		17/09/00	31/01/03	26/11/03	17/11/04	21/10/05	11/01/07
maize	breakfast cereals (cornflakes)	260	260	200	200	180	180
wheat	cookies for children, rusk	n.c.	n.c.	360	360	245	197
	short crust pastries	800	660	575	575	300	300
	almond biscuit	1000	710	710	560	560	416
	diabetic pastries	n.c.	n.c.	1000	1000	545	545
	gingerbread	1000	1000	1000	1000	1000	1000
rye	extruded flat bread, crisp bread	610	610	610	610	590	496

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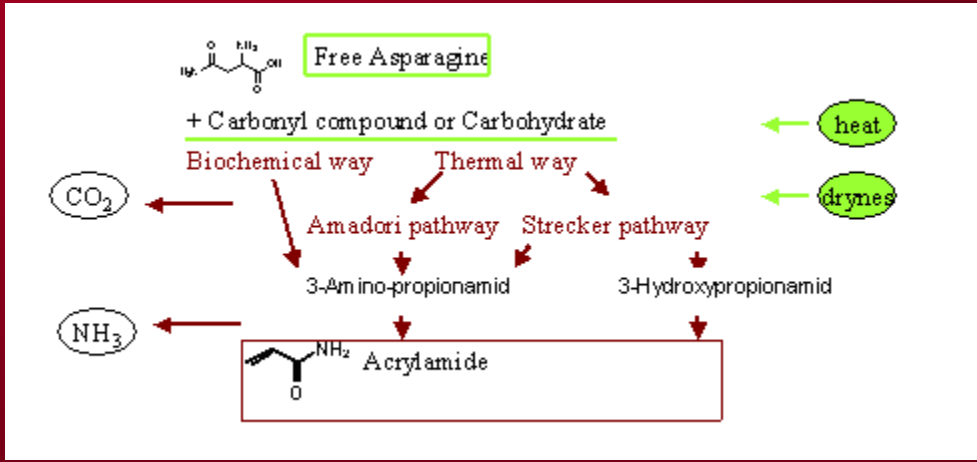
Acrylamide
Formation of acrylamide in food

For the formation of acrylamide several pathways have been proposed:



First pathway discussed is the Strecker pathway about aminopropionamide as intermediate

Beside Strecker pathway with aminopropionamide other ways have been found:

- a biochemical way by decarboxylation of asparagines
- a thermal way (Amadori pathway) with 3-amino-propionamide
- another thermal way (Strecker pathway) with 3-hydroxy-propionamide

➡ In all the ways, free asparagine is the pre-metabolite

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Acrylamide
Formation of acrylamide in food

Strecker pathway with aminopropionamide as intermediate:

- Pathway is characterized by a series of reactions between amino acids, primary free asparagine, and a reducing sugar (fructose or glucose)
- Reactions are part of the Maillard reaction, which also causes the browning of food and development of taste during baking
- Heat (>140 °C) and dryness are important reaction conditions
- The high potential of aminopropionamide for acrylamide formation results from the easy thermal elimination of NH_3
- If asparagine is replaced by other amino acids (cysteine, threonine, methionine) the acrylamide production is quite lower
- Also substitution of glucose, fructose by saccharose or lactose showed a lower potential of acrylamide formation

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Acrylamide
Aim of the project

Known, quite effective technological variations in minimization of acrylamide are

- increasing product moisture
- decreasing baking temperature and baking time

The aim of the project was:

- the investigation of the influences in raw materials to get knowledge about the content of free asparagine
- to show links between free asparagine in cereals and acrylamide in baking or extrusion products

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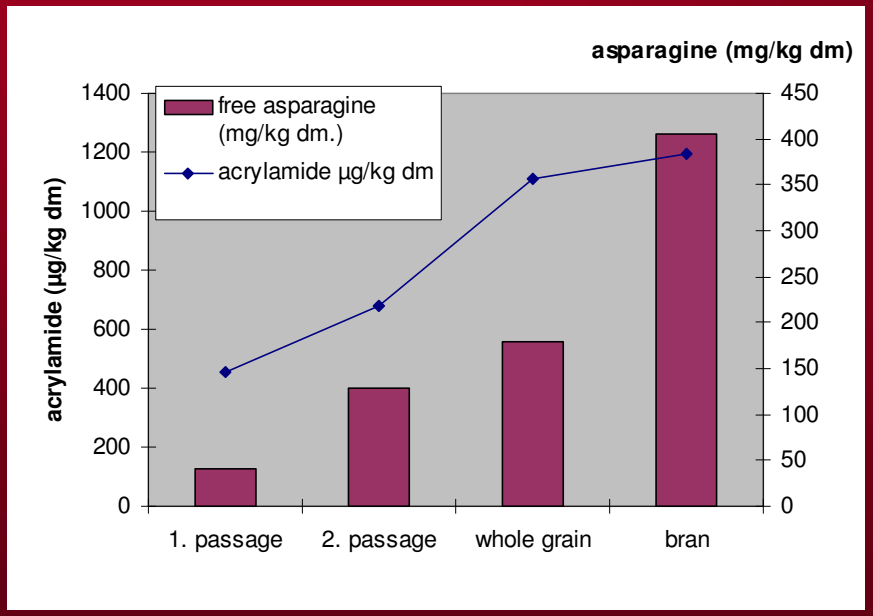
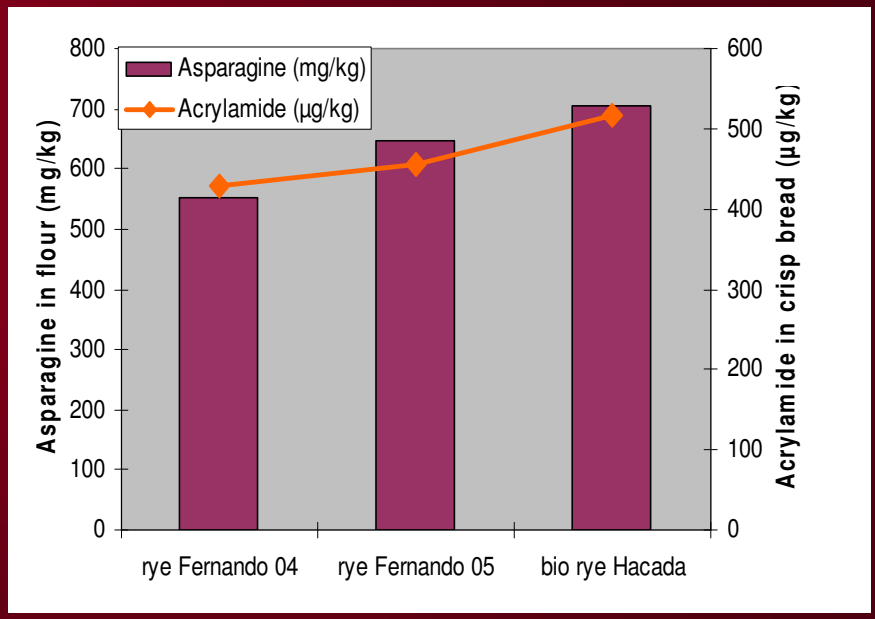
Acrylamide

Link between free asparagine and acrylamide

Study of the link between free asparagine and acrylamide formation:



in a model dough



in industrially produced crisp bread

➔ It has been shown that content of acrylamide is increasing with increasing content of free asparagine in the model dough as well as in the crisp bread

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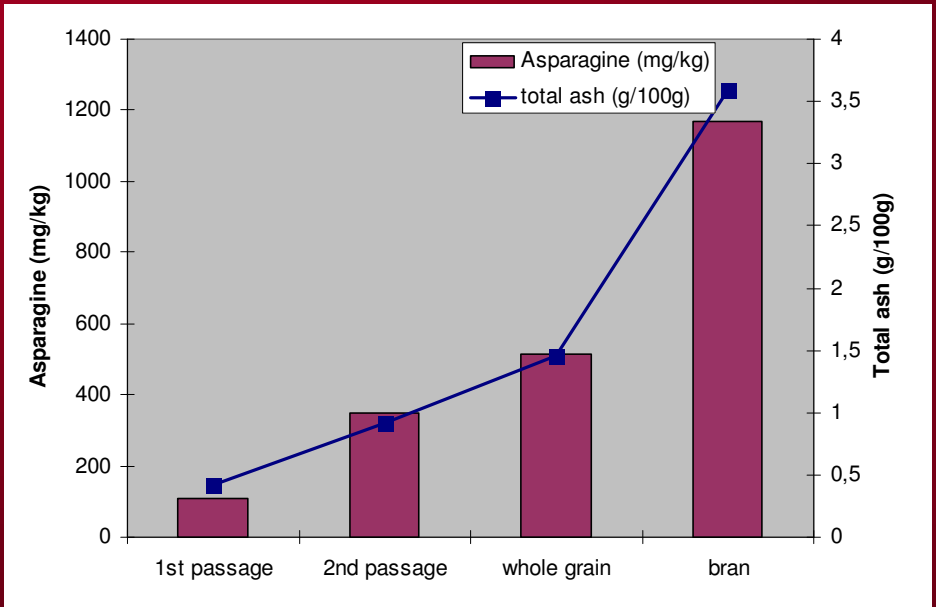
Acrylamide

Distribution of asparagine in different grain layers



- The different types of flour are obtained by including different grain layers during milling process

- They are characterised by different mineral contents.

➡ It has been shown that the content of free asparagine is increasing with increasing ash content

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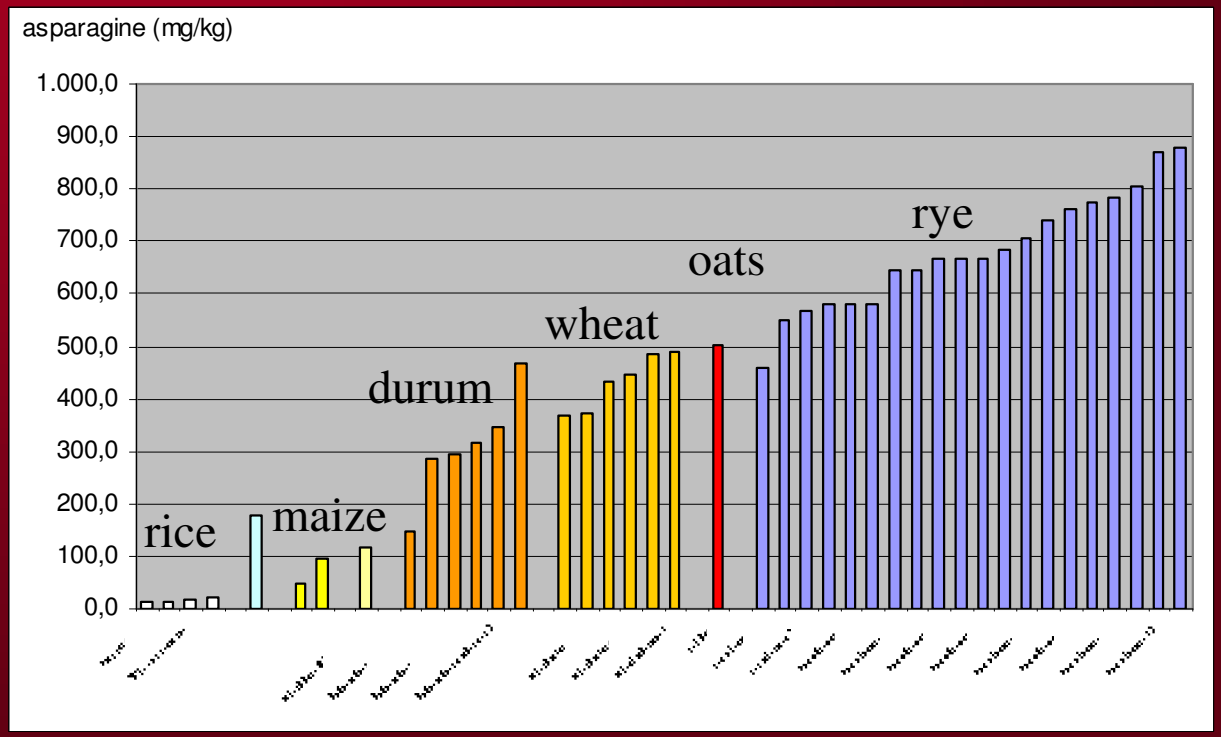
Acrylamide

Distribution of asparagine in different kinds of grain



- There are wide differences in content of free asparagine between the different cereals
- There is an increase from the lowest content in rice and maize to the highest content in rye

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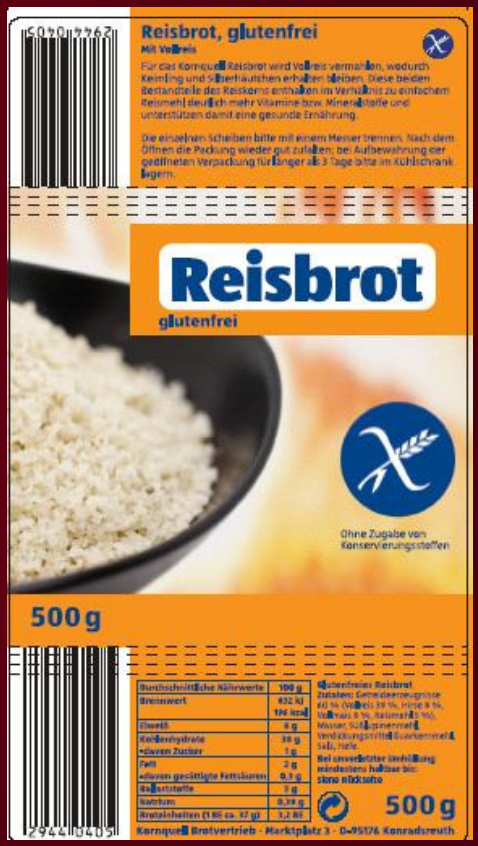
Acrylamide

Lowest asparagine content in rice

The different kinds of grains are used for specific products:

Cereal	mg/kg free asparagine
rice	13 - 20
maize	47 - 95
hard wheat	146 - 467
wheat	370 - 489
oat	504
rye	459 - 880



For extrusion products it is possible to use types of grains with low content of asparagine, like rice or maize. But also for baked goods cereals with a low content of asparagine should be considered as possible ingredient.

One example in this direction is the rice tin bread produced by PEMA Wholemeal Specialties Heinrich-Leupoldt KG, Weißenstadt, Germany:

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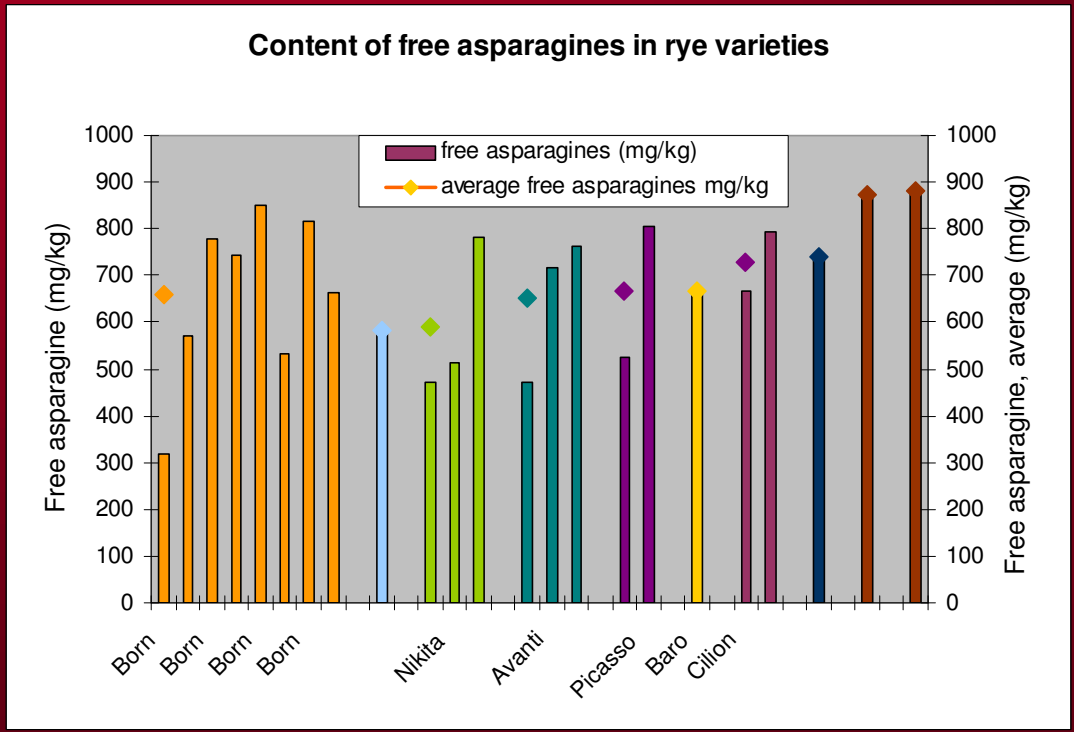
Acrylamide

Distribution of asparagine in different rye varieties



It was shown that rye has a high content of free asparagine.
In the survey on the frequency of acrylamide distribution, crisp bread made from rye belongs to the product group with a high content of acrylamide.

Therefore, efforts were made to select rye varieties with a low content of free asparagine.

➡ But due to the scattering of the asparagine contents in rye samples of one variety it is not possible to select rye varieties of low asparagine content

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Acrylamide
Influence of additional ingredients on the reduction of acrylamide

The formation of acrylamide proved to depend on pH. Acrylamide formation is increasing at alkaline conditions.

Reducing the pH value can reduce acrylamide.

Therefore efforts were made to reduce acrylamide by the addition of organic acids.

The high potential in minimizing acrylamide and a minimum sensory influence on the final product were the criteria for the selection of the kind and concentration of organic acids.

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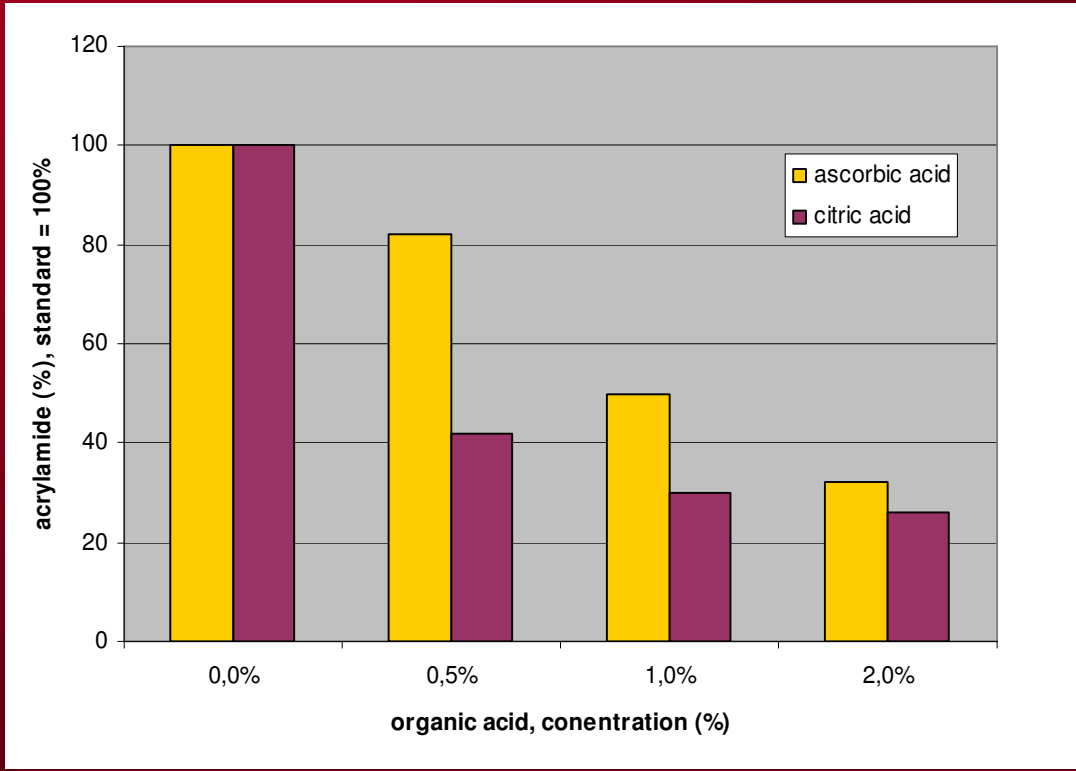
Acrylamide
Influence of organic acids



Influence of organic acids in a model dough

Both acids were effective to minimize acrylamide in baked products to 25 % of standard tests without any organic acid addition

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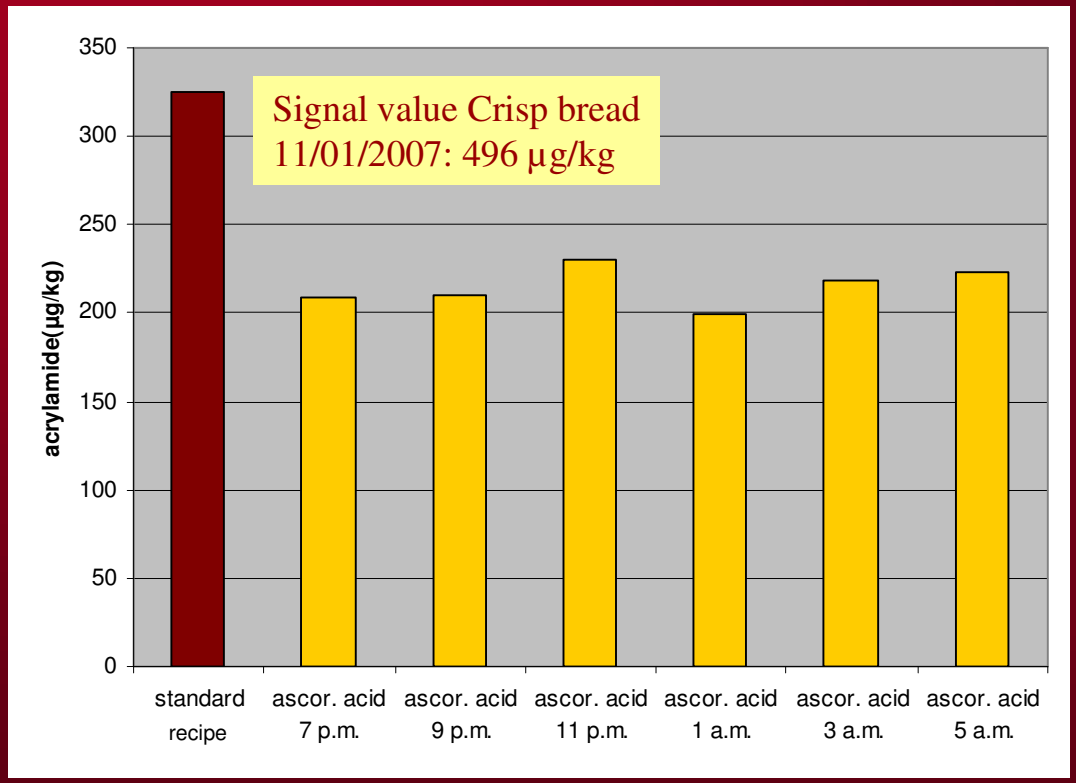
Acrylamide
Influence of organic acids



Influence of organic acid in industrially produced crisp bread

Crisp bread was produced according to two different recipes:
- standard recipe without the addition of ascorbic acid
- recipe with the addition of 1% ascorbic acid

Samples were taken in intervals of two hours during two production periods, i.e. for about 12 hours

➔ A reliable minimizing effect of appr. 30 % was achieved by the addition of 1 % ascorbic acid without any negative effect on the sensory properties

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Acrylamide
Study about acrylamide formation and minimization - Conclusions

Acrylamide can be reduced by

- low content of free asparagine in raw material
  - content of free asparagine in endosperm lower than in bran
  - rice and maize have lower contents than wheat and rye
  - Triticum durum has lower content than Triticum aestivum
  - variety differences show wide scattering
- addition of organic acid (ascorbic acid)
- combining several reducing parameters, if possible

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