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Nordic Innovation Centre

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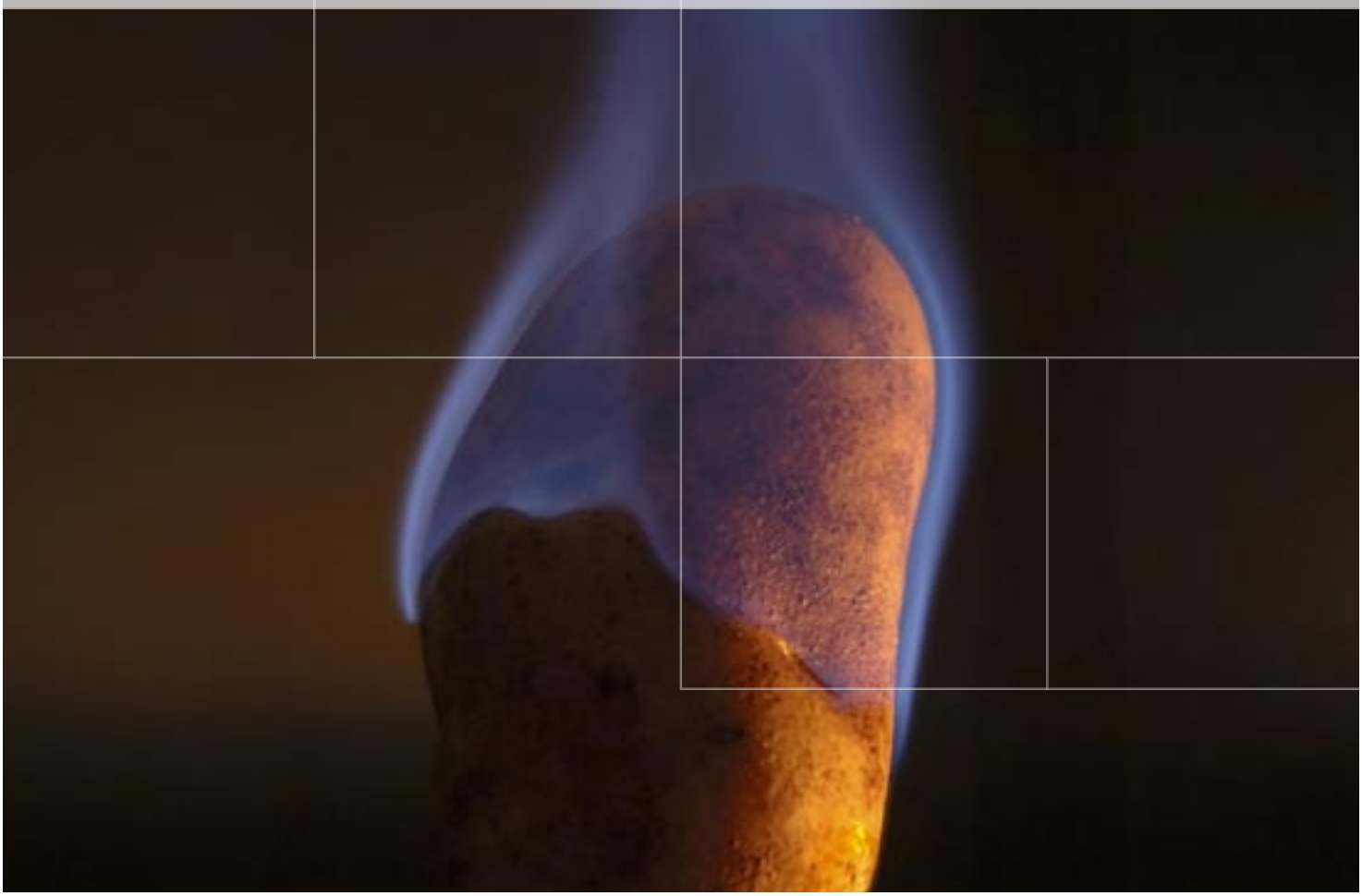
Acrylamide precursors
- Limiting substrates and in vivo effects (NORDACRYL)

- Model experiments and pilot scale production for reducing acrylamide formation
- Effect of sugars and amino acids in heat treated potato and cereals
- Information exchange between Nordic industry and research institutions
- Models for studying uptake and metabolism of acrylamide



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Title:
Acrylamide precursors; Limiting substrates and in vivo effects. (Acronym: NORDACRYL).

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Abstract:
A Nordic 3-year long project financed by Nordic Innovation Centre has included researchers and industries from Norway, Sweden, Iceland, Denmark and Finland. The project has been coordinated by S. H. Knutsen at Matforsk, Norway.

This project has been quite unique since otherwise competing food industries have collaborated by exchanging information related to processes and products.

The work has been restricted to potato and cereal raw materials and has included the investigation of key constituents in the raw materials that form acrylamide upon heating, ways to reduce its formation and the possible biological effects. New technologies have been tested in laboratories, in pilot and industrial scale.

It has been shown that reducing sugars such as glucose and fructose (but not sucrose) are the most common contributors (precursors) to acrylamide formation by heating.
It has further been shown that the constituent that most strongly affects the acrylamide formation in cereals is the naturally occurring free amino acid asparagine (limiting substrate).
For cereals the asparagine content in the flour is quite constant during storage, but might vary between different types of cereals and their milling fractions, highest in the bran.
For some products the additions of glycine or asparaginase contribute to reducing the acrylamide formation during baking.
For potato the limiting substrates are reducing sugars. Their increases during storage then introduce some difficulties and potentially quite large variations in the final products. Sugars in potato can be reduced by hot water treatment (blanching) and thereby lower acrylamide formation upon frying or baking.

The bioavailability of acrylamide has been investigated in a muse model system and some biomarkers have been identified.

Topic/NICe Focus Area:
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Executive summary

Background

The discovery in Sweden in 2002 that acrylamide is formed during heat treatment of starch rich foods caused a lot of concern among consumers, retailers, the food industry and the authorities. Acrylamide is known as a neurotoxin in humans and as a carcinogen in experimental animals and it is classified as a probable human carcinogen by the International Agency of Research on Cancer (IARC). In the Nordic countries the consumption of heated potato and cereal foods is relatively high and there is a strong tradition in eating bread, especially crisp bread. Therefore this project has been focused on acrylamide in cereal and potato products. Ongoing monitoring programmes still focus on the issue of acrylamide in food. Quite recently (June 2007) in Norway the food authorities (Mattilsynet) published a new investigation. It was stated that although the levels for some products had been reduced there is still a matter of concern (www.matilsynet.no). Similar investigations are undertaken in EU and the other Nordic countries.

Scope

In NORDACRYL food industry and researchers joined forces to study the issues related to acrylamide in foodstuffs. The main objectives were to determine how natural occurring compounds in potato and cereals form acrylamide upon heat treatment of foods, and to develop methods for monitoring bioavailability of acrylamide in food to add to the evaluation of possible health effects. This was achieved in laboratory models as well as industrial scale processing experiments.

Impact on the partners involved in NORDACRYL

Industrial partners

During the project period new technologies and methods have been tested in collaboration with the research institution. Some effective methods for reduction have been identified for cereal and bread systems. The industry has now more awareness about the problem and industry R&D is working on it. The identified “tools” for reduction of acrylamide formation are integrated into the processes. Quality control programmes are in place to control the effectiveness of the tools. Especially for Iceland NORDACRYL (Mati prev. IceTec) has been important for the industries to gain knowledge about acrylamide in their products. Otherwise competing industries have established a network with a large degree of open information related to acrylamide and related matters. This is considered as very positive for the industries and is a model that will be utilized in the future for similar problems.

Academic partners

NORDACRYL has helped to contribute to increase the critical mass in the area of acrylamide research within the different institutions. The project has given the researches the possibilities to be updated within a broader field than their traditional specific interests and encouraged multidisciplinary thinking. Publications have been produced and students have been educated. The contacts with industries have increased the possibilities for collaboration in other projects and the possible funding of research. The interaction between health related research and the industry are considered quite new, at least for the involved partners, and is extremely useful for both the researchers and the industries.

Collaboration and information exchange

NORDACRYL was a key platform for connecting Nordic food industries with research activities at Nordic research institutes and universities and national projects as well as with European research projects such as HEATOX and Cost 927. There has also been a continuous exchange of information with National food and health authorities, the European Commission and the European Food Safety Agency (EFSA) and also with the European food industry organisation CIAA. In addition approximately 25 scientific papers have been published in peer reviewed journals from the groups.

Important findings

It is a direct link between the level of heat treatment (temperature and time) and the formation of acrylamide. The formation in general parallels the browning of the products and the formation is highest in the parts of the products that reach humidity less than 98% and for processing above 120ºC. For all systems the important constituents for the heat induced formation of acrylamide are naturally occurring reducing sugars such as fructose and glucose (but not the common table sugar: sucrose!) and the free amino acid asparagine. These compounds (termed: limiting substrates or precursors) are all natural occurring constituents in potato and cereals.

For potato products the limiting substrates are in general the reducing sugars, which vary considerably between cultivars, growing seasons and storage conditions. But also the content of asparagine has an influence, although less than the reducing sugars. In cereal products the potential for the acrylamide formation is mostly determined by the asparagine content of the flour. Although rye contain more free asparagine than wheat, and germ and bran more than sifted flour there is less variation between the varieties and cereal types and no variation induced by storage.

Mitigation strategies are in general coupled to lowering the temperature and/or reducing the processing time but this must be carefully controlled in order to maintain product quality. For potatoes washing steps (blanching) in order to remove sugars are quite efficient but results may be varying and in some cases product quality may be influenced. For cereals asparagine can not be removed but the formation in the products can be reduced by replacing amino based leaving agents, increasing the yeast fermentation time or increasing the thickness and final humidity of the products. Other actions might be adding natural compounds competing with asparagine in the reactions that form acrylamide or alter asparagine in the raw material. The former can be achieved by adding the naturally occurring amino acid glycine and the latter by using the enzyme asparaginase. Since some of these additions might induce sensory effects in certain products and some regulatory aspects are pending some work still remains. Additions of such active constituents are most feasible for cereal based products since they might be added in the dough.

New risk assessments of acrylamide are awaiting additional results from long term animal studies (as performed by FDA), which are expected to be presented in 2008. In addition, more details about the human exposure and internal dose of acrylamide are needed. In a mouse model we studied how urinary metabolites of acrylamide could be used as short time biomarkers of exposure and internal dose. We demonstrated a linear relationship between the intake of acrylamide from crisp bread, experimentally baked to give different content of acrylamide, and the urinary metabolites. The bioavailability was approximately 100%. These urinary biomarkers are valuable tools to determine the real human exposure and to extrapolate toxicological data from animal studies to humans.

Conclusions

- The natural content of sugars and amino acids in the raw materials has a significant influence on acrylamide formation during heating .
- Some varieties of potatoes and cereals tested in the project, but not commercially available, form less acrylamide.
- Acrylamide is minimised by reducing the heat load during processing.
- It can for most cases be reduced but not totally removed.
- Further reduction of acrylamide has been achieved in lab scale experiments by use of additives that compete with the acrylamide formation, modification of fermentation and oven conditions, post drying of potato chips with air.
- Feasibility of such modifications on industrial scale and their effects on overall quality of the products are not yet evaluated.
- The developed method for determination of biomarkers in urine can determine the internal dose as a result of exposure to acrylamide from food

Recommendations

***** Denotes that such collaboration and networking have been established within Nordic industries, universities and research institutes, facilitated by the NORDACRYL network.

Raw materials

- *New varieties of potato and cereals with lower levels of precursors adapted to the Nordic climate must be developed.* Without use of GMO this is a time consuming (> 5 years) project. New varieties must also be suited for industrial production, storage and transport (see below). This is a task for breeders and their collaboration with industries is essential.*
- *Raw materials may in the future be available on the global market for specific uses.* This is a challenge for the Nordic producers linked the above statement.
- *Improved storage facilities and logistics of potatoes should be considered especially for potatoes for general use.* This a complex issue for the food-and transport industry.
- *Other raw materials such as coffee and the effect of combining different raw materials in products should be investigated.* These are tasks for the industry in collaboration with Food scientists.

Research institutions and official research founding

- *Promising principles and methodology must be validated in controlled laboratory experiments.* It is of importance to determine whether a methodology that is effective in one system, can be utilised in different food systems. This applies to some of our findings such as the use of asparaginase and the effect of salt solutions.
- *Human epidemiological studies using biomarkers for acrylamide intake are recommended*. These are important for understanding biological effects and hence necessary for risk evaluation.
- *The metabolism of acrylamide present in common food and the associated risks must be evaluated.*

Industrial Production and collaboration

- *The industry must focus on As Low As Reasonable Achievable* (ALARA).*
- *The NICe induced collaboration regarding the acrylamide issue between normally strongly competing Food industries can act as an innovative work model for future emerging food safety issues*.*
- *New emerging solutions must be tested in real production systems*. Research collaboration with researchers is then necessary.*
- *Industries should consult the CIAA toolbox.* Acrylamide Pamphlets produced on 20 national languages in collaboration with EU. Results and inputs from partners of NORDACRYL have been used in this information.

http://ec.europa.eu/food/food/chemicalsafety/contaminants/acrylamide_en.htm

Consumers and household attitude

- *Excess browning or drying during frying and baking should be avoided.* A golden yellow colour is advisable compared to brown and dark products
- *When preparing fried potato products at home some reduction in sugars and hence acrylamide formation can be obtained if potato slices are boiled for some minutes before frying or baking in the oven.* This is comparable to the present blanching procedures nowadays performed by the potato industries.
- *If possible, select potato with low content of reducing sugar and do not store at cold temperature (below 8ºC) whenever possible.*
- *Consumer advices should be limited until the risk evaluation has been performed*.

Press and information channels

- *The matter is truly complicated and worrying information (especially tabloid type) must be avoided.* Please consult national authorities such as Mattilsynet and Livsmedelsverket and/or relevant research groups.
- *Some care should be taken when simple solutions to the acrylamide problem are communicated.* Take the time to consult researchers and the industry for a critical opinion.

Regulatory authorities

- *The levesl of acrylamide in different foods show a large variation. Also within the same food product considerable variations are found and therefore a significant number of samples need to be included in monitoring programmes*.
- *It is reasonable that the food authorities will continue the monitoring programme of acrylamide in commercial food products.*
- *The approval of the industrial uses of a food grade enzyme (asparaginase) in cereals should be considered immediately*. We have shown that this is a useful method which can lower the acrylamide content considerable in certain products.

The project organisation

The project can be outlined as shown graphically below. There were no deviations from the original plans and the outcomes of the different tasks are summarized in the sections "Results and comments related to the different work packages".

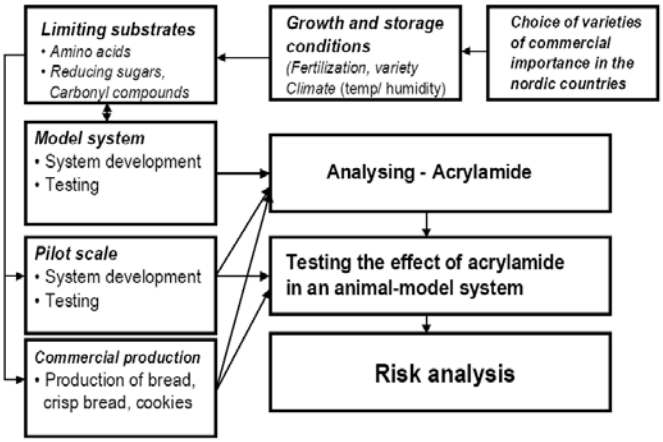

Figure 1 General overview of NORDACRYL as in the proposal.

The project was furthermore organized in different work packages as outlined below.

The work packages

Work pakage	Tasks (Short title)	Task leaders
WP1 Raw materials	Choise of nordic varieties	SW
	Content of limiting substrates	DVFA
	Growt and storage conditions	DIAS
WP2 Production	Model systems cereals	MATFORSK
	Model systems potato	SIK
	Pilot scale (cerals/potao products)	MATFORSK
	Commercial production	
WP3 Analysis	Analyis of acrylamide	NILU
WP4 Healt and risks	Animal-model systems, Risk analysis	NIPH

Table 1 An overview of the different work packages.

The work method

The work within the project was organized by annual meeting and regular contact by phone and EMAIL. There was an open exchange of information related to production parameters, raw materials and results from ongoing monitoring of the acrylamide content of the products performed within the industries. This transparent information flow was shared among large industries otherwise being strong competitors in the Nordic marked. This model was initiated in the first Norwegian Network project in 2002, and was internationally referred to as "The Norwegian model". The network model used and the contacts established can easily be re-used for other or new emerging risks related to food safety and especially process induced toxicants. This is considered by the industrial partners as an innovative working method and an important result by itself.

List of project partners.

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Acrylamide. In short: The compound, the sources and the problem

Acrylamide (AA) (2-propenamide, CAS No. 79-06-01) is a colourless and odourless crystalline solid. Due to its capability to polymerize it is a common used chemical and constituent in such applications as flocculants for water purification, in cosmetics and as a sealing adjuvant in tunnel construction. In relation to the latter the presence of acrylamide in foods was identified in Sweden in 2002. Results from animal tests showed that rat fed with a diet consisting of fried food had a higher content of reaction products between blood and acrylamide (Haemoglobin adducts of acrylamide measured as N-2(-carbamoylethyl valine)) [1]. Then simultaneously, when analyzing heat treated rat food, it was revealed that the content in the food paralleled the content found in the haemoglobin adducts in the rats. Then the Swedish National Food Administration and the University of Stockholm announced that carbohydrate rich foods that are processed at relatively high temperature may contain considerable levels of AA. It was found that the main sources for human dietary exposures to AA were those of coffee, baked cereal- and fried potato products. In the Nordic diet these 3 main groups roughly contribute equally. It is the occurrence of natural constituents such as reducing sugars and the free amino acid asparagine that upon heating reacts into acrylamide.

The presence of AA in foods still causes considerable concern since AA is classified as probably carcinogenic to humans (2A) by the International Agency for Research on Cancer (IARC 1994). AA induces tumours in several organs in mice [2-4] and rats [5, 6] and exerts reproductive-[7, 8] and neurotoxic damage [8-10]. The occurrence of AA in food has been internationally investigated and all old and new data are continuously being validated by such organizations as World Health Organization (WHO), Food and Agriculture Organization (FAO) and Joint Institute of Food Safety and Applied nutrition (JIFSAN), National Centre for Food Safety and Technology (NCFST) and different expert groups. For some comprehensive reviews of the problem please consult [11, 12].

A simplified list of key parameters and important terms

Parameter/term	Comments and a simple explanation of relevant terms
Acrylamide (AA)	2-propenamid. A water soluble reactive compound. May be formed in a heat drive reaction between reducing sugars and the amino acid asparagine.
Fructose	A common reducing sugar (monosaccharide) found in fruits, cereals and vegetables. Together with glucose it forms the common household sugar sucrose. More reactive than glucose towards AA formation.
Glucose	A common reducing sugar (monosaccharide) found in fruits, cereals and vegetables. Together with fructose it forms the common household sugar sucrose.
Sucrose	Common household sugar. Non reducing sugar. Must be degraded (hydrolysed) into glucose and fructose in order to contribute to AA-formation.
Starch	A common polysaccharide based storage molecule for plants. Contains glucose but do not contribute to AA-formation.
Asparagine	One common naturally occurring and abundant free amino acid. React with reducing sugars towards acrylamide. No regular contribution when a building block of proteins.
Asparaginase	An enzyme that can be produced for commercially use that is very promising to prevent AA formation in some systems. Remove an amino group from asparagine and promote formation of "AA neutral" asparagine acid.
Asparagine competitor	An amino acid that can be added in a product when heated. It reacts (i.e. competes) with the reducing sugars but do not form AA.
Acrylamide Precursor	A compound that reacts into AA under certain conditions. The most common ones are reducing sugars (glucose or fructose) and the free amino acid asparagine.
Limiting substrates	The precursor that due to its level in the raw material is the strongest determinant of the AA formation upon heating. For cereals this is asparagine but for potato it is reducing sugars.
Maillard reaction	A complex set of chemical reactions necessary for the formation of AA. It involves the reaction between the carbonyl of the reducing sugar and the amino group of amino acids. The reaction is responsible for browning and also aroma formation. Can not been avoided when food is heated.
Fermentation	In the present context a process involved with controlled yeast growth in bakery products. In a prolonged processing time for bread production the yeast cells will consume reducing sugars and asparagine and thereby reduce the acrylamide formation upon heating
Sweetening	During storage of potato at temperature below 8-10C there is an increase of reducing sugars known as low temperature sweetening due to biological activity in the potato. At higher temperature less reducing sugars are accumulated but potatoes will sprout.
Sprout inhibitor	Approved chemical compounds that are added to hinder sprouting (growing) of the potato when long time stored at temperatures at 8ºC and above.
Reconditioning	Potatoes that have been stored at low temperatures can be stimulated to lower their sugar content by short storage (some weeks) at higher temperature (15 C). This works in some cases.
Bioactivity	This relates to a substance that has an effect on living tissue. In can be due to a reaction with molecules such as haemoglobin in blood or the genetic material. The reactions may be harmful.
Metabolism Metabolites	Chemical constituents (food as well as chemicals) are modified in the organisms, often through a series of enzyme reactions (bio transformation, into metabolites to gain energy or facilitate excretion or detoxification. Some metabolites formed may be more toxic than the starting point.
Glycidamide	A metabolite of AA that can react with DNA or haemoglobin.
Biomarker	A compound that can demonstrate an exposure to a certain compound. It can be the external compound itself or a compound derived from this after metabolism in the body. Related to acrylamide it can be glycidamide or a reaction product between any AA metabolite and constituents of the tissue. A biomarker can be used to determine AA exposure.
Adduct	A molecule formed when AA or an AA metabolite reacts (adding) to a molecule in the tissue.
Short/long time exposure	Long time AA exposure can be calculated from metabolites in the urine whereas long time exposure can be monitored by determining reactions products with blood constituents (Hb-adducts).
Toxicity	How harmful a certain compound is for the organism.
Risk	Can only be determined when exposure, bioavailability and toxicity are known.

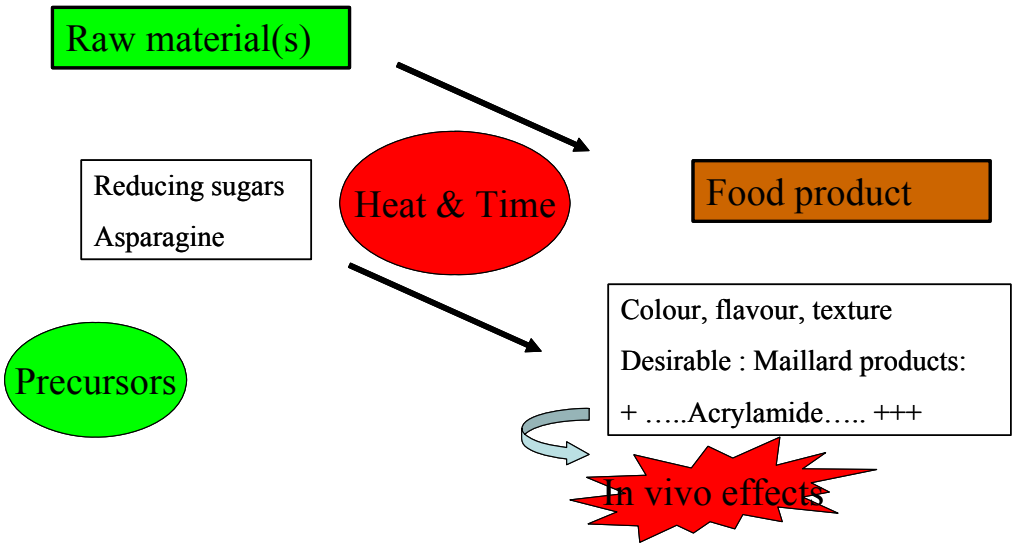

Figure 2 A schematic overview of the formation.

Results and comments related to the different work packages

The project was organized in different Work packages as outlined previously. The main findings as well as the main results related to NORDACRYL are summarized as a whole. When some parts are supplied mainly from a specific partner this is denoted where appropriate. For more details please confer to the list of "*Scientific publications related to acrylamide from the project participants* of NORDACRYL".

WP1 Raw materials

General aspects

During the last years the world wide research related to acrylamide has supplied us with methods and strategies to minimize the formation by adjusting the processes in which food stuffs (i.e. raw materials) are being heat treated. One of the absolute most talented strategies to reduce the amount of acrylamide in food products is to reduce the contents of precursors (e.g. asparagine, reducing sugars) in those raw materials in use and that are practical available in sufficient amounts for industrial uses.

For the Nordic region choosing the optimal cultivars (for the Nordic climate) must be defined and most probably modifications of growth and storage conditions must be undertaken. It might be possible to select varieties that can grow in the Nordic climate without excess accumulation of the acrylamide precursor molecules. It must be noted that this will involve long conventional breeding programmes, especially since GMO technology is not suitable at present. At the same time ways to mitigate the limiting substrates in the raw materials must be known. In the two tables below typical values for precursors in potato and cereals are given.

PRECURSORS IN CEREALS:				
Place etc.	milling fraction (n)	asparagine (range) g/kg dw	glucose (range) g/kg dw	REFERENCE
	WHEAT:			
Europe	whole grain (45)	0.07-0.66		[13]
Sweden	whole grain flour (2)	0.48-0.51		[14]
Sweden	sifted flour (2)	0,14-0,17		[14]
Sweden	bran	1.48		[14]
Sweden	germ (2)	4.88-4.99		[14]
Sweden,	flour Bagarns Bästa,	0.17	0.13	[15]
Europe	flour-whole grain	0.07-0.79		[16]
Switzerland	flour	0.14	0.3	[17]
Britain	flour	0.17	0.54	[18]
Germany	flour (9) 2003-4	0.05-0.25		[19]
Germany	flour (10)	0.15.0.4		[20]
	RYE:			
Sweden	whole grain flour	1.07		[14]
Sweden	sifted flour (2)	0.53-0.68		[14]
Sweden	bran (2)	2.61-3.18		[14]"
Germany	flour (3)2003-4	0.41-0.49		[19]
Britain	flour	0.63	2.1	[18]
	SPELT:			
Germany	flour (2) 2003-4	0.06-0.12		[19]

Table 2 Contents of the precursors asparagine and glucose in cereal material. Supplied by Kit Granby.

Place and time	varieties (n)/ total (n)	asparagine (g/kg ww)	glucose (g/kg ww)	REFERENCE
Sweden 1989, storage all year at 3°C	8/72	0.6-1.4	0.2-5.0	[21]
Sweden 1990, storage all year at 3°C	8/72	0.6-3.0	0.2-5.4	[21]
Sweden 1991, storage all year at 3°C	8/72	0.8-3.2	0.2-4.0	[21]
Sweden 1989-91, storage all year 10°C	mean of 150-200	1.3	0.4	[21]
Denmark 2004 stored all year (ambient temp.>0°C)	2/66	1.2-2.9	1.3-11	Granby, unpublished results
Denmark 2000, storage all year at 8.5°C	1/>20	1.0-3.0	0.1-0.6	[22]
Belgium 2003, stored 6 month at 4°C	3/3	3.9-4.2	1.5-2.4	[23]
Belgium 2003, stored 8 month at 8°C	16/64	1.7-3.6	0.09-1.55 (glu+fruct)	[24]
Switzerland 2002 sampled from market	17/74	2.0-4.3	0.1-2.6	[25]
Switzerland 2003 stored 6-10 weeks at 4°C	12/12		2.7-14 (glu+fruct)	[20]
Switzerland 2003 stored until Nov-Dec at 9°C	14/47	2.5-4.4	0.2-6.7 (glu+fruct)	[26]

Table 3 Contents of the precursors asparagine and glucose in potatoes. Supplied by Kit Granby.

In a separate chapter at the end "*Acrylamide-Precursors – The limiting substrates in the raw materials for acrylamide formation in potato and cereal products*" the status of our knowledge on limiting substrates in potato and cereals raw materials is presented.

Choice of Nordic varieties-potatoes by K. Olsson

Potato:
One aim of the NORDACRYL-project was to analyse potato varieties, which already are commercially grown in the Nordic countries or new varieties, which are under evaluation in official trials for this purpose. Since the potato industry also has the possibilities of importing potato from Europe some additional information is supplied. Some varieties are the preferred choice for French fries such as Asterix and Bintje whereas others are the mostly used for potato crisps such as Saturna and Hulda. The following 13 varieties and breeding clones were included in different trials coordinated by Kerstin Olsson at SW/SLU-Alnarp. Results of the different trials are given in different sections below.

	Origin	Table potato	French fries	Crisps	N-fertilisa-tion trials	Storage trials	Ring-test for sugar analysis
Agria	DE	x	x				x
Asterix	NL	x	x		x	x	
Bintje	NL	x	x		x	x	x
Ditta	AU	x				x	
Fontane	NL	x				x	
Hulda	SW			x		x	
Lady Rosetta	NL			x		x	x
Liva	DK			x			x
Princess		x			x		
Saturna	NL			x		x	x
Superb	SW	x			x	x	
SW 90 102	SW			x		x	x
SW 94 1307	SW	x			x		

Table 4 Potato varieties subjected to various investigations.

Content of limiting substrates

Influence of potato genotype on acrylamide precursors

The levels of acrylamide that can be found in industrially prepared potato crisps and French fries or home made fried potato are related to the amounts of reducing sugars and free asparagine in raw potato. The genotype of the potato has great influence on the content of these substances. In the NORDACRYL-project several potato varieties have been analysed and the precursors showed large differences between genotypes. Thus one way of reducing the acrylamide level in the fried product is to choose varieties with low levels of the precursors throughout storage. Figure 3 represents varieties for industrial crisp production and Bintje, which is often used for making French fries. In this trial the reducing sugars in the crisp varieties varied between 9 and 14 mg/g dry matter (dm) and asparagine between 5 and 14 mg/g dm while the levels in Bintje were 21 and 8 mg/g respectively.

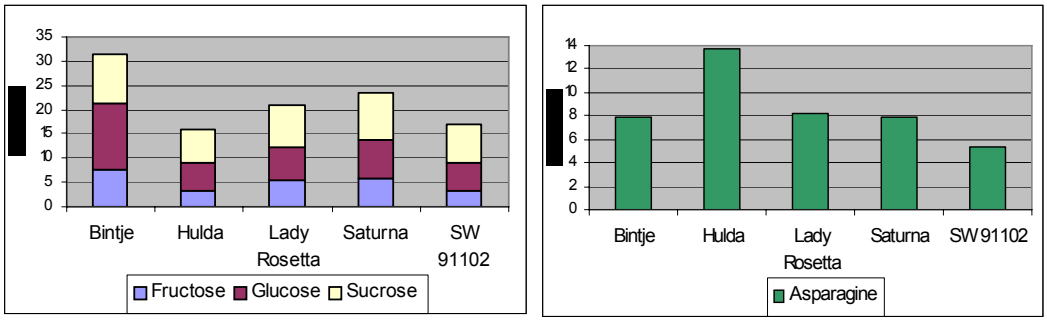

Figure 3Fructose, glucose, sucrose and asparagine, expressed as mg g-1 dm, in Bintje and 4 potato clones used for crisp production. Average content during storage at 4ºC from October to June.

Ordinary household potatoes have traditionally not been demanded to have low reducing sugar content like the crisp potatoes, which should give a golden yellow fried product. In figure 4 the sugar and asparagine levels are shown for Bintje and some new market varieties aimed for ordinary household cooking and frying. The sum of fructose and glucose here varied from 22 to 52 mg/g dm and asparagine from 9 to 11.5 mg/g dm.

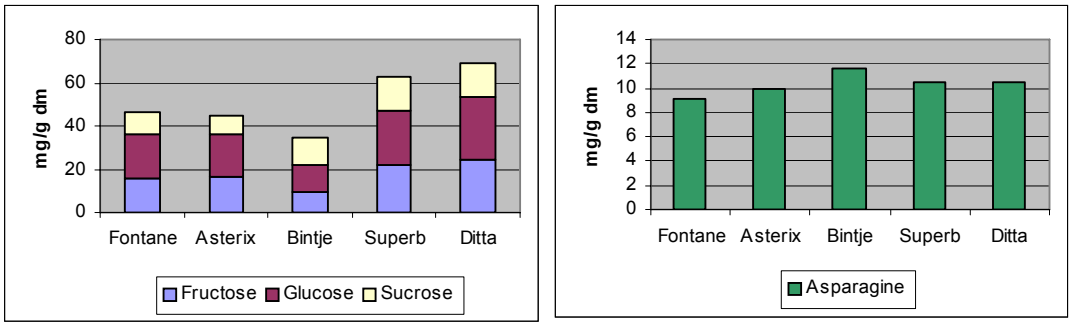

Figure 4 Fructose, glucose and sucrose in potato clones, expressed as mg g-1 dm, in 5 potato varieties used in ordinary household preparation. Average content during storage at 4ºC from October to November.

Influence of storage length and temperature on acrylamide precursors

Storage has great influence on the precursor levels. At 4ºC the reducing sugars, glucose and fructose, markedly increased from November until December but the genotypes did not reach their peak values until March. There was a slight decrease in the levels between March and May. Bintje had the highest reducing sugar levels during the whole storage season. Among the crisp varieties, Saturna and Lady Rosetta showed higher levels than Hulda and SW 91102, which kept below 10 mg/g dm during most of the storage period (fig. 5).

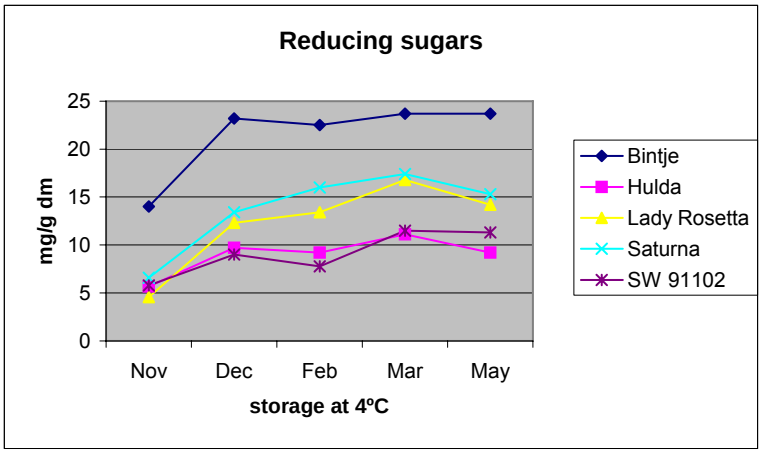

Figure 5 Changes in reducing sugar contents in 5 potato varieties during storage at 4ºC.

At 8ºC the reducing sugar levels were much lower than at 4ºC and all three crisp varieties kept below 10 mg/g dm (fig. 6). The fluctuations were also smaller. There was, however, an increase between December and February followed by a decrease during the last months. Bintje had the highest and SW 91102 the lowest levels throughout storage. Hulda is missing due to lack of material.

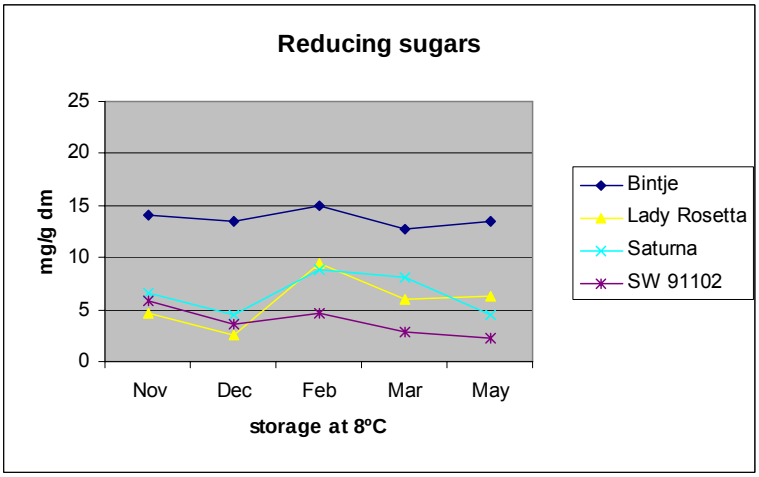

Figure 6 Changes in reducing sugar contents in 4 potato varieties (Hulda is missing) during storage at 8ºC with sprout inhibitor.

The potato clones differed greatly in asparagine levels. Only Hulda showed a clear increase during storage at 4ºC (fig. 7). The storage temperature did not influence on the levels. The fluctuations were also small at 8ºC (fig. 8). Here Hulda is missing because of lack of material.

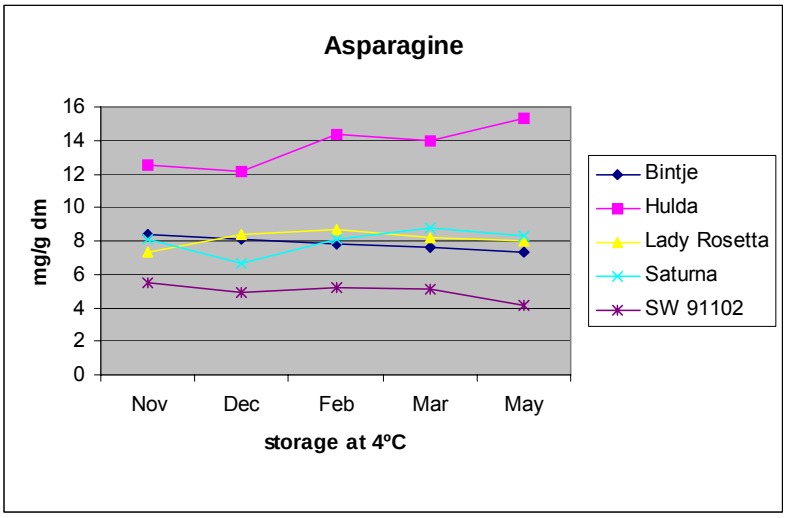

Figure 7 Changes in asparagine contents in 5 potato varieties during storage at 4ºC.

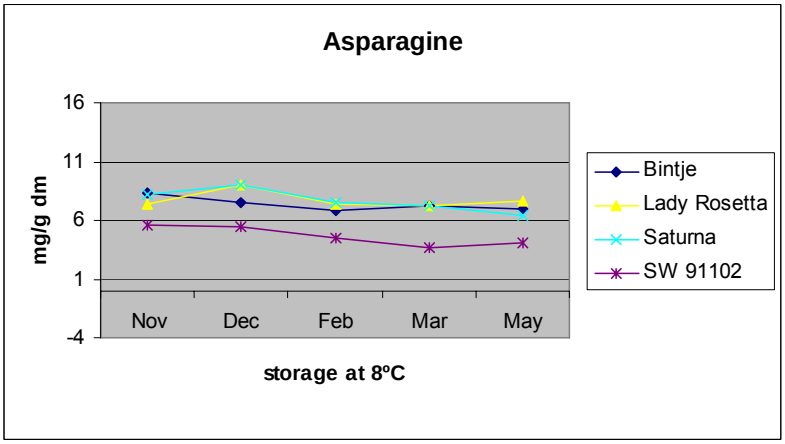

Figure 8 Changes in asparagine contents in 5 potato varieties during storage at 8ºC with sprout inhibitor.

Influence of growing site and N-fertilization on acrylamide precursors

The growing sites Ballingslöv, west of Kristianstad and Götala, east of Gothenburg, differed in both soil and weather conditions. This may have an effect on the physiological age of the tubers at harvest time, which influences the levels of for example sugars and starch. At Götala the contents of reducing sugars were 3-4 times higher than at Ballingslöv in all varieties except Princess where the increase was less pronounced (fig. 9). There were, however, no significant differences in asparagine between the two sites (fig. 10).
The N- regimes did not influence on the levels of reducing sugars nor of asparagine. It was expected that the higher N-supply would increase the asparagine level but the difference between 60 and 120 kg N/ha was probably too small to give effect.

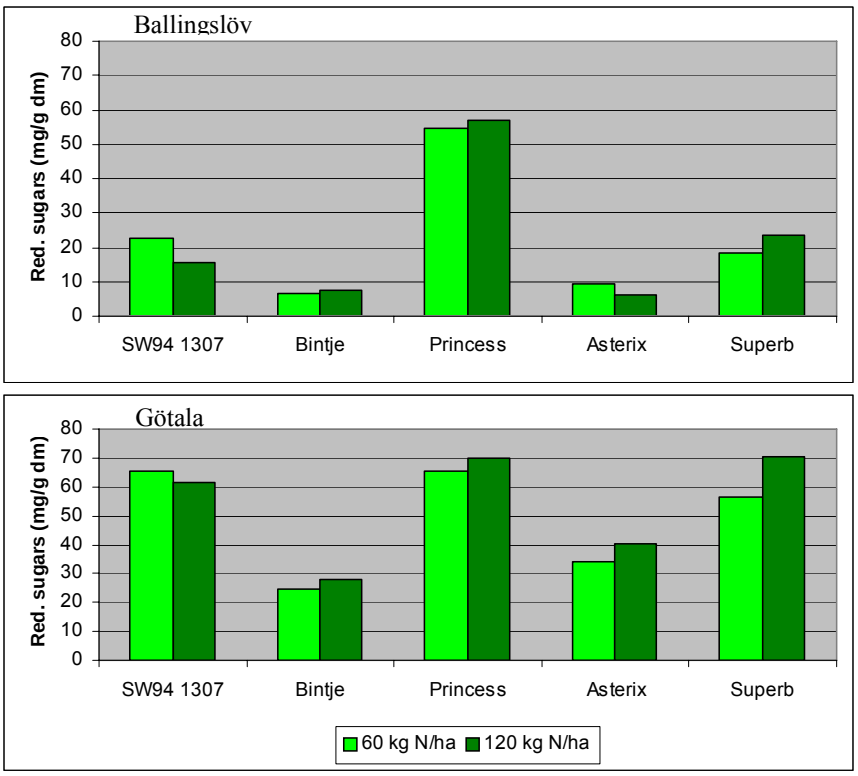

Figure 9 Reducing sugars (fructose and glucose), expressed as mg g-1 dm, in one breeding clone and 4 potato varieties grown at two different sites, Ballingslöv and Götala, and at two different nitrogen supplies, 60 and 120 kg N ha-1.

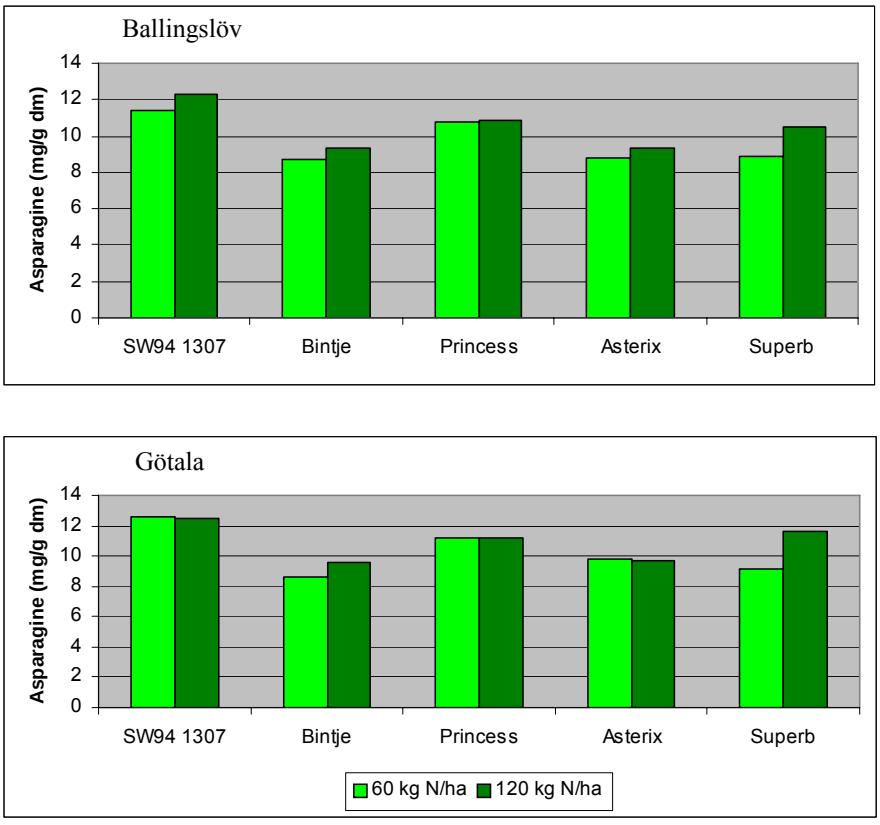

Figure 10 Asparagine, expressed as mg g-1 dm, in one breeding clone and 4 potato varieties grown at two different sites, Ballingslöv and Götala, and at two different nitrogen supplies, 60 and 120 kg N ha^{-1}.

For potatoes there are clear results showing that the content of reducing sugars accumulate during storage. This is most prominent for the conventional optimal storage temperature (4°C) where sprout inhibitors can be avoided. This will give rise to seasonal variation in the final industrial potato based products since potatoes have to be stored during the year.
Although asparagine differs markedly between different varieties there are no clear storage effects or other growth induced effects on this precursor. For potatoes the limiting substrate is therefore reducing sugars.

Choice of Nordic varieties-cereals

Some information related to the precursor content in cereals is given towards the end of this report " Acrylamide -Precursors..". Some batches of rye samples supplied by Lantmannen (Cerealia) were supplied for analysis. As can bee seen the variation in asparagine content is relatively small.

Origin, variety, and harvest year	Asn (g(kg)	Origin, variety, and harvest year	Asn (g(kg)
Dk Avanti 2003	0,99	S Amilo 2003 Haga	0,87
Dk Hacada 1	1,13	S Amilo 2003 Kölbäck	0,91
Dk Hacada 2	1,10	S Amilo 2003 Landskrona	0,87
Dk Picasso 2003	1,26	S Esprit 2003 Bjertorp	1,05
N Darro 2003 Dilling	1,07	S Esprit 2003 Haga	1,03
N Darro 2003 Moss 2	0,96	S Esprit 2003 Kölbäck	0,94
N Darro 2003 Moss1	1,05	S Esprit 2003 Landskrona	0,95
N Darro 2003 Rygge	0,80	S Malmö 2003 1	0,92
N Darro 2003 Råde1	1,01	S Malmö 2003 2	0,95
N Darro 2003 Råde2	0,88	S Malmö 2003 3	0,95
N Darro 2003 Son	1,03	S Mjölby 2003 1	1,14
N Råg 2003 NM	0,62	S Mjölby 2003 2	0,93
S Amilo 2000 Landskrona	1,12	S Mjölby 2003 3	0,92
S Amilo 2001 Landskrona	1,12	S Uppsala 2002	0,90
S Amilo 2002 Landskrona	1,09	S Uppsala 2003 1	0,95
S Amilo 2003 Bjertorp	1,02	S Uppsala 2003 2	0,85

Table 5 The content of asparagine (Asn) in stored rye samples from Sweden (S), Norway (N) and Denmark (Dk) Data supplied by H. Fredriksson.

In general the content of asparagine is higher in whole grain flour than in the sifted fractions. Therefore whole grain products may contain higher content of AA. For some typical Swedish bakery fractions see below.

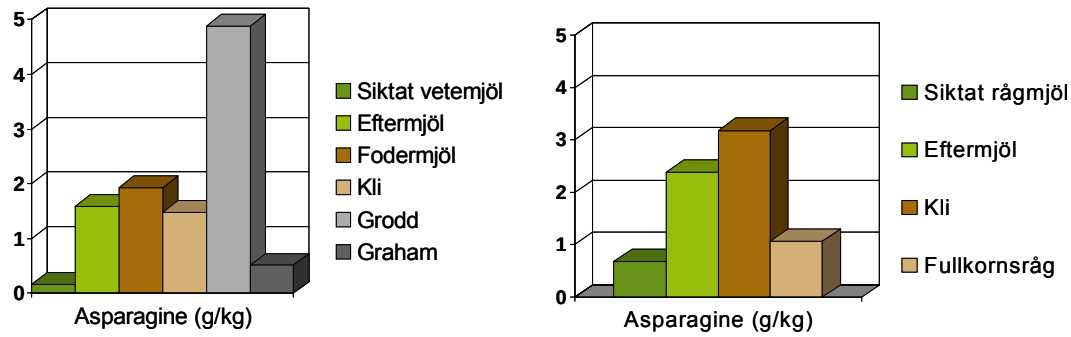

Figure 11 Asparagine content in fractions of rye and wheat. Data supplied by H. Fredriksson.

WP2 Productions

Model systems cereals

In order to study the essential parameters for the acrylamide formation in cereals a starch based model was developed at Matforsk. Starch solutions, added different constituents for testing, were gelatinized, freeze dried and subjected to baking under various conditions (please see our published works [27, 28]. Parallel experiments with crisp bread (Flatbrød) based on rye, salt and water only, confirmed the relevance of the model.

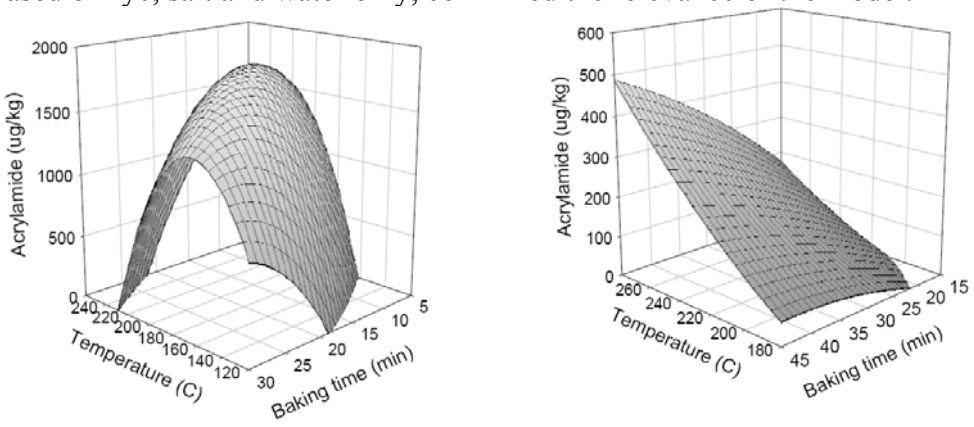

Figure 12 Acrylamide formation in crisp bread (left) and soft bread (right). http://www.if.csic.es/proyectos/cost927/Proceedings-LARNACA.pdf

It was clearly shown that the formation of acrylamide increases with increased time and temperature. For quite dry systems (crisp bread etc) the formation starts at about 120, undergoes a maximum at about 180 °C and levels off at higher temperatures. For common soft bread a temperature for maximum formation is not reached, most probably du to constant cooling and water evaporation from the centre of the breads. The major acrylamide contribution of the latter products is from the crust.

The formation of acrylamide in the bred crust was further studied at SIK, Gøteborg (Hans Lingnert). By advanced sensor technologies parameters such as temperature profiles in inner and outer part of the crust as well as colour, water activity and acrylamide formation were recorded. It was confirmed that the acrylamide formation is most pronounced in the outer part (crust I).

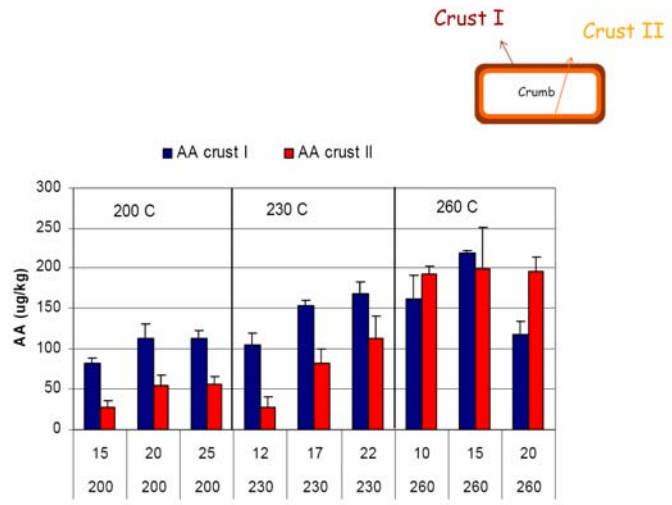

Figure 13 Formation of acrylamide in two parts of the bread crust at different time (10-25 min) and temperature (200-260C). For details see Ahrne et al. [29].

During the initial experiments we were not able to detect a relationship between reducing sugars in the starting cereal material and the potential for acrylamide formation. However, by increasing the asparagine content in the dough a linear relationship was demonstrated in Fig. 14. Indeed asparagine is the limiting substrate for acrylamide formation in cereals.

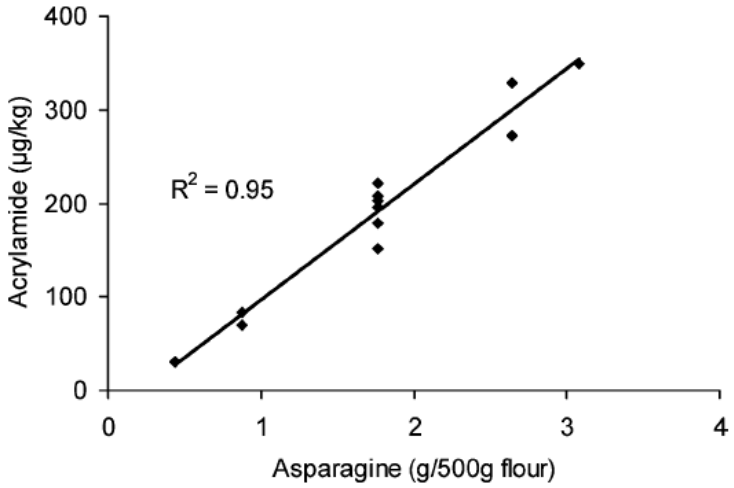

Figure 14 Relationship between acrylamide content in rye crisp bread and added asparagine. Breads were baked at 250ºC for 8 minutes. For details [30].

Model systems potato

For potato the content of acrylamide is as noted heavily linked to the content of reducing sugars. There are no solid evidences that the oil (source or effect of repeated frying) influence the acrylamide formation. The frying conditions (heat, time) are important parameters as well as the final dry matter content of the products. A quite dramatic increase of acrylamide is encountered when arriving at approximately 98% dry matter content and beyond (see figure below)

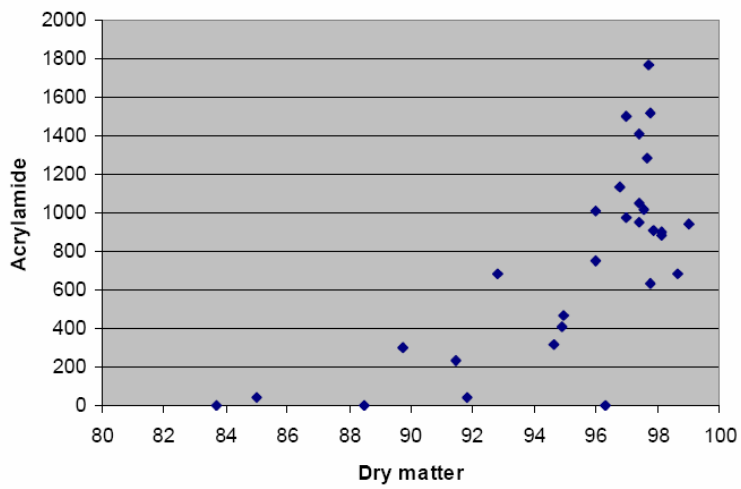

Figure 15 Acrylamide in potato crisps fried to different dry matter content (S.H. Kntsen, unpublished results).

Then it is evident that minute variations in process parameters may have large effect on the formation. In addition to the varying and sometimes uncontrollable sugar content, this is an additional obstacle to understand acrylamide formation. Anyway, careful designed experiments for crisp and French fries products were performed in order to study effect of process parameters and precursor contents in potato (se also *pilot scale* experiments) [31-35]. A model experiment that was useful to demonstrate the complexity of the "potato "system" was based on freeze dried potato materials and oil fried, for constant time and temperature in an oven.

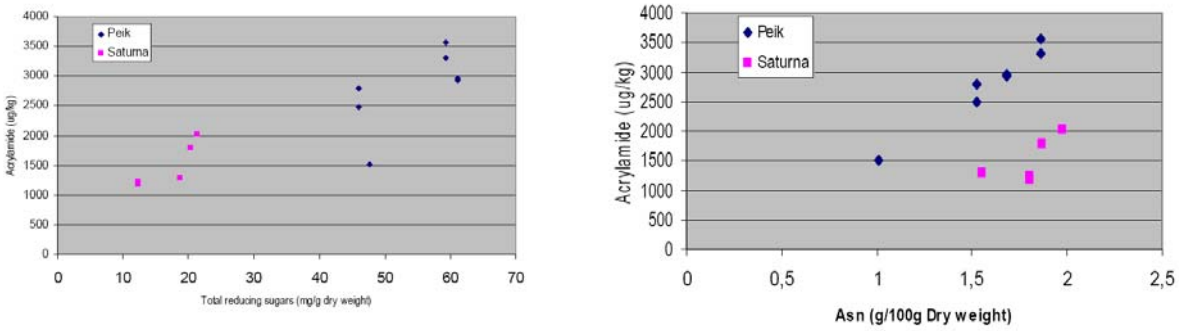

Figure 16 Relationship between precursors and acrylamide formation in unstored potato (S.H. Kntsen, unpublished results).

From dried potato powders obtained from field and storage experiments in Norway it was evident that at time of harvest there was a correlation between acrylamide formation and the content of both reducing sugars and asparagine in the varieties Peik and Saturna. However upon storage the correlation became less evident for reducing sugars and not significant for asparagine. It was suggested that acrylamide might partly due to other pathways than from the established precursors.

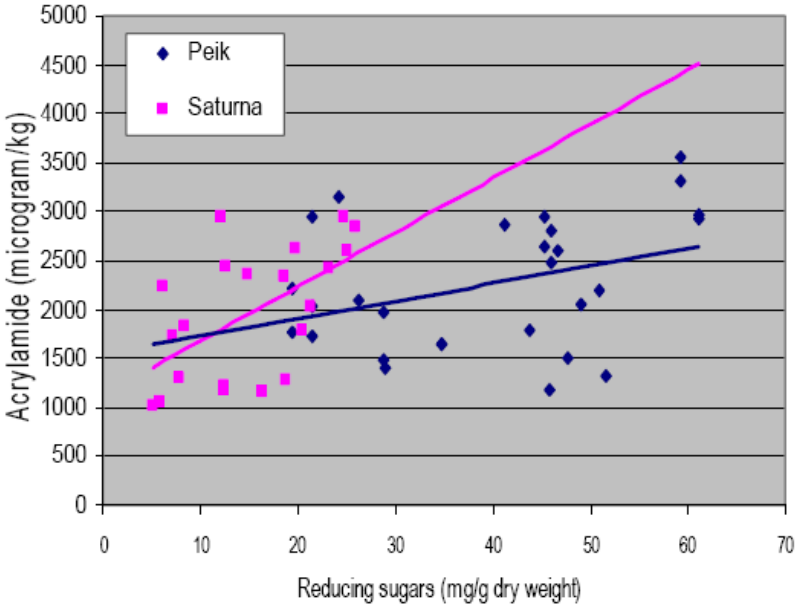

Figure 17 Relationship between acrylamide and reducing sugars in stored Peik and Saturna (S.H. Kntsen, unpublished results).

Pilot scale

Cereal products-baking technology

At SIK alternative baking technologies for bread has been studied. Innovative pilot scale baking by using steam, heating with an infrared source (IR-bak=IR) or high velocity vapour (Impingement baking [IMP]) have been performed. These has been used separately or in combination (IR/IMP) and compared with traditional baking, carefully monitored by sensor technology as noted above.

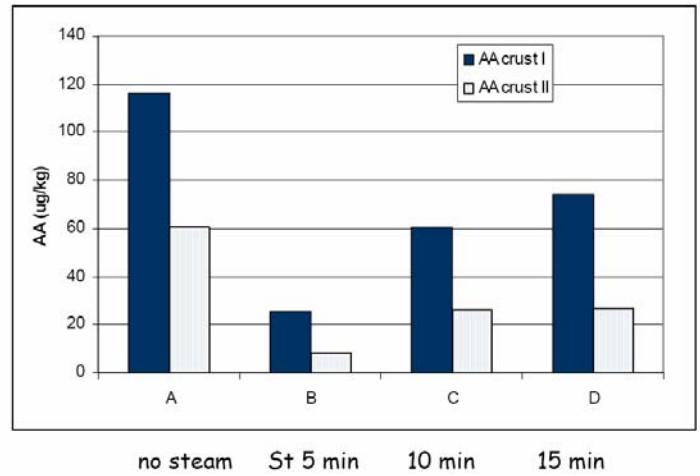

Figure 18 Traditional baking with Steam (*H. Lingnert, unpublished*).

By controlling the steam inlet it was possible to optimize the procedure (15 min) with a marked reduction in AA in the crust but no significant changes in the sensory characteristics compared to traditional baking.

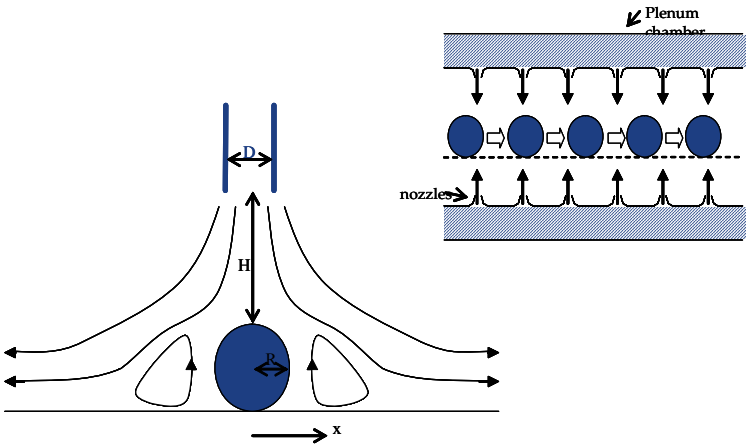

Figure 19 Equipment for impingement baking.

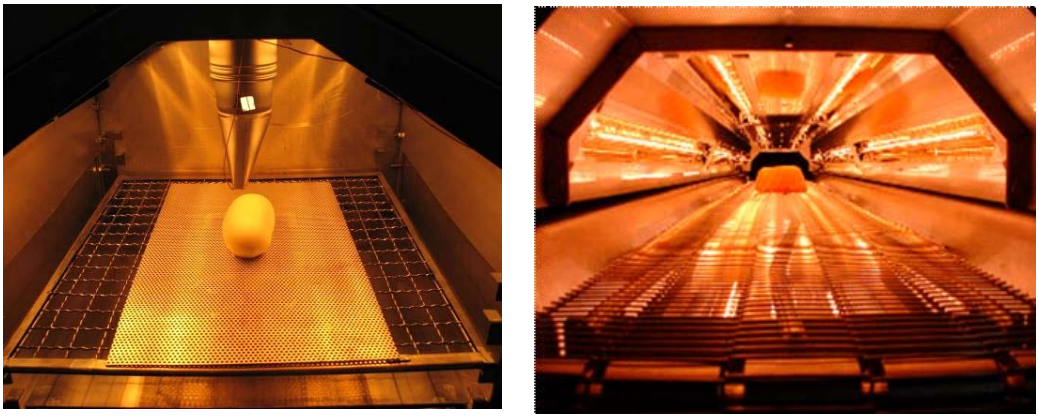

Figure 20 IR baking (left) and combined with impingement methodology (right). *H. Lingnert-unpublished*.

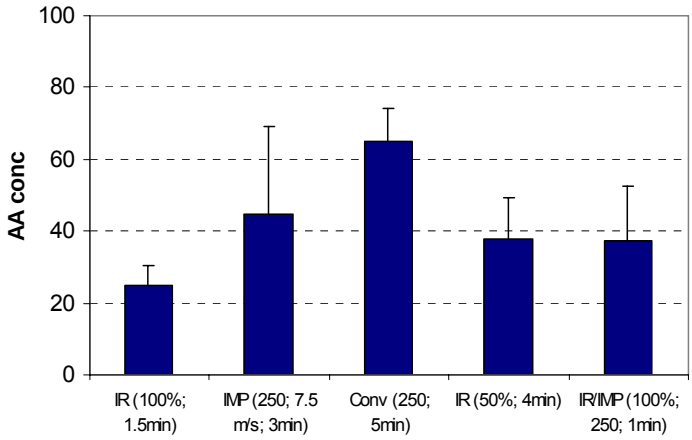

Figure 21 Reduced acrylamide by new baking methods at SIK (H. Lingnert, unpublished results)

These new methodologies give promising results and further work will be undertaken at SIK.

Cereal products- influence of asparagine/asparaginase

As noted in "model systems cereals" the key parameter for AA formation in bread is asparagine (i.e. the *limiting substrate*). In pilot scale bread production performed at Matforsk it was shown that it was possible to reduce asparagine content by transforming it into aspartic acid. The resulting breads had marked lower content of AA. This is simply an enzymatic de-amination of this common amino acid by a, at that time, laboratory scale enzyme from SIGMA chemicals. At the same time it was also found that increasing the content of reducing sugars by other enzymes (amylase or hydrolases) or adding sugars had no significant effect.

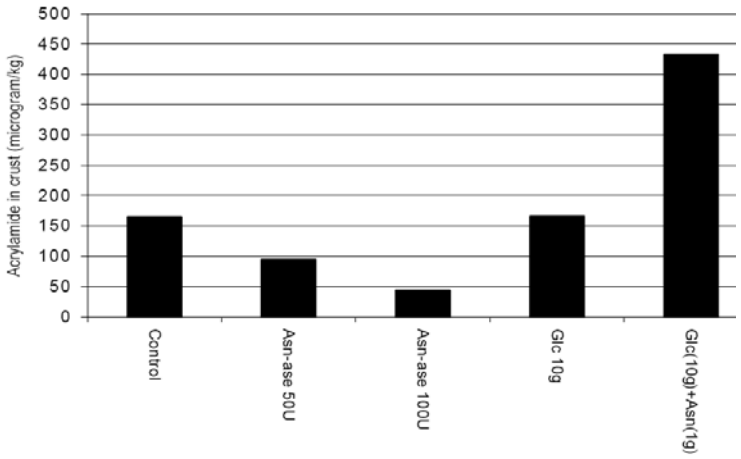

Figure 22 AA content in the crust of breads added asparaginase (50 or 100 units) (Asn-ase), Glucose (Glc) and asparagine (Asn) http://www.if.csic.es/proyectos/cost927/Proceedings-LARNACA.pdf.

This innovative use of enzyme was later repeated in Norwegian cereal based industries at full scale both for bread and crisp bread in collaboration between Matforsk, NORDACRYL and a larger project founded by the Norwegian Research Council (NFR). In NORDACRYL this methodology was used in full scale at WASA for crisp bread (see other part "Asparaginase in crisp bread production at WASA"). As noted the use of asparaginase has minor effects on sensory properties of the products and is very promising for several cereal based products. However, some fine-tuning of the procedures remains to be performed. In all industrial application industrial grade asparaginase enzyme was supplied by NOVOZYMES.

In addition it was demonstrated in Sweden (SLU and Lantmannen -Cerealia) that the content of asparagine, and hence AA-formation, can be reduced by prolonged conventional yeast fermentation. Some work remains to establish the optimum conditions but this is also a promising mitigation strategy for certain products.

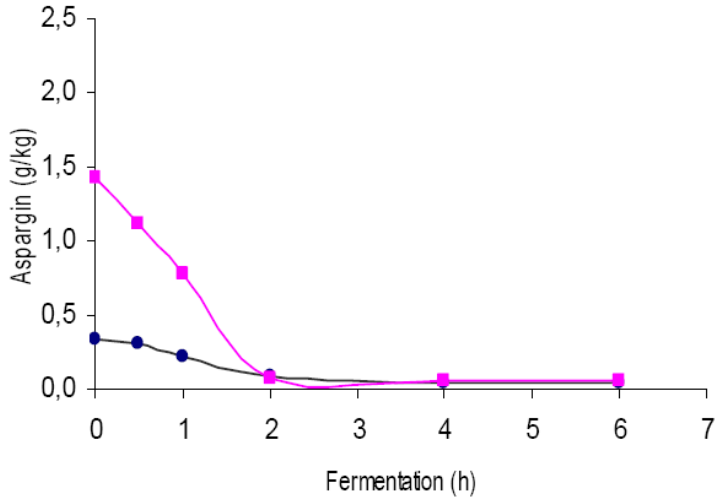

Figure 23 Asparagine is consumed during yeast fermentation of a hveat: graham; 50:50 dough (bottom blue) and wheat: rye bran, 50:50 (upper red). For details see [14].

At present the effect of the use of sour dough is still under investigation. Some initial experiments indicated that this process induced higher content of AA in the final products.

Cereal products- influence adding glycine

Among other amino compounds adding the common natural occurring amino acid glycine was found to reduce the AA formation in several cereal products [28, 31, 36]. One tentative explanation is the effect of a pathway including glycine competing with asparagine in the initial Maillard reactions and hence inhibits acrylamide formation. This has also been found by different workers in Europe and US. This "glycine-methodology" still need some improvements for certain products such as common bread due to negative impact on taste. Addition of glycine has been restricted by only spraying a solution on outer part subjected to crust formation during baking [36]. This involves much less glycine and has minor effect on sensory properties. In fact a consumer test recently performed at Matforsk revealed that "glycine" bread or crisp bread was fully accepted (unpublished ongoing research).

Pilot scale Potato products

In Denmark Granby and co-workers undertook a large investigation in order to monitor the change in precursor content of stored potatoes and the final content of acrylamide in prepared French fries. The results show that the acrylamide content is influenced by the reducing sugar content (fructose is not shown but undergoes a similar variation). However, it can bee seen, comparing Bintje and Sava that acrylamide formation is not strictly coupled to the amounts of precursors only.

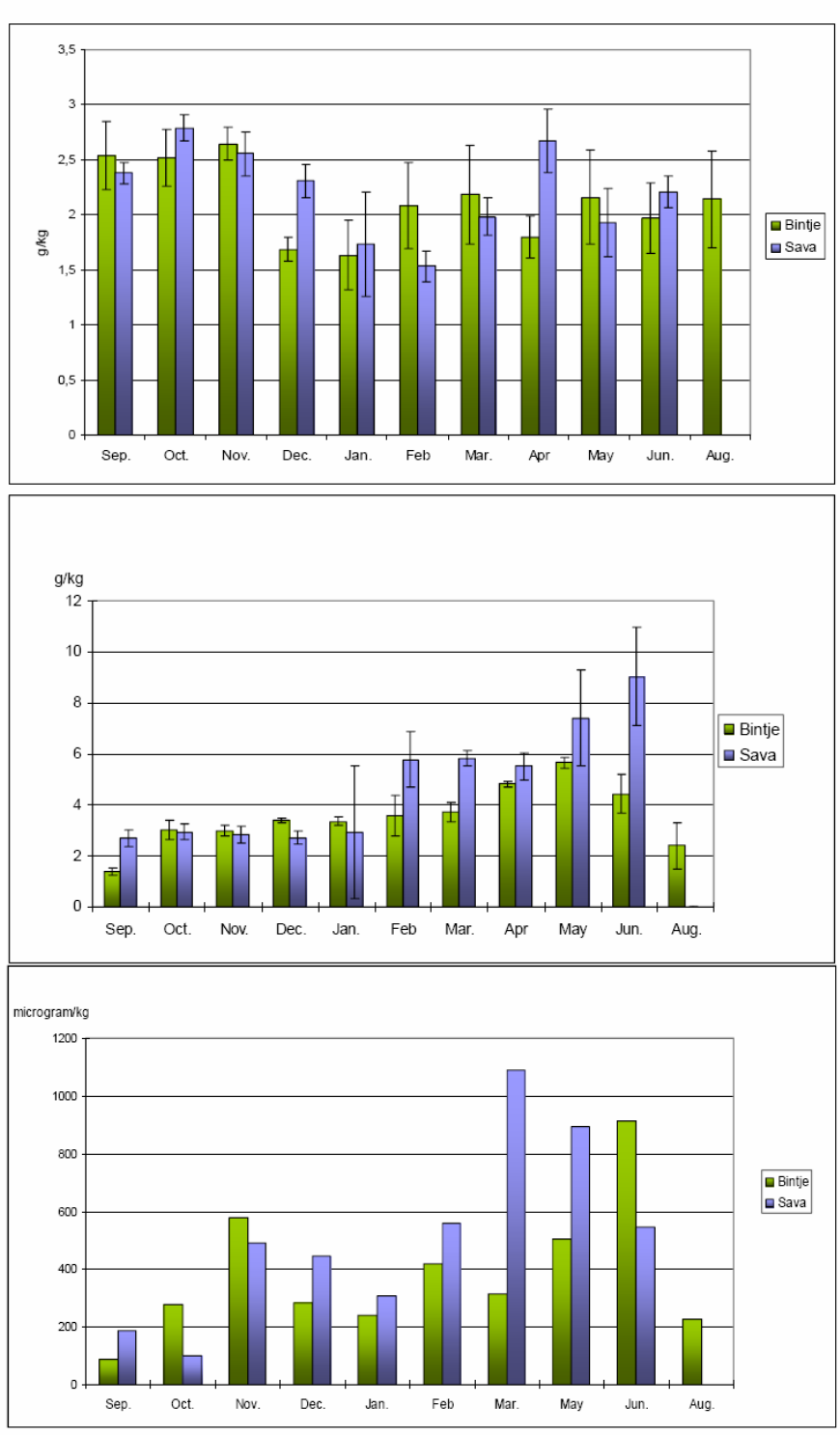

Figure 24 Monthly variation in asparagine (upper) glucose and final acrylamide content (bottom) in industrial scale prepared French fries.

Potato products-effect of amino acids and other acids

As for cereals removing, modifying or introducing a competitor to asparagine will have an effect on acrylamide formation during frying. At Matforsk it was shown that blanching in a solution containing glycine or glutamine (another amino acid that will compete with asparagine) reduced the content of acrylamide in crisps by 30%[28]. Additional reductions were obtained by immersing in acetic acid solutions (90%) soaking or blanching in citric acid solution (50%) [31]. Again some sensory effects were recorded and the procedures have to be

corrected to each different product and most probably modified according to the available raw material at the time of processing.

Potato products-effect salt solutions and other additions

The effects of acidic solutions are attributed to protonation of the amino group of asparagine and thereby reducing its reactivity towards acrylamide. Also dipping in salt solutions of NaCl [37]or $CaCl_2$ [38] has been applied by different workers indicating mitigation effects. In a collaboration between GRO industries UMB and Matforsk a $CaCl_2$ dipping step during production of French fries gave products with lower content of AA (unpublished results). A fully reasonable explanation has not been given, but at least the effect of Ca^{2+} might be linked to interaction with pectin in the cell wall of potato. However, since the effect of $CaCl_2$ is large in simple model systems containing asparagine and sugars[38], other completely different mechanisms must be important. If the mitigation effect of $CaCl_2$ can be understood in such systems this might also have an implication for cereal based systems.

Potato products-effect of the use of asparaginase

Some trials have been undertaken to study the use of asparaginase in potato systems. It is evident that the delivery of an enzyme into the potato tissue is a challenging task. However, immersing potato slices in an asparaginase solution at 40ºC has indicated that uses of this enzymic approach might have some effect in special products (Granby, Pedreschi et al., unpublished works). Collaboration with enzyme producers (NOVOZYMES), researchers, industry and equipment producers, such as PPM, is crucial in order to gain knowledge and practical solutions regarding this approach.

Commercial production

During the commercial production of potato based products such as crisps and French fries the problems associated with the variability of the raw materials (*i.e. Potato*) have to be dealt with. The access to large scale facilities for higher temperature storage promoting lower sugar contents varies. The problems related to sugar accumulation is at largest for production in the spring based on stored potatoes. The suitability of a potato batch for frying is commonly determined by use of a “frying test”. Although some feasible methods for sugar analysis (see “analysis”) are at hand the problems of representative samplings are considerable and therefore the “fry test” is preferred. Due to the variability in sugar content between seasons and within seasons the acrylamide content in potato products show an annual variation. In general at present the industries focus on removal of dark products in their production and control of the frying processes.

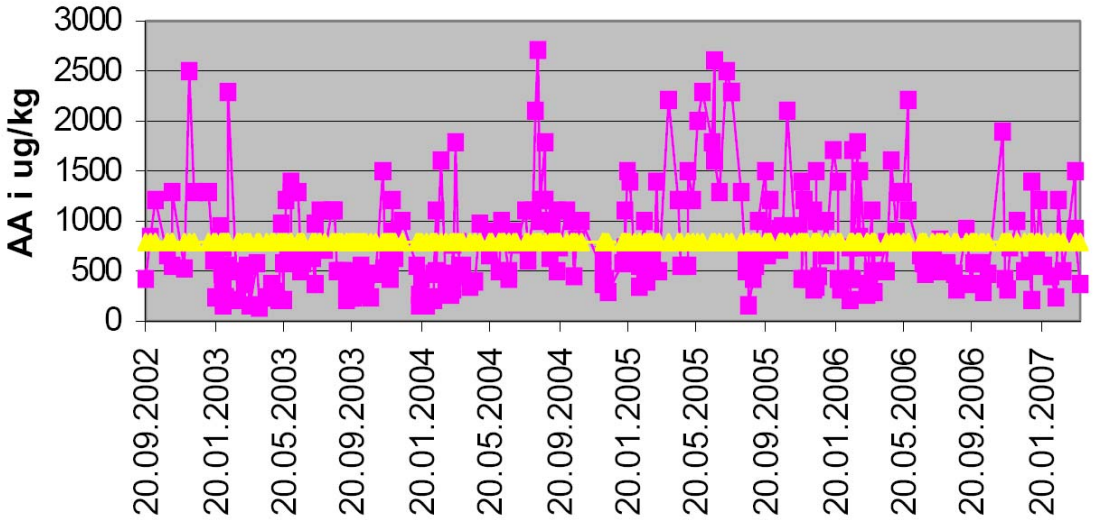

Figure 25 Annual variation in a typical potato crisp products

The method used to reduce the content of the precursors is the procedure known as blanching. Here the potato material (after cutting to the appropriate size and geometry) is subjected to a hot water treatment (80°C) for some minutes and parts of the sugars in the outer parts are removed. This might also apply to asparagine but effect of this is less known. Earlier this method was used to control browning of the products. For some products sugars were even added in the blanching water, but at present, due to AA formation this is avoided. Today most production lines have one or more blanching steps.

Apart from the content of reducing sugar the frying conditions are crucial for the formation of acrylamide. Some reduction in frying temperature and a slightly higher water content in the final products will contribute to lowering the acrylamide values. However, this might influence sensory properties and storage stability. For the fundamental aspects of crisp productions some diagrams supplied from PPM are enclosed below.

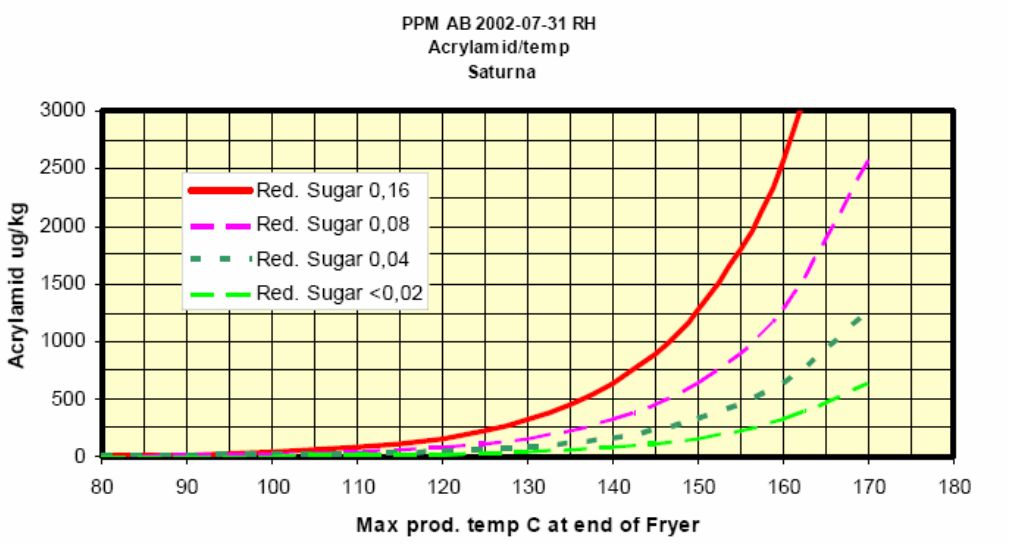

Figure 26 Temperature profile in fryer and acrylamide formation. Supplied from R. Haraldsson, PPM).

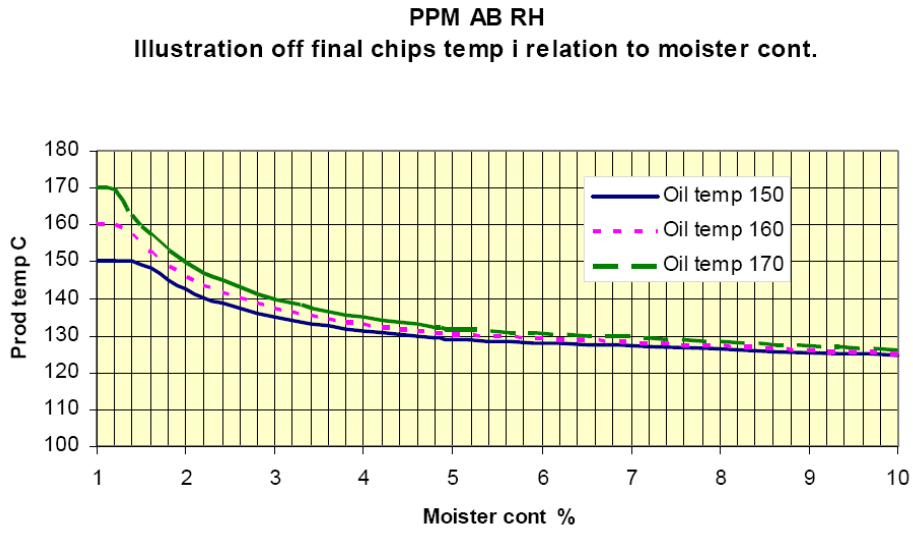

Figure 27 Final moisture content and final frying temperature for potato crisps.

PPM - the food industry and NORDACRYL

PPM is a supplier of machinery to the food industry and have special interests and experiences related to deep fried potato products such as crisps (chips) and French fries (pommes frites). The contacts established within NORDACRYL are considered essential in our efforts to make machinery to meet the demands of the food industry, which even includes the option of lowest possible contents of acrylamide. The contacts established in this network and the fruitful discussions during project meetings have been very useful for future planning and innovations regarding our new equipments.

Our contribution to the project has mostly been to communicate our experiences and practical knowledge obtained in process lines produced by PPM and given the opportunities to perform experiments in our laboratory and pilot equipments. During full scale trials at KIMS Norge we have been part of planning of experiments and tuning of the process parameters.

a) Post drying of semi fried chips by utilizing the PPM de-fatting equipment based on superheated steam
b) Chips with somewhat higher water content have been produced in the KIMS factory in Norway on 1500 kg/h line for a later post drying at MATFORSK

Some changes in taste and texture compared to the standard products were noticed.

PPM has investigated the future possibilities in utilizing asparaginase in crisp production, held meetings with NOVOZYMES and performed certain immersing tests. A practical problem that has to be solved for traditional chips will be the prolonged time for treatment in the enzyme solution (20 min, 40C) compared to conventional blanching.

PPM is also a supplier of blanching equipments and a system for rapid dipping of sliced potatoes between blanching and deep frying. Such equipments have now been installed in several of our production lines.

Blanching in PPM type MTB-blancher

Our MTB blancher is at present more and more frequently used by our customers, not only for colour adjustments but more important as a device to reduce acrylamide formation. A blanching in water for 3 min at 80 °C, with a simultaneous wash out of reducing sugars has contributed to a reduction in the acrylamide content in the average range of 30-40%. This

numbers are valid in Scandinavia. It is important to note that there is a large variation in the degree of acrylamide reduction related to the geographical location. By a South European customer only a small reduction in acrylamide was achieved.

Addition of NaCl and $CaCl_2$

The addition of salt in the water solutions change the osmotic potential and in order to establish equilibrium the water is transported out of the potato whereas the salt will move in. It is reasonable to suggest that the salt and the simultaneous wash out of reducing sugars and low molecular weight compounds raise the overall dry matter content. This will change the frying history and the product temperature in the critical phase towards the end frying (se figures 26 and 27 above). In full scale production up to 50% reduction of acrylamide has been recorded when blanching has been performed in water containing 1% table salt. This has again been achieved in Scandinavia. In this context is should be stressed that the later addition of salt during seasoning is therefore reduced, giving products with the same end content of salt.

Calcium salts ($CaCl_2$) has also been utilized, but then in lower concentrations to avoid contribution to taste. Ca^{2+} tends to influence the pectin bindings (possibly cross links) in the cell walls and harder and more brittle chips are obtained. A direct consequence is that the porosity is reduced and hence the fat absorption and the fat content of the final products.

Deep frying, CF model program

An important equipment development has been performed at PPM related to the techniques for deep frying of potato crisps in the CF500 and CF3000 models. These production lines have a production capacity from 500 to 3000 kg/h potato product. The temperature profile has been adjusted towards a high temperature in the beginning and the middle of the frying and a lower ending temperature. This has demanded some improvements of the fat circulation systems.

*CATZ system (**c**old **a**djustable **t**emperature **z**one) system*

This makes it possible to a more accurate temperature reduction in the final frying phase. The system is now the standard in the deep fryers. An additional option is CATZ, with a separate circulation pump, further improves the possibilities for oil flow and means of adjusting the temperature.

Temperature control

Traditionally the oil temperature is regulated after the heat exchanger and this determines the temperature of the inlet oil. The temperature at the end phase in the fryer will be influenced by the temperature difference towards the heather, the water content of the potato and the actual raw material loading. In order to control the acrylamide formation a very constant temperature is aimed at the critical end phase of frying (see figures above). PPM has therefore constructed a"cruiser control" ASTA (auto set point temp adjustment) that keeps the end temperature constant and automatically adjusts the set point of the inlet temperature. This facilitates work for the personnel involved in the frying and reduces the risk of temporary peaks of the temperature in the products, and hence boosts in the acrylamide formation.

Concluding remarks from PPM

NORDACRYL has increased our knowledge of several aspects that govern the direction of our product development. This can not be pinpointed to one certain specific product or revolutionary findings, but given us increased understanding of the complex parts in the frying process. It has been a fruitful forum where I (R. Haraldsson and then PPM) have had

the possibilities to discuss the actual process and chemical problems with skilled colleagues in the project group. In our work related to world wide export of deep frying equipments we have noticed the puzzling fact that some methods that work in a certain place do not work in another place, and vice versa. This is for sure a direct result of the variation in the raw material. The potato is complex and there is still a lot of research to be undertaken.

PPM AB / Roland Haraldsson (translated from Swedish by S. Knutsen)
(Questions related to the industrial equipment of potato frying may be addressed directly by EMAIL to Roland.Haraldsson@intl.fmcti.com).

Ongoing tests and plans

At present new technologies are being tested in an ongoing collaboration with PPM, KIMS and other industrial and academic partners. The network formed in NORDACRYL will keep working after the official closing of the project. This include full scale experiments related to

- Post-drying of semi fried potato crisps
- Blanching or dipping potato in $CaCl_2$ solutions in order to reduce acrylamide formation
- Use of asparaginase in potato processing
- Testing of storage facilities for potato
- Searching for of new industrial scale usable varieties
- Exploit the possibility of rapid methods (Online/IR) in quality control in the production of potato products

Asparaginase in crisp bread production at WASA

Background

The amino acid asparagin and reducing sugars are forming acrylamide during the Maillard reaction. Asparaginase is an enzyme that degrades asparagin and consequently has the potential to reduce the formation of acrylamide quite dramatically. Asparaginase was received from NOVOZYMES which at present (June 2007) is finalizing their production line for large scale commercial grade enzyme solutions. Asparagine was added in different concentrations to demonstrate if the acrylamide content can be reduced in the bread. The trial, performed at WASA in Filipstad in cooperation with NOVOZYMES (Denmark), was factory trials to confirm earlier findings[39].

Process

The bread was baked with the cold bread process in the test bakery of WASA, Filipstad. The flour used was the rye flour used for cold bread in Celle, RPM. The asparaginase arrived frozen in plastic containers to WASA. The samples were kept in the freezer until the afternoon before the day of the test. The samples were thawed in the fridge over night and the asparaginase was then diluted in different amounts into 100 ml water the next morning. The remaining enzyme was put into the freezer again. The solutions were stored in the fridge until they were used in the dough making during the day. The solutions were dissolved in the total amount of dough water before dough preparation.
It was decided to run three different amounts of enzyme. The reason for not testing a larger number of different amount was that time was needed to adjust the oven during baking to receive the right water content in the breads. The pH of the first dough without enzyme was 6.3.

Dough	Amount (Units/10 kg flour)	Amount (g/10 kg flour)	Water content after oven (%)	Water content after drying (%)	Height of 10 cakes (mm)	Evaluation of bread
1 (2)	0	0	4,67	3,20	48	Good bread with light colour of the surface and the interior.
2 (2)	5000	0,35	5,24	3,29	48	No significant differences from the reference.
3 (2)	40 000	2,80	4,60	3,78	46	No significant differences from the reference. Slightly poorer interior.
4 (2)	100 000	7,00	5,20	3,34	48	No significant differences from the reference.
5 (1)	0	0	4,65	-	48	Good bread like the reference but the bread was bent because there was not enough time to adjust the oven.

Table 6 Different doses of asparaginase in the dough.

The parameters on the line were very stabile during the first doughs. The difference that could be noted when the asparaginase was added was a lower the pressure on the air mixing machine because the volume weight was increasing. This happened at every new concentration of asparaginase. The temperatures in the oven were also lowered for every new concentration of asparaginase to keep the water content of the bread after oven between 4.5 and 5.5 %. The parameters on the line were varying during the day when asparaginase was mixed into the dough. They were not as stabile as without asparaginase.

Results of acryl amide analysis

The samples sent for analysis were from dough 1, 2, 3 and 4. Two cakes each of the samples were sent three days after baking in separate plastic bags. The cakes were not sawed into slices. The samples arrived in the lab after 6 days and the analysis report was finished 13 days after baking. The method used was LC-MS/MS.

Dough	Code	Asparaginase (units/kg flour)	Acrylamide in the bread (µg/kg)
1	7	0	430
2	2	500	97
3	6	4000	100
4	4	10 000	110

Table 7 Asparaginase dose and acrylamide formation (unpublished results).

Since the results are based on only one sample per dough statistics cannot be used in this trial but it can be seen that the amount of acryl amide is lowered and that there is no difference in acryl amide content whether 500 units/kg or 10 000 units/kg flour have been added. The results show that during this trial it was enough to use 500 units/kg flour of asparaginase and maybe an even lower amount can be used with the same effect.

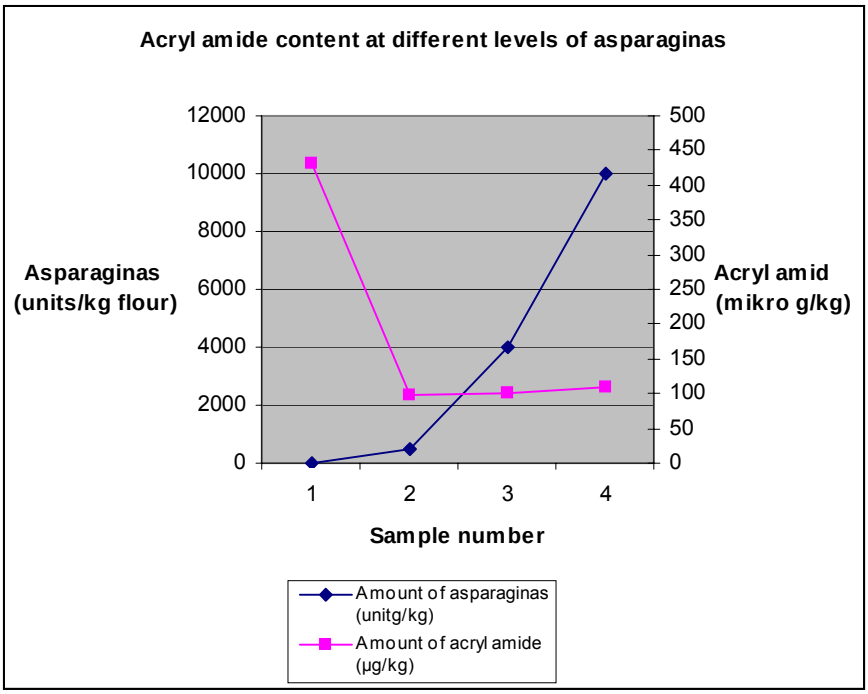

Figure 28 The addition asparaginase and the reduction of acrylamide in crisp bread.

The amount of acryl amide in the product is normally about 400 µg/kg and the reference in this trial is on the same level.

Conclusion
The results of this trial are very promising since it was possible to lower the acryl amide content substantially. It was possible to lower the content of acryl amide in the samples to about 25% of the amount in the reference. The parameters on the baking line had to be adjusted during baking but it was possible to receive bread that was as good as the reference. The optimum dosage for the asparaginase in the doughs baked in the test bakery is less than 500 unit/kg flour. The lack of a dose-response relationship indicates that the system is saturated with enzyme and suggests that a small amount of asparaginase is needed. Similar results were obtained in Norway for crisp bread on a 500 kg scale and for conventional breads. The failure of a complete removal of acrylamide formation, even at depleted asparagine, can be explained by the findings that acrylamide can be formed directly from protein (gluten) by heating and pyrolytic formation. [40].

Supplied by L. Holmgren, WASA

Commercial uses of precursor modifying enzymes-Comments
At present (June 2007) asparaginase is on the GRAS (Generally Recognized As Safe) and is approved by the FDA. There are at least 2 companies who can supply the product. The regulatory situation in EU is not clear and European industries await the EU-legislation as well as the price and a quantitative adequate food grade delivery. This enzyme has a clear potential in several applications. For potato products there are still some obstacles in order to ensure contact between the enzyme (asparaginase) and the substrate (asparagin).
There are at present no commercial available enzymes to convert reducing sugars into Maillard inactive compounds such as sugar alcohols or other carbohydrate derivatives which would have been another option.

Special products varieties from Iceland (supplied by Olafur Reykdal)

Varieties and results

The potato varieties used in Iceland for production of French fries are Golden-Eye and Premier. Other varieties have been rejected because of small size or other reasons. The cool climate can influence the sugar content of potatoes, a cold period before harvesting would increase the concentration, resulting in more acrylamide in the product. Limited analysis of sugars in Icelandic potatoes shows values comparable to reported values in other countries.

The only cereal grown in Iceland is barley. Arve is the only variety used by the baking industry although several varieties are possible candidates. The composition of Icelandic barley is not well known but measurements indicate normal level of free asparagine but quite high levels of reducing sugars. Increasing the proportion of Icelandic barley did not influence the level of acrylamide in a baking experiment.

Acrylamide was measured in French fries produced in Iceland in 2002 before the NORDACRYL project started and the level of acrylamide was high. In the project the processing was studied to reduce acrylamide. When acrylamide was measured in Icelandic French fries 2006 the concentration was below the detection limit of 20 µg/kg. French fries were sampled after frying in the processing plant (first frying).

A few Icelandic traditional bakery products were expected to contain high levels of acrylamide. This was among the reasons for Icelandic participation in the NORDACRYL project. The products of special concern were the fat bread baked on a hot plate at a temperature above 250 °C and the deep-fried wheat bread. Experiments were carried out to study the concentration of acrylamide in these products. Acrylamide in flat bread was comparable to reported values for bakery products from other countries. Only by increasing both time and temperature did the acrylamide level increase considerably. Acrylamide concentration in the deep-fried bread was low and considerably lower than in French fries fried both in the factory and at home. The short frying time explains the low acrylamide concentration in the bread.

WP 3 Analysis

Comparison of methods for analysis of reducing sugars

In the Nordic countries laboratories use different methods for the analysis of acrylamide substrates, i.e. glucose, fructose and free asparagine. Methods and protocols for the analysis of acrylamide substrates were exchanged and discussed among members of the project. In order to compare the different analytical methods used, a number of potato samples, differing in their natural content of acrylamide substrates were prepared, freeze-dried and sent for analysis in the laboratories of the different project members.

Partner	Principle and methodology	References
DFVF	Simultaneous analysis of free amino acids and reducing sugars by HPLC-MS-MS	[41]
DIAS	Enzymatic and spectrofotometric analysis of reducing sugars	[42, 43]
SW	Gas chromatography of derivatised reducing sugars	[21]
Matforsk	HPLC analysis of sugars in 60% methanol extracts	[31]

Table 8 Comparison of different methods for sugar analysis.

Sample	Variety	Temp. ºC
1	Lady Rosetta	4
2	Agria	8
3	Lady Rosetta	8
4	Liva	4
5	Agria	4
6	Saturna	8
7	Liva	8
8	Bintje	8
9	Saturna	4
10	SW 91 102	4
11	Sw 91 102	8
12	Bintje	4

Table 9 The potato varieties and had been stored at 8 or 4 ºC.

Although very different analytical methods have been used in the various Nordic research laboratories, the analytical results obtained were rather consistent and reproducible, as shown in the plots of figure 29.

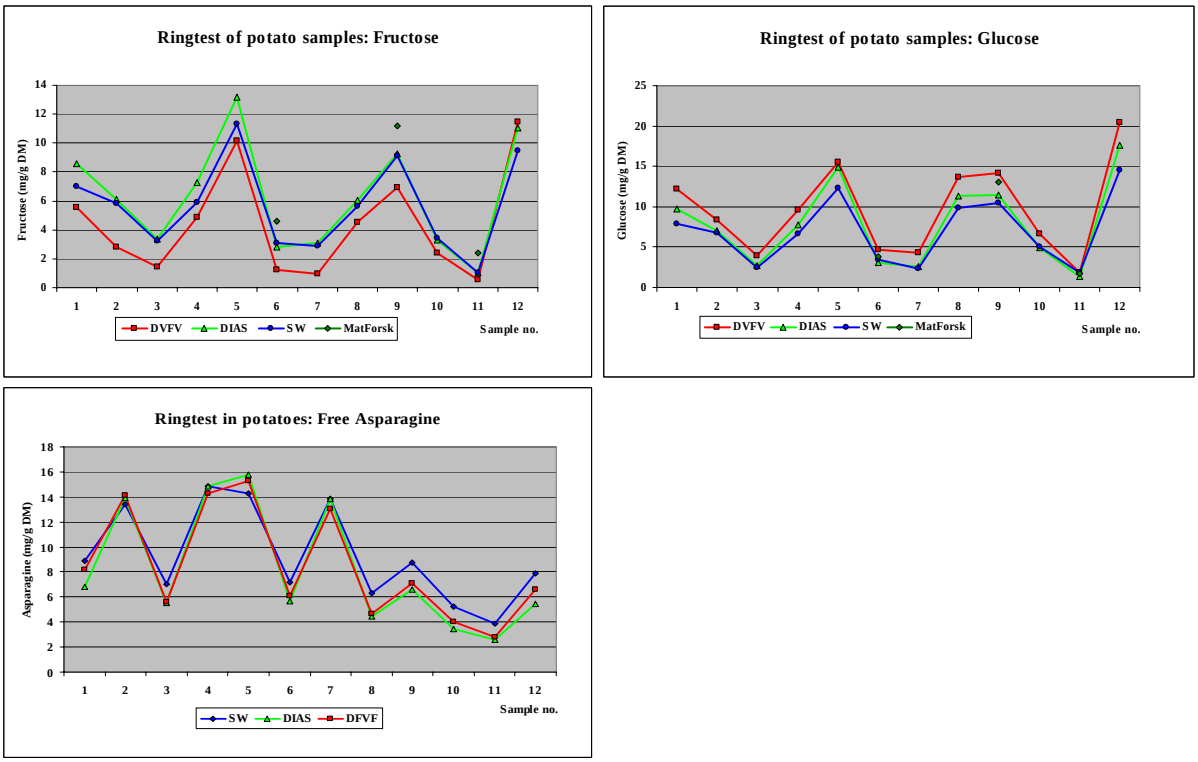

Figure 29 Fructose, glucose and free asparagine in dried potato.

During the storage of potato the increase of fructose parallels that of glucose which in general can be monitored by simple commercial available kits, such as glucose in blood. Then the day to day variation of the limiting substrates (sum of glucose and fructose) can be monitored by simple means by the preparation of a representative sample of potato juice.

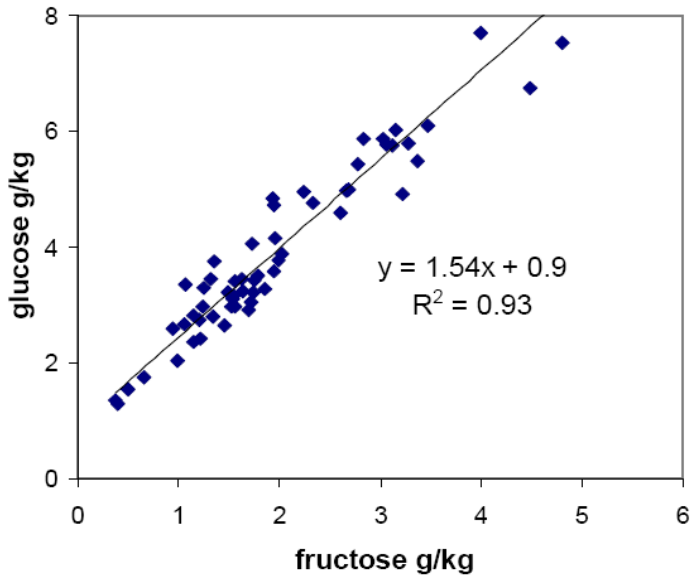

Figure 30 Relationship between fructose and glucose in potatoes. (Unpublished results K. Granby).

A new simple method for analysis free amino acids

Analysis of free amino acids in cereals products

Initially results obtained in different laboratories for amino acids in cereals showed quite deviating values. SLU undertook the task to improve and define a fast and reliable method.

Free amino acids were extracted from cereals products using 50 % ethanol to prevent solubilisation of polysaccharides and other viscous polymers and to avoid starch gelatinisation [44]. The extracts were analyzed by GC after ion-exchange solid phase extraction and chloroformate derivatisation using Ez-Faast technology (Phenomenex). Free amino acids in cereal products could be analyzed within 1 h of extraction and determination, with good separation between peaks and repeatable retention times (for a chromatogram see below). A relative correction factor for each amino acid was established. The matrix did not affect the results and the method was repeatable for most of the amino acids (coefficient of variation was in the order of 10 %). Different fractions and products of wheat, rye, oats, and barley were analyzed. The bran contained more free amino acids than did the other analyzed fractions of cereals. Germ fractions, which are known to be very high in free asparagine, were not analyzed.

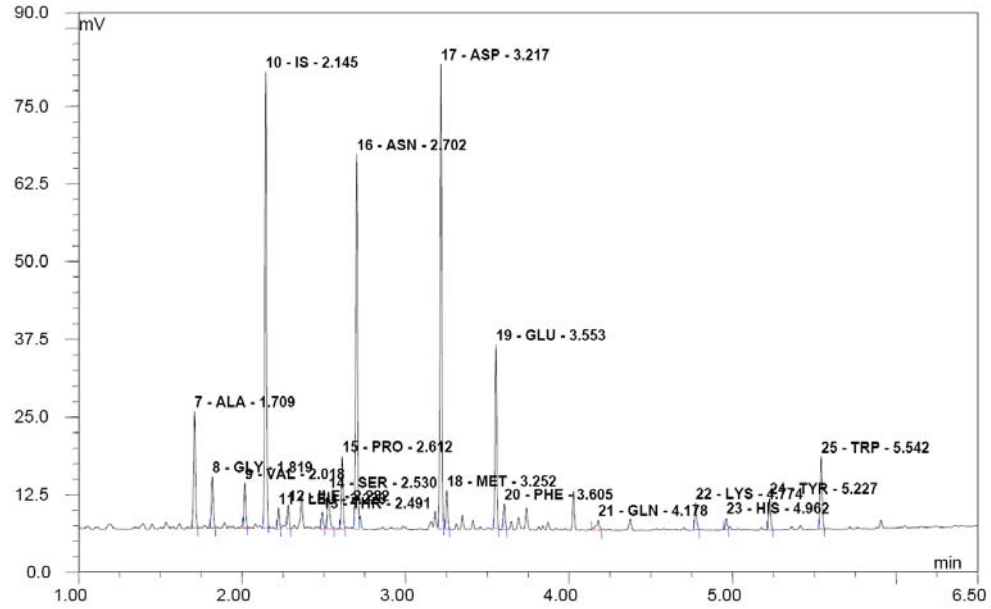

Figure 31 A GC chromatogram of Ez-fast method adopted to cereals at SLU. For details see [44].

Analysis of acrylamide by chromatography with MS-detection

In general NILU (Norway) analysed the potato based products whereas EVIRA (Finland) handled the cereal based sample. EVIRA also handled some special products from Iceland. The methodology for detection of acrylamide in the HPLC instrumentation was different (see below) but sample preparation, which is a crucial part, was similar for the two laboratories. No results are presented her since the analytical data are utilized in the different works sited elsewhere in this document. In short the methodology used was:

At NILU:

Acrylamide was analyzed by a method similar to that of Rosen and Hellenas (2002), using high resolution time of flight mass spectrometry instead of tandem mass spectrometry [27]. In short the processed samples were homogenised in water containing d3-acrylamide internal

standard. Acrylamide was extracted by sonication and the extract was purified by Carrez reagents, centrifugation, SPE extraction and membrane filtration. Acrylamide was separated from the sample matrix by a high performance liquid chromatography system (Agilent HP-1100) system equipped with a Micromass LCT orthogonal Time-Of-Flight (TOF) mass detector equipped with a Z-spray ion source operated in the atmospheric pressure chemical ionisation positive mode APCI (+). Limit of detection depended on instrument tuning and ion source contamination and corresponds, typically, to 10–30 microgram/kg acrylamide in the sample.

At EVIRA:

The LC-MS/MS method has been validated and applied for routine analysis of acrylamide in various foods. It is a modification of the methods presented by Rosén and Hellenäs [45]. The LC-MS/MS is operated in a positive electrospray mode. The Multiple Reaction Monitoring (MRM) mode is used for ion detection. Quantification is performed using internal standard method. The MRM transitions used for identification and quantification of acrylamide are m/z 72>55, 72>27 for acrylamide and m/z 75>58 for acrylamide-d3. The detection limit of the method is 25 microgram/kg. The relative standard deviations for repeatability and between-day variation are below 15% in different food matrices.

Rapid methods for acrylamide analysis based on NIR

In order to study the formation of acrylamide in potato crisps during processing, an experimental design was set up. The design variables were drying time (6 levels), frying temperature (2 levels) and frying time (8 levels). The design contained 36 samples, which were analysed for acrylamide contents using LC high-resolution mass spectroscopy (LC-HRMS) at NILU, and fat contents using the Soxhlet apparatus. Prior to analysis, all potato crisp samples were ground and analysed on near-infrared (NIR) spectrometer. The acrylamide contents were modelled by: (i) design variables using multiple linear regression, (ii) NIR spectra using partial least squares regression (PLSR) and (iii) design variables and NIR spectra in combination using a novel technique combining least squares regression on the former, and PLSR on the latter. NIR spectra alone or in combination with the design variables gave better prediction models for acrylamide than the design variables alone. This implies that the spectra contain chemical information that is not purely a result of the processing variables that were investigated in this experiment. NIR spectroscopy is proposed as a possible tool for screening and identification of potato crisps with high acrylamide content [46]. At present ongoing collaboration with KIMS and Matforsk has been continued in order to investigate the possibilities of real online IR-measurements of fat, dry matter and acrylamide.

WP 4 Health and risks by J. E. Paulsen and co-workers

Bioavailability of acrylamide in mice

Metabolism of acrylamide

Early studies in rats showed that AA was mainly excreted in the urine (71% within 7 days) [47]. A major urinary AA metabolite in both rats and mice is N-acetyl-S-(3-amino-3-oxopropyl)cysteine, a mercapturic acid AA derivative (MA-AA) [48, 49]. An alternative metabolic pathway is the CYP2E1 dependent oxidation of AA, from which the genotoxic

epoxide glycidamide (GA) is formed [50]. GA reacts readily with DNA and other macromolecules [51, 52] and this is probably the main pathway responsible for the carcinogenic effect of AA. GA may be further metabolised by epoxide hydrolase to glyceramide [53] or conjugated to glutathione. Following a stepwise conversion, it is excreted as the urinary mercapturic acid derivatives N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl)cysteine (MA-GA_3) and N-acetyl-S-(carbamoyl-2-hydroxyethyl)cysteine (MA-GA_2) [48].

Intake of acrylamide, internal dose and urinary biomarkers

The average daily intake of AA for adult humans, based on food consumption data from 17 different countries, has been estimated to be between 0.3-2.0 µg/kg bw in adults and even higher in children [12, 48, 54]. For the risk assessment of AA it is important to determine the bioavailability, systemic exposure and conversion rate of AA from food to the genotoxic epoxide GA. It is important to determine the internal dose in humans and rodents, since current cancer risk extrapolation is based on rodent studies. However, so far the bioavailability of AA from foods is uncertain. In previous studies on rodents, the bioavailability of AA has been investigated following oral administration of AA in aqueous solutions or in diets fortified with AA [55, 56]. Bioavailability of AA as it occurs in the matrix from regular foods has to the best of our knowledge not yet been investigated. Recently, NIPH developed a method to determine the mercapturic acids of AA and GA in human urine[57]. We used these as biomarkers for dietary AA intake in a clinical study and found immediate changes in the urinary AA derived mercapturic acids following consumption of foods rich in acrylamide[58]. We therefore wanted to apply this method in mice to study the AA bioavailability from foods. Crisp bread, which is a significant AA source in the in the Nordic countries, was chosen as experimental diet.

Objectives within NORDACRYL

The objective of the present study was to: (i) determine the bioavailability of AA from a regular food matrix by using an experimental crisp bread diet baked to contain different concentrations of AA, 0.19, 1.02, 2.65 mg/kg; (ii) explore whether urinary metabolites of AA and GA could be candidate biomarkers of AA intake and internal dose.

Determination of urinary metabolites

Urinary mercapturic acid metabolites from AA were determined by liquid chromatography with positive electrospray-ionisation tandem-mass spectrometry (LC-MS/MS) as previously described [57]. Urinary GA (NIBC-GA_3) was determined using the same LC-MS/MS instrumentation. The radioactivity in the samples was counted with a Packard Tri-Carb 1900CA liquid scintillation analyser. The counting efficiency was controlled by a Packard automatic ^{14}C-quenching standard.

Molar relationship between urinary AA metabolites and AA exposure from dietary crisp bread

To achieve a steady-state level of intake and urinary excretion, the C57 BL mice were initially given crisp bread diets or control diet for 3 days. After moving the mice to metabolic cages (Figure 32) the feeding regime was continued and the 24 h intake and excretion of AA as urinary metabolites for each cage was measured for 4 days.

Figure 32 Mice placed in a metabolic cage. The mice were given 3 types of experimentally baked crisp bread containing 191 µg/kg, 1020 µg/kg, or 2650 µg/kg of AA. The steady-state 24 h intake and excretion of AA as urinary metabolites for each cage was measured for 4 days.

Plotting all the 24 h records as molar intake of AA from crisp bread versus total molar excretion of urinary AA metabolites it was found a good linear relationship between AA intake and excretion (se following figure).

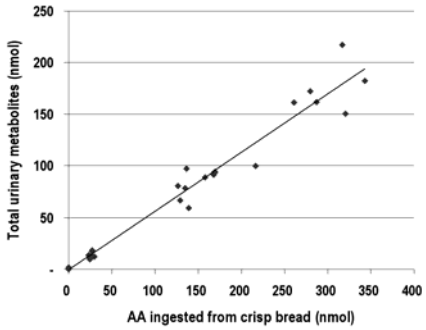

Figure 33 The molar relationships between the 24 h records of AA ingested from crisp bread and the total excretion of urinary AA metabolites.

The mean fraction of the ingested dose recovered as urinary AA metabolites was 55±8% (± SD) for the different crisp bread diets, and the molar proportions between the urinary metabolites showed a similar pattern for the different doses (Figure below).

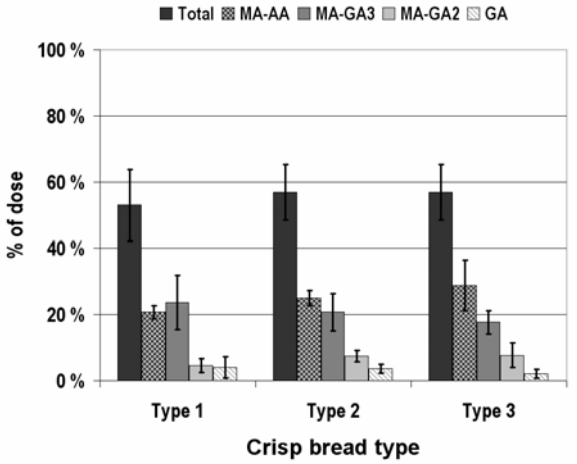

Figure 34 The mean fraction of the ingested dose recovered as urinary AA metabolites for the 3 types of crisp bread ingested.

Molar relationship between urinary AA metabolites and AA exposure from sc injection
After 1 day of acclimatisation in the metabolic cages, the mice were given AA sc at doses of 0, 0.05, 0.5, 5 and 50 mg/kg bw, and the 24 h excretion of AA metabolites was measured the subsequent 3 days. Again, a good linear relationship was found between the molar amount of AA administrated and the total molar amount of urinary AA metabolites excreted, even in the large dose range investigated (Figure below). No AA metabolites were observed in the urine of the controls.

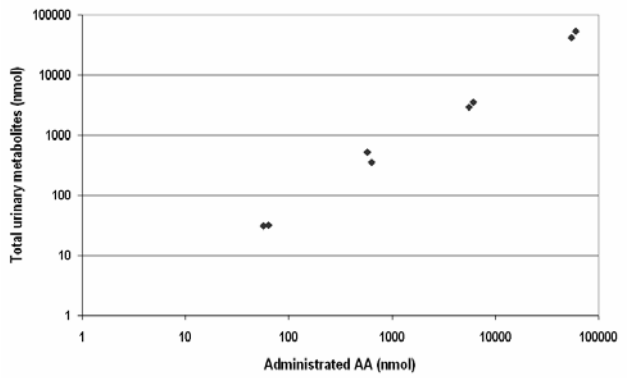

Figure 35 The molar relationships between AA given sc and the total excretion of urinary AA metabolites.

For the three lowest doses injected the mean fraction of the injected dose recovered as urinary AA metabolites was calculated to be 54±3% (Figure 5). The recovery of the highest dose was significantly higher than that of the lower doses, and the metabolite pattern differed considerably from that of the lower doses with a much higher proportion of MA-AA relative to MA-GA_3. This could be due to a saturation of CYP2E1 mediated conversion of AA to GA.

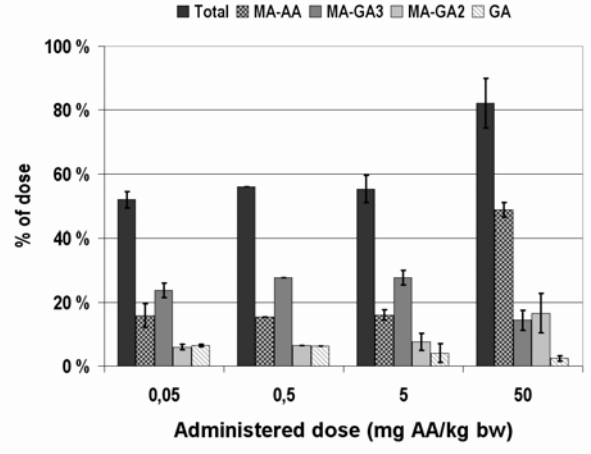

Figure 36 The mean fraction of the sc injected AA dose recovered as urinary AA metabolites.

The cumulative urinary excretion curves, expressed as recovery (%) of injected AA dose, shows that the major part was excreted the first 24 h (Figure below).

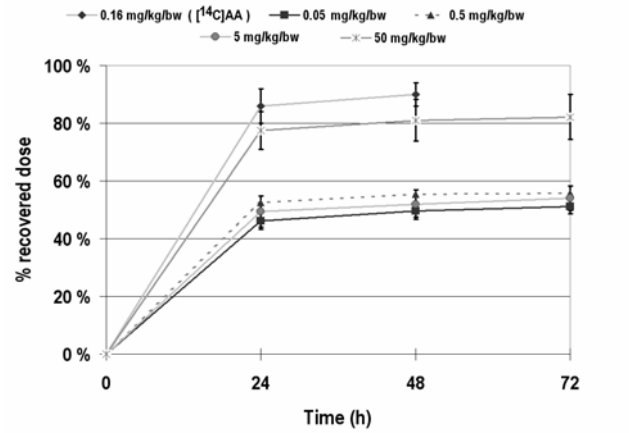

Figure 37 Cumulative excretion expressed as recovery of injected AA dose.

The bioavailability of AA from crisp bread

Since the amount of AA administered sc can be considered to be completely systemically available, the bioavailability of AA from crisp bread could be defined as the amount of AA recovered as urinary metabolites from crisp bread feeding (55 %) relative to the recovered amount of AA as urinary metabolites from sc AA administration (54 %). Since the recoveries of urinary metabolites from mice with dietary exposure to AA and mice sc administrated AA were found to be practically identical, the bioavailability of AA from crisp bread is approximately 100%.

Fate and recovery of [^{14}C]AA from sc injection

Since only about 54% of the lower doses of the AA administered sc was recovered in urine as mercapturic acid metabolites and GA, we investigated the fate of the remaining 45%. Therefore, mice were given a dose of radio-labelled [^{14}C]AA sc. Urine, faeces, liver and blood samples were analysed by liquid scintillation counting. The recovery of radioactivity in the urine was 92 % at 48 h (Figure below). About 2% of the activity was recovered in faeces, liver, intestinal contents, blood and wash water of the cage (Table 1). The total radioactivity recovered from the administration of [^{14}C]AA was calculated to be 94% (±4 SD). The discrepancy between recovery in urine from sc [^{14}C]AA administration and recovery in urine from sc AA administration strongly suggest a significant contribution of other urinary metabolites not determined by the methods used in the present study.

Samples	24 h Average (%)	SD (%)	48 h Average (%)	SD (%)
Urine	86	6	92	4
Faeces	0.7	0.4	0.84	0.06
Liver	-		0.57	0.01
Blood	-		0.08	0.01
Intestinal content[a]	-		0.12	0.03
Rest activity[b]	-		0.59	0.03
Total	87	6	94	4

[a] *coecum and colon contents*

[b] *Activity in 10 ml H_2O used washes the metabolic funnel in the cage*

Table 10 Distribution of activity from [14C]AA.

Molar relationship between urinary GA metabolites and GA exposure from sc injection
In order to examine the metabolism of GA and the amounts excreted as parent compound and as mercapturic acid metabolites, mice were given GA sc at doses 0, 0.05, 5 and 50 mg/kg bw. The major metabolite found in urine was MA-GA_3. The recoveries of the determined urinary metabolites were dose dependent: 40%, 55% and 66% for the doses 0.05, 5 and 50 mg/kg bw, respectively (Figure below)). The minor amounts of MA-AA found in several urine samples can be attributed to an AA impurity in the commercial GA (98% purity).

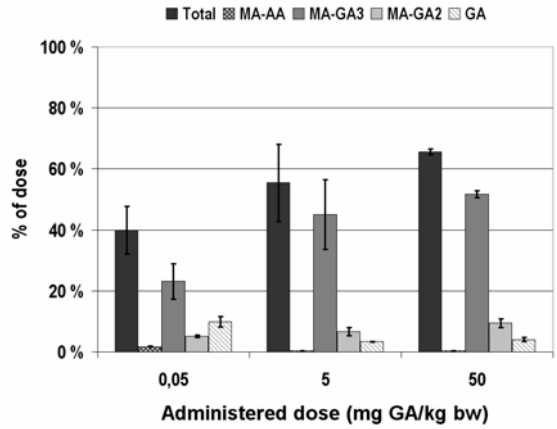

Figure 38 The mean fraction of the sc injected GA dose recovered as urinary GA metabolites.

A linear relationship was found between the amount of GA administered and the amount of urinary metabolites within the exposure range investigated (Figure 8). The recovery of only 40% to 66% of the given GA at increasing dose levels suggests the formation and urinary excretion of additional GA metabolites not accounted for by the method used in this study, possibly 2,3-dihydroxypropionamide (glyceramide) (previously determined by NMR (Sumner *et al.* 1992). Our LC-MS/MS method is not suitable for the determination of this highly polar compound, which could be responsible for the discrepancy in recoveries recorded.

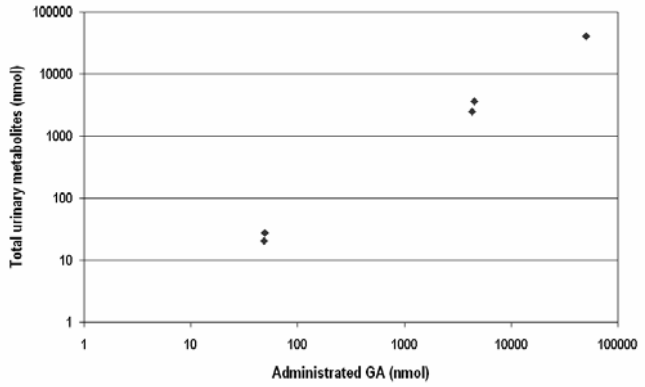

Figure 39The molar relationships between GA given sc and the total excretion of urinary GA metabolites.

Conclusion from animal studies

In the present study, we analysed urinary metabolites of AA and GA in C57BL mice after dietary and parenteral exposure to AA. The bioavailability of AA from an experimental crisp bread diet, baked to achieve different dose levels of AA, was determined to be approximately 100% by comparing the amount recovered as urinary metabolites from steady state feeding of the crisp bread diet (intake/day : urinary metabolites/day) with the amount recovered as

urinary metabolites from a single dose of AA injected sc (dose injected : total amount metabolites excreted in 3 days). There was a remarkable linear relationship between AA exposure and the total amount of urinary metabolites in the dose range 0.024 – 5 mg/kg bw/day independently of mode of administration. Furthermore, similar molar proportions between the urine metabolites were observed up to 5 mg/kg bw/day. Although the urinary metabolites detected only represent ~55% of the dose administrated, they appear to be good biomarkers of AA intake and the internal dose.

For the risk assessment of AA it is important to determine the conversion rate of AA from food to the genotoxic epoxide GA, and to compare the conversion rate in humans to that in rodents, since current cancer risk extrapolation is based on rodent studies. By injecting GA it was possible to investigate the fate of systemically available GA as urinary mercapturic acid metabolites. The linear relationship between systemic GA and the total amount of these metabolites indicates that they may function as biomarkers of internal GA dose in the present model. However, for comparing the internal GA dose between species, which may vary in enzyme characteristics, it seems necessary to determine all the urinary metabolites, including glyceramide.

Risk characterisation

Based on previous toxicity data from animal studies and intake data from the human population the current risk evaluations of acrylamide indicate a lifetime cancer risk of acrylamide at the level of 1-2 x 10^{-3} [12]. The cancer risk associated with food borne acrylamide exposure is therefore probably low compared with the total cancer risk (10-15%) associated with diet. However, for other chemicals a risk level to consumers compatible with that of acrylamide would not be ignored. Actions to reduce the risk by reducing acrylamide in foods and thereby food borne exposure are therefore warranted and generally agreed upon. New risk assessments of acrylamide are awaiting additional results from large scale animal studies (as performed by FDA), which are expected to be presented in 2007-8. In addition, more details about the human exposure and internal dose of acrylamide are needed.

Acrylamide-Precursors – by Kit Granby

The limiting substrates in the raw materials for acrylamide formation in potato and cereal products.

Background:

One of the strategies to reduce the amount of acrylamide in food products is to reduce the content of precursors (e.g. asparagine, sugars) in the raw material, by choosing the optimal cultivars (for the Nordic regions) and by modifications of growth and storage conditions. It might be possible to select varieties that can grow in the Nordic climate without excess accumulation of the acrylamide precursor molecules. For this strategy to succeed, the ways to mitigate the limiting substrates in the raw materials must be known. In the following the status of our knowledge on limiting substrates in potato and cereals raw materials is presented.

POTATOES

Influence of storage and varieties on the levels of limiting substrate (reducing sugars).

In 2004 it was suggested [59] that one of the ways acrylamide formation in potato products can be controlled is by the use of raw products with low sugar (and to a lesser degree, asparagine) content. From many subsequent studies it is generally concluded that reducing the reducing sugars is the most efficient way to reduce the acrylamide in the potato products. An

exception from that general conclusion is for products based on mashed potatoes, where treatment of the potato slurry with the enzyme asparaginase will remove asparagine, and almost eliminate the acrylamide of the prepared potato product. In the study by [59] the contents of asparagine in raw potatoes (different cultivars bought in Canada) ranged from 1.5-11.4 g/kg ww, glucose from 0.07-6.3 g/kg ww, and fructose from 0.07-6.1g/kg ww (n=66). The lowest acrylamide formation (60 µg/kg) was from raw potato with 3.5 g/kg ww asparagine, 0.27 g/kg ww glucose and 0.27 g/kg ww fructose. The highest acrylamide formation (1823 µg/kg) was from raw potato with contents of asparagine at 8.6 g/kg ww, glucose at 4.8 g/kg ww, and fructose at 4.2 g/kg ww.

A three years study on contents of asparagine and sugars in eight potato clones stored at 3°C and 10°C [21] showed that storing temperature had no effect on the asparagine concentration, but the average glucose and fructose contents was four-five times higher at 3°C. Both sugars showed the highest contents during the winter. The varieties Hulda, Lady Rosetta, Saturna and 5 clones made at Svalöf Weibull AB were compared. For asparagine large variation with genotype were found for year 90 and 91 but not 89, however with about the same ranking between varieties. The average concentration ranged from 3.9 g/kg dw in clone B to 10.2 g/kg dw in clone E. Glucose ranged from 5.5-13.9 g/kg dw and fructose from 3.8 –11.8 g/kg dw. Breeding potatoes clones with low asparagines levels might be of importance to reduce the high acrylamide levels in the products.

In Denmark [22] performed a three years study on the limiting substrates in Saturna potatoes, the variety preferred in Denmark for crisp production. At the storage temperature 8.5 °C during the seasons 2000/2001 the glucose and fructose levels increased all the season from ca 0.5 g/kg dw up to ca 3 g/kg dw. During 2001/2002 the reducing sugars peaked at about 1 g/kg dw during the winter and during 1999/2000 the reducing sugar levels decreased after May. The levels of asparagine varied among the six research field between 5-15 g/kg dw but with no seasonal variation.

In a study of the varieties Bintje, Ramos and Saturna stored at 4°C and 8°C no significant increase in the levels of asparagine or sucrose was seen after 24 weeks [23]. The asparagine level ranged from 1.5-1.9 g/kg dw. The reducing sugar content in potatoes stored at 8°C did not increase significantly during the storage, however when stored at 4°C the glucose and fructose levels increased from ca 1 g/kg dw to ca 10 g/kg dw for Bintje and Ramos and ca 6 g/kg dw for Saturna. The influence on storage time and temperature on the acrylamide formation in fried potatoes was also tested and the changes in acrylamide formation could mainly be explained by the sugar content of the potatoes (R^2=0.84, n=160). The intention with cold storage is to prevent sprouting. However at the same time it has a negative impact on the formation of the acrylamide in the fried potato products. The formation of reducing sugars during cold storage is partly a reversible process and reconditioning of the potatoes at a higher temperature before use (at 15°C for 3-5 weeks) reduced the glucose and fructose levels from 10 to 1-2 g/kg dw [23]. It was not possible to have a complete reconditioning to the reducing sugar level before the reconditioning. This investigation was in accordance with results by Isherwood [60] and Iritani [61], who argued that only sugars formed during cold storage could be reconditioned and e.g. sugars formed due to senescent sweetening could not be reduced. When reconditioning the potatoes, Chlorpropham was used as sprout inhibitor, as the potatoes without sprout inhibitor showed sprout or root formation. The usage of sprout inhibitor did not influence the levels of precursors in the potatoes [23].

In a study on 17 potato cultivars grown in Switzerland in 2002 glucose and fructose were found to determine the acrylamide formation [25]. The acrylamide contents were most strongly correlated (R^2=0.91) to the formula (0.5*glucose + fructose)*asparagine [g(kg^2]. The large difference between cultivars in potential for acrylamide formation was primarily related

to the sugar contents. The concentrations asparagine contents ranged from 2.0-4.3 g/kg ww, (~ 10-22 g/kg dw)-lowest in Appell and Naturella. The glucose contents varied more, ranging from 0.1- 2.6g/kg ww (~ 0.5-13 g/kg dw) – lowest in Lady Claire, Lady Rosetta, Markies, Marlene and highest in Nicola, Naturella, Appell, Charlotte and Desiree (Table.1). The potential for acrylamide formation ranged from 80 µg/kg (cultivar Panda) to 1700 µg/kg (Charlotte) and 2020 µg/kg (Nicola).

In a study by using the variety Tivoli the raw potato contained 10 ± 1 g/kg dw (2 ± 0.2 g/kg ww), glucose 5.1g/kg dw and fructose 6.8 g/kg dw [34]. The acrylamide contents in chips fried at 150°C and 190°C after blanching were well correlated with asparagine (R^2= 0.94;0.90) and glucose (R^2= 0.97; 0.85) in the raw potato. Davies [62] and Hippe [63] reported asparagine contents in potatoes of 5-30 g/kg dw for different varieties, locations, storages and fertilization.

Agronomic factors influencing the precursor levels in potatoes:

Besides the genotype, the reducing sugar contents of the potatoes depend upon different agronomic factors: environmental conditions, climate, cultural practices during growth, type of soil and fertilizer conditions. Hence it is difficult to compare different studies. In a study on 16 potato varieties grown in Belgium the authors [64] did not find that different soil types reflected the reducing sugar levels. For 96 samples analysed they found a strong correlation between reducing sugar contents and acrylamide formation(R^2=0.82). The levels of reducing sugars varied quite a lot from 0.4- 6.8g/kg dw. Hence it may be useful to screen the potato varieties with respect to reducing sugars to select the varieties suitable for frying.

Climatic conditions during growth have impact on the acrylamide levels in the potato products. If the weather is cold before the potatoes are harvested the reducing sugar levels may increase.

Concerning the fertilizer status, when less fertilizer is given the free amino acid levels decreased, but this resulted in and reverse effect since the reducing sugar levels increased [64].

Reduction of limiting substrates before preparation of fried potato products:

The levels of the asparagine or reducing sugars in the raw potatoes may be reduced by blanching or treatment with different solutions e.g. enzymes (asparaginase), other amino acids, acids , salt etc. before the potato products are prepared. Blanching has shown to reduce the levels of both reducing sugars and asparagine in the potato pieces considerable and by that reduce the acrylamide in potato products like French fries or crisps [34, 35, 37, 64, 65]. Treatment with asparaginase also reduces the acrylamide in potato pieces to some extent (Pedreschi et al. unpublished.). However the best effect of the treatment with asparaginase on potato products is on mashed potato products as all the asparagine present is supposed to be available for reaction with the enzyme.

Variety	Grown in:	Crisps	French Fries	Reducing sugars g/kg dw *	asparagine g/kg dw *
Ampera	S				
Arielle	S				
Asterix	N,S		N	4.7	7.2
Bintje	S		Dk, N	2.5	13.3
Ditta	S				
Fontane	S			1.3	13.7
Lady Claire		N		0.4	16.4
Laila	N		N		
Liva		N			
Marietiema	S				
Novara	S				
Peik	N,		N		
Rapido	S				
Rocket	S				
Roko	S				
Satina	S				
Saturna	Dk, N, S	Dk, N		0.8	13.9
Sava	Dk		Dk		
Solist	S				
Spey	S		N		
SW 1214	S				
Tivoli	Dk	Dk			
Ukama	S				

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Table 11 Potato varieties used for production of chips and prefabricated potatoes (French fries) or for home preparation.

CEREALS:

The influence of milling fractions etc. on the levels of the limiting substrate (asparagine).

Variations in free asparagine and reducing sugars in cereals are less researched but asparagine is found to be the limiting substrate in cereal products. Surdyk *et al. [15]* investigated the effects of asparagine and fructose on acrylamide in wheat bread (flour with asparagines 0.17 g/kg ww, glucose 0.13 g/kg ww and fructose 0.05 g/kg ww. Added asparagine (1-7g/kg ww) dramatically increased the acrylamide level in the crust from 80 µg/kg (background) to between 600 and 6000 µg/kg while added fructose did not significantly influence the acrylamide content. Surdyk *et al.* [15] found a strong correlation between acrylamide and colour for different baking times and temperatures. However adding of asparagine increased acrylamide but did not significantly change colour.

The clear relation between asparagine level and acrylamide formation has also been demonstrated in other bread model systems [18, 27] and it may be concluded that levels of asparagine in flour used for bread production influence the acrylamide formation.

Fredriksson et al. [14] found that the contents of free asparagine are lower in sifted wheat flour i.e. 0.14- 0.17 g (kg dw)$^{-1}$ compared to whole grain flour i.e. 0.5 g kg^{-1}(dw) or bran. Rye showed higher figures: sifted flour 0.6 g kg^{-1}(dw) and whole grain flour: 1.1 g kg^{-1}(dw). Not only milling has been found to reduce the levels of the limiting substrate asparagine. It was found that yeast fermentation depletes a major part of the asparagine in dough (>80%), while sourdough fermentation reduce the asparagine content to a smaller extent [14]. During baking parameters such as water activity, heating time and temperature,

surface to volume ratio, the presence of additives and pH also influence acrylamide levels [14, 15, 66].

Agronomic factors influencing the precursor levels in cereals:
Claus et al. [40] investigated agronomic factors related to the acrylamide formation in cereal products. They showed that nitrogen fertilization resulted in elevated amino acid levels resulting in increasing acrylamide levels from 11 to 56 µg/kg. Nine wheat, two rye and two spelt varieties harvested in 2003 and 2004 formed the basis of the study. Breads produced from 2003 flour showed significantly higher acrylamide levels than those from 2004 flour, which was ascribed to favourable light and temperature conditions in 2003 enhancing amino acid and protein contents. Sprouting of the grain also resulted in significantly higher acrylamide levels. Due to elevated asparagine contents, acrylamide in rye were general higher than in wheat and spelt. The rye flour contained 0.41-0.44 g asparagine/kg , the spelt 0.06-0.12 g asparagine/kg and the wheat 0.05-0.25 g asparagine/kg. Additional S-fertilization did also influence the acrylamide levels [40].

Rye Variety	Wheat Variety
Agronom	Hereword
Askari	Kraka
Avanti	Kris
Caroass	Ritmo
Carotop	Pentium
Dominator	Hussar
Gamet 90M	Flair
Hacada	Windsor
Matador	Stakado
Picasso	Trintella
Recrut	Cortez

Table 12 Rye and wheat varieties used for production of different products.

Scientific publications related to acrylamide from project participants.

Ahrne, L., Andresson, C.G, Floberg,P. Rosen, J. and Lingnert, H.(2007). *Effect of crust temperature and water content on acrylamide formation during baking of white bread: Steam and falling temperature baking.* LWT - Food Science and Technology, 1708-1715.

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The Nordic Innovation Centre initiates and finances activities that enhance innovation collaboration and develop and maintain a smoothly functioning market in the Nordic region.

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