

Book of Abstracts



3-5 August 2009
Viterbo, Italy



Welcome

Dear Workshop Participant,

Cereals&Europe organises, in cooperation with the University of Tuscia, Viterbo, Italy and with the support of several companies for the 8th time this traditional three day workshop for PhD students and young professionals, working in the field of cereal science and technology. The aim of the workshop is to enable (young) people, new to the field of cereal science to present a broad range of cereal related topics, including breeding, biotechnology, ingredients- functionality, processing, nutrition....

This workshop also intends to facilitate the dissemination of and to stimulate the discussion about state of the art topics in the field. It is also specifically designed to initiate networking with the PhD students who will be the future of the cereal community.

The beautiful city Viterbo and the typical Italian atmosphere will certainly catalyse to achieve the goals of this workshop.

We wish you all an interesting and interactive (scientifically and socially) workshop !

Organising Committee

Domenico Lafiandra

Peter Weegels

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Participants

FIRST_NAME	NAME	COMPANY	E_MAIL
Gábor	Balázs	Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science	gbalazs@mail.bme.hu
Josef	Baumann	Novozymes	JBAU@novozymes.com
Norbert	Boros	Institute of Food Science, Quality Assurance and Microbiology, Faculty of Agriculture, University of Debrecen	norbert.boros@hotmail.com
Gabriella	Bottega	DISTAM University Degli Study di Milano	gabriella.bottega@unimi.it
Linda	Botticella	Department of Agrobiolgy and Agrochemistry of University of Tuscia	e_botticella@unitus.it
Zsuzsanna	Bugyi	Budapest University of Technology and Economics	bugyi@mail.bme.hu
Irene	Della Ghezza	Barilla	I.DellaGhezza@Barilla.it
Liesbeth	Derde	Lab of Food Chemistry & Biochemistry, KULeuven	liesbeth.derde@biw.kuleuven.be
Annelies	Duyvejonck	Lab of Food Chemistry & Biochemistry, KULeuven	annelies.duyvejonck@biw.kuleuven.be
Eleonora	Egidi	Department of Agrobiolgy and Agrochemistry of University of Tuscia	eleonoraegidi@unitus.it
Diya	Eldin Mohamed Ali Ibrahim	Sudanese Standards & Metrology Organization (SSMO).	rahbamohamed563@hotmail.com
Tímea	Gelencsér Bachrathyné	Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science	gelencser@mail.bme.hu
Angelica	Giancaspro	Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari	angelica.giancaspro@libero.it
Gaëtan	Gombaud	Department of Agrobiolgy and Agrochemistry of University of Tuscia	gaetangombaud@unitus.it
Anita	Hirte	TI Food and Nutrition, Wageningen University	anita.hirte@wur.nl
Mária	Hódsági	Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics	hodsagi@mail.bme.hu
Domenico	Lafiandra	Department of Agrobiolgy and Agrochemistry of University of Tuscia	lafiandr@unitus.it
Lieve	Lamberts	Lab of Food Chemistry & Biochemistry, KULeuven	lieve.lamberts@biw.kuleuven.be
Jenni	Lappi	Fppd and Health Research Centre	jenni.lappi@uku.fi
Roberta	Lupi	Department of Agrobiolgy and Agrochemistry of University of Tuscia	robertalupi@unitus.it
Valentina	Narducci	Istituto Nazionale de Ricerca per gli Alimenti e la Nutrizione	narducci@inran.it
Laura	Oliver Hernández	TI Food and Nutrition, Wageningen University	laura.oliverhernandez@tno.nl
Roel	Orsel	CSM	Roel.Orsel@csm.nl
Daniela	Panichi	Department of Agrobiolgy and Agrochemistry of University of Tuscia	panichi@unitus.it
Frederica	Paoletti	Department of Agrobiolgy and Agrochemistry of University of Tuscia	f.paoletti@unitus.it
Anna	Patsioura	Department of Environmental Engineering Technical University of Crete	annapatsioura@yahoo.gr
Silvia Paola	Perrone	Universita Degli Studi di Parma	silvia.perrone@email.it
Moustafa	Saad	UMR-IATE	saadm@supagro.inra.fr
Alireza	Sadeghi Mahoonak	Department of Food Science and Technology, Gorgan University of Agricultural Sciences and Natural Resources	sadeghiaz@yahoo.com
Julien	Saint Paul	CSM	Julien.saint-paul@csminnovation.com
Outi	Santala	VTT Technical Research Centre of Finland	ext-outi.santala@vtt.fi
Valeria	Scala	CRA-QCE	valeriascala@hotmail.com
Adalgisa	Schiavulli	Department of Environmental and Agro-Forestry Biology and Chemistry, University of Bari	gisella_s@libero.it
Francesco	Sestili	Department of Agrobiolgy and Agrochemistry of University of Tuscia	francescosestili@unitus.it
Juhani	Sibakov	University VTT TECHNICAL RESEARCH CENTRE OF FINLAND	juhani.sibakov@vtt.fi
Mariana	Soto Cámara	IFAPA	mariana.soto.ext@juntadeandalucia.es
Filip	Van Bockstaele	University College Ghent, Ghent University	filip.vanbockstaele@hogent.be
Aliette	Verel	Kraft	Aliette.Verel@kraft.com
Peter	Weegels	Sonneveld Group BV	p.weegels@sonneveld.com

Presentations

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PROGRAM		
	Monday August 3	
8.00	Depart student housing	
8.15	Breakfast	
9.15	Start registration	
9.30	Welcome	
	Session 1	Methodology, Physical aspects and Rheology
9.50	Peter Weegels	Stress, Strain Hardening and Large and Fast Deformations during Dough Proofing
10.10	Filip Van Bockstaele	Effect of mixer type, mixing time and dough formulation on the rheological properties of bread dough
10.30	Laura Oliver Hernández	Water sorption in starch and gluten films
10.50	<i>Coffee Break</i>	
11.10	Anita Hirte	How does protein content influence crispness of bread
11.30	Tímea Gelencsér	Monitoring of enzyme catalysed starch gel hydrolysis by the rapid visco analyser (RVA)
11.50	Marianna Soto Cámara	Near Infrared Spectroscopy: an interesting tool to monitor variety mixtures in wheat milling industry
12.10	Moustafa Saad	Application of X-ray photoelectron spectroscopy on the characterisation of surface chemical composition of wheat flour
12.30	Zsuzsanna Bugyi	Determination of allergenic proteins in wheat flour based food model systems - a scientific cooperation within MoniQA Network of Excellence
13.00	<i>Lunch</i>	
	Session 2	Wheat components and nutritional aspects
14.00	Mária Hódsági	Investigations of the effects of different storage conditions on native and resistant starches
14.20	Jenni Lappi	Degradation of fibre by colon fermentation in subjects consuming whole grain foods
14.40	Juhani Sibakov	Novel dry fractionation technology to produce oat ingredients with high beta-glucan and protein concentration
15.00	Gábor Balázs	Complex quality approach of wheat - analytical properties and possible applications of lab-on-a-chip technology in the protein profiling
15.20	<i>Coffee break</i>	
15.40	Roberta Lupi	Characterization of seed allergens present in transgenic and untransformed wheat lines
evening	<i>BBQ!</i>	

	Tuesday August 4	
8.30	Depart student housing	
8.45	Breakfast	
	Session 3	Agronomy, Breeding and Genetic Modification
9.30	Domenico Lafiandra	Keynote presentation
10.10	Irene Della Ghezza	SIGRAD: an integrated project for the Italian durum wheat-pasta production chain
10.30	Norbert Boros	Influence of nitrogen fertilizer treatments on winter wheat (<i>Triticum aestivum</i> L.) flour characteristics and their effect on dough quality
10.50	<i>Coffee Break</i>	
11.10	Angelica Giancaspro	A novel non-antibiotic/non herbicide selection system for durum wheat transformation
11.30	Eleonora Egidi	Proteomic analysis of transgenic durum wheat lines transformed with mutated m- and s-type LMW-GS
11.50	Frederica Paoletti	Modulation of starch granule distribution in durum wheat by silencing isoamylase-1 genes
12.10	<i>Lunch</i>	
14.00	Linda Botticella	A TILLING project applied to wheat starch improvement
14.20	Valentina Narducci	Quality parameters of conventional, partial waxy and full waxy grains of the Italian durum wheat cultivar Svevo
14.40	<i>Tour around the facilities</i>	
15.20	<i>Coffee Break</i>	
	Session 4	Ingredients and effects of processing
15.40	Josef Baumann	Keynote presentation
16.00	Liesbeth Derde	The action pattern of amylases on α -limit dextrins
16.20	Anna Patsioura	Study of enzymatic hydrolysis & characterization of oat flour hydrolyzates
evening	<i>dinner</i>	

	Wednesday August 5	
	Session 4	Ingredients and effects of processing
8.30	Depart student housing	
8.45	Breakfast	
9.30	Outi Santala	Enzymatic modification of wheat bran arabinoxylans at reduced water content
10.10	Annelies Duyvejonck	Flour properties as a basis for bread and cookie quality
10.30	Gabriella Bottega	The Debranning of Common Wheat as Innovative Tool in Milling
10.50	<i>Coffee Break</i>	
11.10	Julien Saint Paul	Comparative effects of two ozonation treatments On wheat flour technological properties
11.30	Lieve Lamberts	Parboiling of rice: changes in colour characteristics
11.50	Kraft Award	
12.15	adjourn	
12.30	<i>Lunch</i>	

Presentation Abstracts

Stress, Strain Hardening and Large and Fast Deformations during Dough Proofing

Peter L. Weegels

Innovation&Development/European Bakery Innovation Centre, Sonneveld Group BV,
Papendrecht, The Netherlands

During proofing bread dough seems to slowly moving and expanding. A close look inside the dough shows the opposite: deformation rates of 4-5 orders of magnitude larger than reported appear to occur. In view of these fast deformations strain hardening of dough as an important quality parameter of dough rheology will be discussed. Recent insights in rheological models will be applied on dough. The expansion of dough and effect on the increases in stress and the benefit of strain hardening will be demonstrated in a model.

Effect of mixer type, mixing time and dough formulation on the rheological properties of bread dough

F. Van Bockstaele^{1,2}, I. De Leyn¹, M. Eeckhout¹, K. Dewettinck²

¹Department of Food Science and Technology, Faculty of Biosciences and Landscape Architecture, University College Ghent, Voskenslaan 270, Ghent, Belgium

²Laboratory of Food Technology and Engineering, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium

Dough rheological properties are a key factor in the processing of bread products. Not only do they influence final product quality (loaf volume - crumb structure), they also have a major impact on the machinability of the dough. Next to flour quality and water absorption level, also the mixing process (type of mixer and mixing time) influences dough rheological properties. However, the effect of the mixing process on fundamental rheological properties is not yet well described. The effect of mixer type (farinograph and pin mixer) and mixing time on dough rheological properties determined by frequency sweeps (0.1-100Hz), creep-recovery and uniaxial extension experiments was investigated. A standard Belgian bread flour (12%db protein) was used and experiments were performed on a flour/water mixture, and on a mixture which also included salt (1.5%) and ascorbic acid (25 ppm). Mixing time and mixer type only slightly influenced G' and G'' . However, increasing the mixing time caused a decrease of the phase angle δ in the lower frequency range (0.1-1Hz) for both mixer types. This effect was not observed when salt and ascorbic acid were added to the doughs. The maximum creep strain of water-flour doughs decreased when mixing time was increased. Also, mixer type had a significant effect on maximum creep strain as mixing in the pin mixer caused a steeper decrease than mixing in the farinograph. Salt and ascorbic acid made the doughs more stable which resulted in a more constant maximum creep strain during mixing. Finally, also uniaxial extension tests were strongly changed by mixing time and formulation.

Water sorption in starch and gluten films

L. Oliver^{1,2}, C. Primo-Martín², M.B.J. Meinders^{2,3}

¹Instituto Universitario de Ingeniería de Alimentos para el Desarrollo, Universidad Politécnica de Valencia, Valencia, Spain.

²TI Food and Nutrition, Wageningen, the Netherlands

³Wageningen University and Research Centre, Wageningen, the Netherlands

Understanding water sorption in amorphous bio-polymers, like starch and gluten, is important in order to control the water transport in food systems. Food quality features, like e.g. crispness and caking, are affected by the water transport. In amorphous bio-polymer systems, the main mechanisms involved in water migration are water concentration gradient and polymer relaxation. It is possible to observe these mechanisms by performing experiments that traverse large ranges of temperature, water concentration, and polymer molecular weight or by varying the characteristic time of an experiment. In this work, water sorption of gluten and starch films were studied using gravimetric step-change sorption experiments. For both materials, films of different thicknesses were used with the aim of varying the characteristic diffusion time. Water adsorption was continuously measured while relative humidity (RH) was step-wise increased. In order to locate regions of Fickian diffusion, experimental results were compared with a Fickian diffusion model and an empirical (exponential) model. It turned out that the water sorption kinetics was described better by the Fickian diffusion model when the polymer is in the glassy state. Near the glass rubber transition, the exponential model described the water sorption kinetics better. Moreover, for gluten films in the glassy state, the diffusion constant appeared to be independent on the thickness. These results indicate that water-sorption kinetic in the glassy state may be controlled by the water concentration gradient (Fickian diffusion), while near the glass rubber transition it may be controlled by the water concentration gradient as well as matrix relaxation (non-Fickian, anomalous, visco-elastic diffusion). Diffusion constants of starch films were independent on the thickness only for very low RH, not presenting any relation with the glass rubber transition. This suggests that the starch films may present higher complexity than gluten films and that additional parameters related to e.g. the amount of gelatinization and/or crystallinity may influence the water sorption.

How does protein content influence crispness of bread

Anita Hirte ^{1,2}, Jorien Peters ², Rob Hamer ^{1,2}, Cristina Primo-Martín ¹

TI Food and Nutrition¹, Wageningen University², The Netherlands

Crispness is one of the most important sensory characteristics on which consumers base their appreciation of certain types of breads. Unfortunately, fast loss of crispness occurs just after baking, since water migrates from the relatively wet soft crumb to the dry crispy crust. This water migration through the open cellular matrix causes a fast and undesired decrease of the quality and shelf life of bread. The aim of our work was therefore to influence crispness retention of breads by using flours of different protein contents (9.1 % and 16.9 % protein). Crispness and water content of crust were evaluated during storage at a relative humidity of 40 % for 4 hours after baking. Both the water content of the crust and the crispness were significantly different between breads with different protein contents. Breads with lower protein content had a significant lower water content of crust and a significant longer crispness retention. These breads emitted during mechanical fracturing a higher number of sound pulses, a property known to relate to crispness. Furthermore, breads prepared with the two different flours had different water content of the crumb and had different morphologies. For example, the crumb of breads with low protein content is denser. To discern which one of these aspects is responsible for the differences in water transport and crispness retention we made an effort to distinguish effects from water content, morphology and crust permeability. The results of our work show that the driving force, i.e. the difference in water content of crumb and crust, has a large influence on crispness retention and that the decrease of protein content in breads is a successful tool to improve the quality of breads in terms of crispness retention.

Monitoring of enzyme catalysed starch gel hydrolysis by the rapid visco analyser (RVA)

T. Gelencsér, D. Szabó, A. Salgó

Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Budapest, Hungary

Monitoring of starch gel enzymatic hydrolysis is generally carried out in vitro using buffered enzymes and special incubation conditions. By sampling in function of time the disappearance of the substrate or the appearance of the products can be investigated. Amylolytic procedures, however, are usually complicated and preparations need lots of time and manual work, therefore methods correlated to hydrolysis may play notable role. Previous studies have used NIR and FT-IR spectroscopic methods for monitoring the starch digestion, now in this research RVA has been used to follow up this process. The enzyme catalysed disruption of starch gel and the fall of viscosity in RVA may show strong relationship with the amount of glucose liberated during the hydrolysis.

In the RVA Std1 profile with modifications was used to get the starch gel, in the next step buffered alpha-amylase solution was added (1, 1.5, 2, 3, 4, 6, 8, 10 Unit in 50 µl phosphate buffer, pH=6.9) and the fall of viscosity was followed up in a 3 h long period of time.

Starch gel enzymatic hydrolysis in centrifuge tubes using the same enzyme solutions was also carried out and the amount of liberated glucose was measured after 30, 60, 90, 120 and 180 minutes. Measurements were evaluated and mathematical models were fitted to describe the measured data. Correlation between raw data (viscosity and glucose) as well as the parameters of the most adequate mathematical models was calculated.

Results showed that the disruption of starch gel can be described by exponential equation and the correlation between viscosity and liberated glucose values is strong (above 0.8 in all cases). The common correlation coefficient between methods is 0.79 highlighting the adequacy of RVA in the monitoring of starch hydrolysis.

Near Infrared Spectroscopy: an interesting tool to monitor variety mixtures in wheat milling industry

Soto M. , Font R., Del Río-Celestino M., España F. and Domínguez J.

IFAPA- Centro Alameda del Obispo. Apartado 3092, E-14080 Córdoba, Spain.

Nowadays, milling industry is more demanding on the quality and safety of their products. Over the years, cereal researchers have been interested on how visible and near infrared spectroscopy (Vis-NIR) could be applied for characterizing wheat varieties with precision as well as to discriminate among them; the success of the technique has been shown to be highly dependent on the wheat varieties used. The aim of the present work was to provide a rapid and accurate method for differentiate wheat varieties, seeking to avoid wheat adulteration.

Five different wheat varieties of soft wheat (*Triticum aestivum* L.) grown in different locations of Andalusia (Southern Spain) under different climatic and edaphic conditions over four years (2004-2008), were scanned on an NIRS spectrophotometer (model 6500 Foss- NIRSystem, Inc., Silver Spring, MD, USA) in the reflectance mode over the 400 nm to 2500 nm region and recorded as $\log(1/R)$ at 2 nm intervals, using the WinISI III version 1.5 spectrum analysis software program for the development of mathematical models (PLS) able to discriminate among sample spectra.

All the five wheat varieties used in this study were correctly classified in their respective classes, so indicating that Vis-NIR analysis could be an useful tool for wheat classification and discrimination.

Application of X-ray photoelectron spectroscopy on the characterisation of surface chemical composition of wheat flour

M. M. Saad ^a, C. Gaiani ^b, M. Mullet ^c, J. Scher ^b, B. Cuq ^a

^a Montpellier SupAgro - INRA, UMR 1208 Unit for Emerging Technology and Polymer Engineering, 2, place VIALA, 34060 Montpellier CEDEX 1, France

^b LIBio, Laboratoire d'Ingénierie des Biomolécules, Nancy Université, 2 avenue de la Forêt de Haye, B.P. 172, 54505 Vandoeuvre Les Nancy Cedex, France

^c LCPME, Laboratoire de Chimie Physique et de Microbiologie pour l'Environnement, UMR 7564 CNRS – Nancy Université, 405, rue de Vandoeuvre, 54600 Villers Lès Nancy, France

The surface of wheat flour particles is supposed to play a basic role as the interface to particle-particle and particle-water interactions. So far, a lot of studies have been carried out to characterize the bulk chemical composition of wheat flour, but only few works focused on the surface chemical composition.

In this work we develop a method to quantify the relative coverage of components on the surface of wheat flour particles by using the X-ray photoelectron spectroscopy (XPS). The analyses of XPS are carried out on fractions separated from the flour main components (protein, starch, arabinoxylan, and lipids). Simplified flours, obtained by mixing protein and starch (separated from the native wheat flour) in addition to several native flours are also tested by XPS.

XPS results are analyzed in order to identify the main elements (O, C, N, and S) on the surface of particles of the examined samples and also to determine their relative concentrations. Based on these concentrations, we calculate the relative coverage of components for all flours by using a matrix. XPS is able to distinguish polysaccharides, protein and lipids. As expected, it is not able to distinguish starch and arabinoxylan due to the similar chemical structure. For the tested simplified flours, the calculated relative coverage and the bulk chemical composition are similar. However, for the native flours it can be observed that the relative coverage is different from the bulk chemical composition. Compared to bulk chemical composition, an overrepresentation of protein and lipids and underrepresentation of polysaccharides on the surface of particles are reported. Results are discussed with regard to the potential mechanisms of wheat flour milling, that is to say the formation of wheat flour particles.

Determination of allergenic proteins in wheat flour based food model systems - a scientific cooperation within MoniQA Network of Excellence

Zs. Bugyi, S. Tömösközi

Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest, Hungary

Food allergy is an abnormal immunological response to certain proteins in food. The statistical data shows that within the adult population the prevalence of food allergies is 3-4%, in case of children prevalence is 4-8%. Usually the only treatment of food allergy is the total avoidance of foodstuffs which contains the allergenic proteins. According to the EU directives, the most important 14 components of foods which can cause allergic reactions (“big fourteen”) must be labelled.

There are many difficulties to keep away the allergenic components from the whole food chain. One of them is to find and apply suitable analytical methods and to evaluate the obtained results. Today, many dedicated and user-friendly immuno-based methods (ELISA, dipstick) are used in the every day practice. DNA-based methods are also available and HPLC-MS is also used in research labs. However, during the validation of methods and the quality assurance processes of results, the main problem is originated from the behaviour of these proteins in real food matrices, which is not exactly known. The effects of protein degradation during the food processing on the analytical results are also questionable. This issue is one of the hot topics of the activity of the EU funded Network of Excellence, MoniQA (Monitoring and Quality Assurance in the Food Supply Chain). As the Hungarian member of this network, the aim of our work is to examine such processed food matrices which contain the allergen components in dedicated amount. The examined allergen components are milk and egg, the processed food matrix is wheat flour based cookies.

In the preliminary phase of the work a proper recipe was evaluated and the first model products were made. The analytical examination of these samples was carried out by using indirect competitive and sandwich ELISA methods. As a result of analytical measurements we observed a large-scale decrease of the protein amount after baking. This phenomenon may have different explanations: a total or partial heat denaturation which can cause lower extractability, minor modifications of the protein structure which can cause less detectability during the ELISA method or interactions between the observed protein and other food components. To study these assumptions is the main goal of our work – which requires the application of other analytical and chemical approaches.

Acknowledgements: This work is carried out with the financial support of MoniQA project and the fellowship for PhD students awarded by the government of Hungary. The ELISA measurements were implemented with the professional support of Hungarian Central Food Research Institute.

Further information: <http://www.moniqa.org>, <http://biokemia.bme.hu>

Investigations of the effects of different storage conditions on native and resistant starches

M.Hódsági, T. Gelencsér, Sz. Gergely, A. Salgó

Department of Applied Biotechnology and Food Science, Budapest University of Technology and Economics, Hungary

One of the main problems recently is obesity in the developed countries. The most consumed foods are rich in carbohydrates. These products are easily digestible resulting in high glucose level in the bloodstream. A promising solution in lowering blood glucose level after eating can be the consumption of food prepared with resistant starch (RS) additives, resistant starch namely resists the digestion of amylolytic enzymes.

The investigations of the starches during storage are important because the changes in their structure can cause differences in availability, digestibility and other parameters as well. Our aim was to investigate the changes of different native and resistant starches during storage at different temperatures followed by enzymatic (hydrolysis by alpha-amylase), spectroscopic and physical methods.

The starches were stored in a fridge at +4°C and in an incubator at 22°C. The digestibility measurements were carried out with two parallel samples in a half-year period, sampling every month, and once a half year later (one year long study). Additionally, the starches were cooked as well, at 100°C, 5 minutes long and then they were digested. The dry matter content and the water absorption capacity of the starches were also determined. Furthermore, the samples were scanned with near infrared spectroscopy during the storage procedure.

The results showed that the different storage temperatures as a factor did not have any significant effects on the values of the water absorption capacity, the enzymatic digestibility and the near infrared spectra of the starches. However, significant differences in the digestibility of the starch samples were observed during the storage, in both uncooked and cooked cases, but no tendencies could be traced. The spectroscopic method has the potential to highlight the changes of the starches.

Degradation of fibre by colon fermentation in subjects consuming whole grain foods

J. Lappi¹, U. Holopainen², H. Mykkänen¹, M. Kolehmainen¹, K. Poutanen^{1,2}.

¹Food and Health Research Centre, Department of Clinical Nutrition, School of Public Health and Clinical Nutrition, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland; ²VTT, P.O. Box 1000, FI-02044 VTT, Finland

Increased consumption of whole grain foods is associated with a reduced risk of type 2 diabetes in epidemiological studies. However, mechanisms underlying the association are unclear. It has been suggested that fermentation of insoluble cereal fibre by colonic microbiota has a role in decreasing the risk of type 2 diabetes, and phenolic compounds bound to the aleurone cell walls may be involved. Release of phenolic compounds is dependent on the ability of colon microbiota to degrade fibre.

The aim of the present study is to investigate the degree of degradation of fibre in subjects consuming whole grain based diet.

A 12-week intervention study was conducted with two groups of subjects with metabolic syndrome, of which one consumed whole grain based diet, and the control group consumed refined grain foods. Faecal spot samples were collected from the subjects in the beginning and at the end of the intervention, and frozen in -20°C.

Degradation of fibre in freeze-dried faecal samples will be analysed microscopically. Five representative samples will be chosen from the group of subjects consuming whole grain based diet both in the beginning and at the end of the diet period. In addition, two control samples will be chosen from the subjects consuming refined grain foods. The samples will be fixed, embedded in methylacrylate resin, and sectioned for staining. For microscopic examination, protein and cell walls in the samples will be visualised with Acid Fuchsin and Calcofluor White, respectively. In exciting light, protein appears red, cell walls light blue, and starch remains unstained.

Test breads consumed by the subjects on whole grain based diet will also be microscopically analysed. The degree of degradation of fibre within and between subjects will be qualitatively defined by comparing the microstructure of fibre in breads with that in faeces. The degree of degradation of fibre will also be compared with changes in microbiota of the faeces, and content of polyphenol metabolites. We hypothesise that degree of fibre degradation is associated with changes in microbial composition favouring fibre degradation and release of polyphenol metabolites.

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Novel dry fractionation technology to produce oat ingredients with high beta-glucan and protein concentration

J. Sibakov,¹ O. Myllymäki,¹ V. Hietaniemi,² J.-M. Pihlava,²
A. Kaukovirta-Norja,¹ K. Poutanen¹ & P. Lehtinen¹

¹VTT Technical Research Centre of Finland, P.O. Box 1000, FI-02044 VTT, Finland

²MTT Agrifood Research Finland, FI-31600 Jokioinen, Finland

Oat is an ideal raw-material for healthy and functional food products due to its high content of both soluble and insoluble dietary fibre. The insoluble fibre consists mostly of mixed linkage (1→3)(1→4)-β-D-β-glucan, which has been proved to have beneficial effects on serum cholesterol as well as on postprandial blood glucose and insulin level attenuations. Nutritionally, oats contain high amounts of lipids with beneficial fatty acid composition, proteins rich in valuable amino acids, as well as essential vitamins and minerals. However, production and use of high-fibre oat fractions is technologically challenging due to susceptibility of β-glucans to depolymerisation and formation of viscous gels at very low concentrations.

In the present work, a dry fractionation method to obtain high purity β-glucan concentrates was developed. The utilisation of other oat fractions: protein, starch and lipids was also studied. The oat flour was first defatted by supercritical CO₂, and then milled and air-classified to increase the amount of β-glucan in the bran fraction. We conducted the process both in pilot- and in industrial scale. Removal of lipids prevented the formation of lumps, especially during the milling. In addition, lipid removal enabled more effective separation of bran, starch and protein particles, and the β-glucan concentration of the bran fraction was able to be increased to 35 %. The process was optimised in terms of the mass yield of bran fraction and its β-glucan concentration. Other valuable fractions obtained were: a protein concentrate with over 70 % of protein, a starch enriched fraction with 70-80 % of starch and an endosperm β-glucan concentrate with 40 % of β-glucan. Overall, the process was economical, as expensive and energy-consuming drying operations were avoided.

Complex quality approach of wheat - analytical proprieties and possible applications of lab-on-a-chip technology in the protein profiling

G. Balazs¹, A. Harasztos¹, A. Bagdi¹, S. Tomoskozi¹, F. Bekes²

¹ Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Budapest, Hungary, ²CSIRO, Food Futures National Research Flagship, Canberra, Australia

Nowadays the expected quality of cereal and wheat based products trends toward to breed new species with unique and functional properties. These challenges in research require more *complex approach of quality* and more *efficient and innovative solutions* in analytical works. Numerous promising aspects exist for describing the complex term of quality, and the required chemical information (starch, protein and other minor component – such as arabinoxylan (AX), alkylresorcinols (AR) – content and quality) which is the base of the desirable raw material selection. In analytical point of view, beside the conventional methods (such as SDS-PAGE, RP and SE-HPLC etc.), using a new rapid, and automated techniques to determinate the protein composition is get more and more important.

Microchip capillary electrophoresis seems to be a valuable and flexible analytical methodology at present time. With merging of conventional capillary electrophoresis and microfabrication, the commercially available *lab-on-a-chip (LOC) technology* has a significant promise to improve the capability of analysis for various biological molecules, like protein in this study.

In our research work, we applied and optimized a one step extraction based method for LOC, and to get detailed information about the method capacity, and the important analytical parameters, namely: accuracy, precision, selectivity and robustness were measured, and compared with the traditional SDS-PAGE - using *wheat samples*. The sample preparation with micromill (which was developed at our department earlier) found to be more suitable comparing with the previous described methods, and the analytical parameters were suitable for our purpose. The method was applied for *wheat variety identification* and additionally other cereal and pseudo-cereal samples were also studied (barley, millet, quinoa, and amaranth). The newest result of our work is the protein characterization and comparison of different varieties of *sweet sorghum seeds*. These results and their possible application in the selection of varieties will also be presented.

Furthermore, an optimized sample preparation method was developed, for the determination of wheat glutenin alleles with LOC, *focusing on the LMW-GS*. According the result, the different allele combinations were partly identifiable. We would like to apply these results to get detailed information about the correlations of glutenin subunit composition and functional qualities of cereals, and about the G X E (Genotype x Environment) interactions. According this purpose, beside the LOC, other methods is applied (RP and SE-HPLC, GC), and minor components (like AX), will be determinate to get more information about the *correlation of the chemical composition and the complex quality (technological and functional) of wheat*.

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Characterization of seed allergens present in transgenic and untransformed wheat lines

R. Lupi^{1,2}, F. Pineau¹, G. Deshayes¹, DA. Moneret-Vautrin², Y. Popineau¹, S. Denery¹, D'Ovidio R.³, Lafiandra D.³, Anderson O.D.⁴, S. Masci³, C. Larré¹

¹ UR1268, INRA BIA, Rue de la Géraudière, BP 71627, 44316 Nantes, France

² Clinical immunology and allergology, University hospital, 54035 Nancy, France

³ Dipartimento di Agrobiologia e Agrochimica, Università degli Studi della Tuscia, Via S. Camillo de Lellis s.n.c, 01100 Viterbo, Italy

⁴ USDA-ARS, WRRRC, Albany, CA, USA

Wheat is one of the most important crops in the world in terms of human nutrition. Both technological and nutritional properties of wheat flours and wheat-derived foods are determined by kernel proteins. As regard health aspects, wheat belongs to the group of six foods, identified by the Codex Alimentarius, as eliciting IgE-mediated allergic responses, such as skin pathologies, gastrointestinal and respiratory disorders, these later mostly determined by the soluble protein fraction. It is general opinion that there is an increase in wheat allergic pathologies. The problem of allergy to plant proteins has received increasing attention also in regard to the issue of “substantial equivalence” of genetically modified (GM) plants. Since the first discussions on strategies to assess the food safety of GM crop plants, assessment of GM plants and derived tissues has been based on comparisons with their traditionally bred counterparts.

As in other crops, many strategies are explored to produce genetically modified wheat lines for research purposes aimed at increasing technological and nutritional quality. A specific transformed bread wheat line strongly over-expressing a low molecular weight glutenin subunit, in comparison with the corresponding untransformed genotype, showed a strong decrease of the soluble protein fraction, likely as a compensatory response to transgene over-expression, involving, in general, a significant decrease in the amounts of polypeptides belonging to the prolamin superfamily (Scossa et al, 2008). The objective of this work is to get information on the impact of such a gene transfer event on the expression of endogenous allergens.

Wheat proteins are composed of three classes according to their solubility : the water/salt-soluble albumin/globulin (A/G), the ethanol-soluble gliadins (Gli) and the glutenins (Glu). Among these fractions, the albumin/globulin fraction is involved in food allergy to wheat as well as in baker's asthma. In this study, sera from children and adults with clinically documented wheat allergy are used. Our investigation focuses on the comparison of the A/G fractions obtained from this particular genetically modified wheat (GM-Wheat) and the untransformed line, along with other wild-type bread wheat genotypes. For the comparison we have used ELISA test, 2D and 1D immunoblots on A/G fraction and Gli, Glu fractions respectively. Analysis will be completed with identification of allergenic polypeptides by mass spectrometry. This work should lead to the characterization of the potential allergenic risk of this GM-wheat compared to other non-transgenic lines.

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SIGRAD: an integrated project for the Italian durum wheat-pasta production chain

I. Della Ghezza¹, M. Atallah¹, H. Lavorano², M. Silvestri¹

¹ Grains and Food Plants Research Unit, Barilla G. e R. F.lli S.p.A., Italy

² H&L Strategies

S.I.GRA.D (Inter-professional Durum Wheat Society) is a Consortium that aims at the production and exchange of high quality Italian durum wheat. Partners of this Consortium are several wheat producers and co-operative storage centres, one seed company, four industrial milling companies and two professional organizations. The S.I.GRA.D. Consortium originated from a government financed project that aims to improve the competitiveness of the durum wheat production chain: in order to achieve this goal, different kinds of activities have been funded: EDP applied to the supply chain, Communication & Promotion, Commercial Agreement and Scientific Research. Barilla is involved in coordinating R&D in the fields of Food Safety and Environmental Sustainability.

The studies in the Food Safety area have been focused on different issues, like cadmium accumulation in plants, raw material traceability, monitoring of micotoxins and pests control. In more detail, the specific objects of the projects are: 1) the evaluation of the genetic variability of durum wheat lines for the inclination to cadmium translocation and accumulation, and the development of molecular markers correlated to cadmium absorption; 2) the realization of a traceability system of the wheat production chain, to allow the complete traceability of the raw material from the field to the mill, and a study to optimize sanitary controls along the production chain; 3) the development of a new methodology for the selection of durum wheat lines tolerant to *Fusarium spp.* and the development of knowledge and new techniques for the detection of toxins T-2 and HT-2, to be used for monitoring along the production chain; 4) the evaluation of the effectiveness of different methodologies for the control of infesting insects in storage facilities.

The second research area coordinated by Barilla is Environmental Sustainability. In this field, the project has focused on the utilization of durum wheat waste biomasses, and specifically, the development of co-generation systems for the production of thermal and electric energy from durum wheat straw and milling by-products.

Influence of nitrogen fertilizer treatments on winter wheat (*Triticum aestivum* L.) flour characteristics and their effect on dough quality

N. Boros, M. Borbély, Z. Győri

Institute of Food Science, Quality Assurance and Microbiology, Faculty of Agriculture, University of Debrecen, Debrecen, Hungary

The application of constant flour qualities is of decisive importance for the milling and baking industries. Different baking products require different flour qualities. The rheological characteristics of dough are determined by the amount and proportion of gliadin and glutenin proteins, and their significance lies in the fact that they affect both the processing ability of the dough and the quality of the end product. Probably the most significant factor in determining the baking quality of winter wheat is the genetic background, as it primarily accounts for the amount and quality of gluten proteins. However, other factors, such as cropping year, site, mineral fertilization also have considerable importance.

In our study we have examined the effects of mineral fertilization on the extensigraph characteristics of winter wheat (*Triticum aestivum* L.) flours. In total, 192 flour samples were analyzed for extensigraph properties. The applied treatments were the following: a control, 30 kg ha⁻¹ nitrogen, 22.5 kg ha⁻¹ P₂O₅ and 26.5 kg ha⁻¹ K₂O and the double, threefold, fourfold and fivefold amount of these doses in four repetitions. Flours were from four diverse cultivars (Öthalom, Lupus, Saturnus, and Sixtus) grown at six nitrogen fertilizer levels over two years. The average wet gluten content of cultivars was very different (25.7, 29.3, 33.4, and 29.6%, respectively). Dough properties were determined by the Brabender Extensigraph according to Approved Method 54-10 (AACC International 2000) after a 135-minute rest period.

The rheological features of the studied wheat cultivars were very different from one another. Lupus and Saturnus could be characterised with excellent extensigraph parameters, while the other two cultivars (Öthalom and Sixtus) are only average cultivars in respect of rheological properties. We have found that mineral fertilization improved significantly the extensigraph properties. The measured extensigraph parameters (area under the curve, extensibility, resistance to extension, and maximum resistance to extension) had improved by rise of additional mineral fertilizer ratio to the 120 kg ha⁻¹ N + P,K fertilization level, but the further increase of fertilizer ratio did not improve the rheological parameters, except for the extensibility of the dough.

A novel non-antibiotic/non herbicide selection system for durum wheat transformation

A. Giancaspro*, A. Gadaleta*, D. Rosellini**, F. Veronesi**, A. Blanco*

*Department of Environmental and Agro-Forestry Biology and Chemistry, sect. of Genetics and Plant Breeding, University of Bari, Via Amendola, 165/A -70126 Bari, Italy

**Dipartimento di Biologia Vegetale e Biotecnologie Agroambientali e Zootecniche, Università degli Studi di Perugia, Borgo XX Giugno 74 - 06121 Perugia, Italy

The majority of selectable markers employed in transgenic plants production are genes conferring to the plant cells a resistance to toxic compounds such as herbicides or antibiotics. However, the presence of these genes in crop plants grown in open field or destined to human and animal consumption is useless as they serve only in the selection phase, and “unsafe ” because of their potential harm to environmental and human health.

In the present work a novel alternative selection system was proposed for the first time in durum wheat biolistic transformation, which employs a gene conferring resistance to a phytotoxic chemical different from antibiotics and herbicides. This “safer” marker is the *hemL* gene from *Synechococcus* strain GR6, coding for a mutant form of the enzyme GSA-AT (glutamate-semialdehyde aminotransferase) which is insensitive to the phytotoxin gabaculine. The *hemL* gene could be a good candidate for a safer selection system as it is present in all plant species and it has never been used as an herbicide. In this work the efficiency of the *hemL* gene as a selectable marker was compared with the conventional *bar* gene from *S. Hygroscopicus* which confers resistance to Bialaphos herbicide. A study on gabaculine toxicity was conducted to investigate the phytotoxic effects on wheat calli embryogenesis, and to establish the best concentration for the transformant selection. A co-transformation experiment was carried out on durum wheat calli by delivering the plasmids pAPCK-*hemL* and pAHC20-*Bar*, carrying the *hemL* and the *bar* genes respectively, and separately performing selection with gabaculine and Bialaphos. The *hemL* proved a very efficient selectable marker for durum wheat transformation, in fact it performed as well as the *bar* gene in the selection of the transformed plants, and it allowed to recover a 100% selection efficiency.

Proteomic analysis of transgenic durum wheat lines transformed with mutated m- and s-type LMW-GS

E. EGIDI¹, S. MASCI¹, M. JANNI¹, F. SESTILI¹, A. CERIOTTI², R. D'OVIDIO¹, D. LAFIANDRA¹

¹DABAC, University of Tuscia, Viterbo, Italy; ²IBBA, CNR, Milan, Italy

Gluten, the major protein fraction of grain, confers the unique properties of elasticity and extensibility on wheat doughs, that make them suitable for the production of bread, pasta, cakes, etc. These properties are mostly due to the presence of seed storage proteins, called prolamines. The prolamines are divided into two groups: gliadins and glutenins. These latter are complex polymers held together by intermolecular disulfide bonds and are divided, according to their molecular weight, in high (HMW-GS) and low (LMW-GS) molecular weight subunits, whose structure and size greatly influence gluten rheological properties.

The LMW-GS represent a very abundant and heterogeneous fraction and their allelic variability is correlated with the quality of dough, especially in durum wheat. Among the various classes of LMW-GS present in wheat grain, the most abundant are LMW-m and LMW-s, so called according to the first amino acid of mature sequence (methionine for LMW-m and serine for LMW-s).

The two classes of LMW-GS differ mainly for the presence of three additional N-terminal amino acids in the LMW-m types. The comparison between the amino acids sequences of the signal peptides of the two types has suggested the possibility of a different maturation process. It has been noted in fact, that LMW-s have an asparagine residue at position 23, which is replaced by a threonine residue in the LMW-m. Thus we have hypothesized that the presence of the Asn residue in LMW-s types could determine a differential processing at the N-terminal end, that might generate the cleavage of the peptide MEN by an asparaginyl endoprotease.

In order to shed light on this mechanism, we have taken into consideration two proteins: a presumed LMW-m (LMW-B1133) and a LMW-s (LMW-42K) types. The corresponding coding genes were mutated in position 23 in order to have a threonine instead of asparagine and viceversa. The mutated gene versions (B1133T23N and LMW 42KN23T) along with the wild type ones (LMW-B1133 and LMW-42K) were previously used for transient heterologous expression in *Nicotiana benthamiana* plants, but these experiments did not give any reliable result, because of difficulties in obtaining sufficient amounts of heterologous polypeptides for their analysis.

For this reason, the same genes have been used to transform, using the biolistic method, plants of durum wheat (cv. Svevo), and two different tags (His and FLAG tags) were added at the C-terminus, in order to facilitate the recognition and purification of the encoded proteins.

Data about the characterization of these transgenic plants are presented.

MODULATION OF STARCH GRANULE DISTRIBUTION IN DURUM WHEAT BY SILENCING ISOAMYLASE-1 GENES

PAOLETTI F., SESTILI F., JANNI M., BOTTICELLA E., D'OVIDIO R.,
LAFIANDRA D.

Department of Agrobiological and Agrochemistry of University of Tuscia, Italy

RNA interference, starch, durum wheat, debranching enzymes

Reserve starch represents 70% of the wheat seed and it is physically organized in semicrystalline structures called granules localized in the stroma of the plastids. Two types of granules, different in shape and size, are present in wheat: type-A with a larger dimension (10-100 μ m); type-B with smaller size (1-10 μ m). Starch Granule size distribution is dependent on the plant genotype and it has been seen to strongly affect starch chemical-physical properties. The two types of granules have been shown to confer different functional properties to the flours and semolinas. For instance in the bread making process granule-A are believed to be responsible of increasing the resistance to mixing, while type-B increases the extensibility.

In barley two natural mutants defective in the expression of the gene coding isoamylase showed starch granules abnormal both in shape and in size. In potato, reduction of the the activity of the two isoamylase, *Stisa-1* and *Stisa-2*, has resulted drastic changes in granules size distribution with the presence of very small granules not observed in the wild-type genotype.

In this work to modify the structure and distribution of starch granules in durum wheat, a transgenic approach has been undertaken. In particular, RNA interference has been used to silence *isoamylase-1* genes (*Iso-1*). A DNA amplicon corresponding to the region between exon II and exon VI of the gene *Iso-1* has been isolated by RT-PCR from durum wheat and introduced in both sense and antisense orientation in the RNAi vector. The two complementary fragments are interspaced by a sequence corresponding to the third intron of the *SBEIIa* gene playing the role of loop. Moreover the construct contains an endosperm-specific high-molecular-weight glutenin (Dx5) promoter.

In total 800 embryos of durum wheat cultivar Svevo have been transformed by the biolistic approach in order to silence the genes coding the isoamylases. In future, the effect of the elimination of the isoamylasic activity on granule size distribution will be evaluated.

A TILLING PROJECT APPLIED TO WHEAT STARCH IMPROVEMENT

BOTTICELLA E.* , SESTILI F.* , PHILLIPS A.** , LAFIANDRA D.*

*) *Department of Agrobiological & Agrochemistry, University of Tuscia, Italy*

**) *Rothamsted Research, Harpenden, United Kingdom*

Starch branching IIa, high amylose starch, TILLING, High Melting Resolution , wheat

A new challenge to develop novel wheats has been recently identified in the manipulation of starch composition. The modulation of amylose/amylopectin ratio greatly affects wheat flour nutritional and processing properties. Currently many efforts focus on the objective of increasing the amylose fraction in cereal starches. Higher amylose content correlates with an increased amount of “resistant starch”, a particular starch fraction, considered to have beneficial effects on human health lowering the risk of important diseases .

The two starch components are synthesized from a common substrate, ADP-glucose, by two distinct pathways. Amylose production involves one enzyme – Granule Bound Starch Synthase I (GBSSI) - whereas three classes of enzyme, known as starch synthases (SSs), branching (SBEs) and debranching enzymes (DBEs) take part to the synthesis of amylopectin.

Starch branching enzymes IIa are one of the main targets to increase amylose content in cereals. Recent studies, in bread (Regina *et al.*, 2006) and durum wheat (Sestili *et al.*, 2009) have shown that the RNA interference technology can result efficiently in the loss of SBEIIa functionality, leading to a drastic change of the amylose/amylopectin ratio compared to the wild type.

TILLING -Targeting Induced Lesions In Genomes- represents an effective reverse genetics tool for the production of novel allelic variants functional to crop improvement. In TILLING the combination of traditional chemical mutagenesis with high-throughput detection methods of point mutations has originated an attractive strategy both for functional genomics and breeding applications overcoming limits associated with transgenic issues.

In this work genotyping of a TILLING library of bread wheat has been carried out to identify novel allelic variants in the three homoeoalleles *wSBEIIa* (located in A, B and D genomes) with the aim to negatively affect the enzyme activity in starch biosynthetic process.

The targeted regions of the three *SBEIIa* genes have been analyzed in approximately two thousand wheat lines by High Resolution Melting technology, based on the comparison of the melting behaviours of the PCR fragments analyzed. Forty eight mutant genotypes have been characterized for *wSBEIIa-A*, ten for *wSBEIIa-B* and 25 for *wSBEIIa-D* confirming a mutation frequency of 1 SNP for each 40 kb associated to the TILLING library.

Worthy of note two SNPs causing the truncation of the gene product have been identified for *SBEIIa-A* gene and one analogous mutation for *wSBEIIa-B* gene. For all three homoeologues different mis-sense mutations causing the change of conserved residues located in catalytic domains of SBEIIa enzymes have been identified. Combining those mutations believed to affect enzymes functionality will be carried out to develop novel wheat genotypes with a highly increased amylose content.

Quality parameters of conventional, partial waxy and full waxy grains of the Italian durum wheat cultivar Svevo

**V. Narducci¹, F. Mellara¹, V. Turfani, G. Colaprico², M. Urbano², B. Margiotta²
and M. Carcea¹**

¹ Istituto Nazionale per la Ricerca sugli Alimenti e la Nutrizione (INRAN), Roma, Italy

² CNR Institute of Plant Genetics (CNR-IGV), Bari, Italy

The introduction of the “waxy” character in wheat plants represents a subject of interest in cereal science and technology because of the peculiar characteristics possessed by waxy wheat. Waxy starch is composed essentially by amylopectin with only traces of amylose and, as a consequence, the nutritional and the technological properties of waxy wheat are deeply affected. The development of waxy xy lines from wheat, and the assessment of their characteristics and of their functional properties in relation to specific end-products are the object of extensive studies worldwide. Our study aimed at characterizing waxy durum wheat lines derived from an Italian cultivar widely used in the manufacture of traditional Italian wheat products. The Italian cv Svevo, commonly used for pasta making thanks to its good gluten qualities, was crossed with the Japanese bread wheat cultivar Kanto 107 lacking the Wx-A1 and Wx-B1 proteins. Electrophoretic selection of backcrossed generations permitted the production of the entire set of partial waxy lines along with the total waxy in durum wheat.

Grains of the conventional cv., of the two partial waxy lines and of the full waxy line, coming from two harvests (years 2007 and 2008), were analysed for their protein, ash, soluble and insoluble fibre content, SDS sedimentation index, dry gluten, gluten index, α -amylase activity, resistant starch content and thousand kernel weight.

As regards kernel characteristics, the thousand kernel weight was higher for the waxy-7A line and for the conventional cv., while it was lower for the waxy-4A and for the full waxy lines. Ash content was lower in the conventional cv. instead, higher in the full waxy line and intermediate in the two partial waxy lines. The samples had increasing protein content in the order: waxy-4A, conventional, waxy-7A, full waxy.

As regards protein quality indicators, the relative SDS sedimentation index was not found to be different in the samples, whereas the gluten index was halved in the full waxy line with respect to the conventional cv and to both partial waxy lines.

As regards parameters related to starch, α -amylase activity was higher in the full waxy and in the waxy-4A lines, lower in the conventional cv. The resistant starch content was very low in all samples and therefore it was impossible to detect any difference.

Similar behaviours are reported in the literature for ash content, α -amylase activity and thousand kernel weight in other durum wheat cultivars and full waxy lines.

The action pattern of amylases on β -limit dextrans

Derde, L., Fraiponts, A., Putseys, J., Gomand, S., Delcour, J.A.

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Leuven, Belgium

The shelf life of wheaten bread can be extended by addition of certain amylases to dough. Two anti-firming amylases are a maltogenic amylase from *Bacillus stearothermophilus* (BStA) and a maltotetraose forming exo-amylase from *Pseudomonas saccharophila* (G4). However, the difference in their underlying mechanism and the way these are reflected in different effects on bread crumb firming and crumb resilience are far from being fully understood. In the present study, the action pattern of these and other amylases (endo- α -amylases from *Bacillus subtilis* and porcine pancreas and a thermostable exo- β -amylase from *Clostridium thermosulfurogenes*) were studied. The amylases were characterised and their action pattern on amylose and amylopectin was determined by measuring the decrease in iodine staining capacity as a function of the production of reducing sugars. Different non-random hydrolysis patterns were found for the amylases. Secondly, β -limit dextrans were produced to study the hydrolysis behaviour of amylases on the inner chains of amylopectin structures. To this end, a suspension of *waxy* maize starch was incubated with β -amylase until hydrolysis was complete. The remaining β -limit dextrans were incubated with the amylases of interest. The degree of hydrolysis was monitored as a function of time. In addition, the produced hydrolysis products and the remaining β -limit dextrin fine structure were analysed by High Performance Anion Exchange Chromatography and High Performance Size Exclusion Chromatography, respectively. Differences in hydrolysis products and residual β -limit dextrin fine structure were found for the various amylases. These differences reflect the action pattern of the amylases.

Study of enzymatic hydrolysis & characterization of oat flour hydrolyzates

A. Patsioura, V. Gekas, A. Lazaridou, C. Biliaderis

Laboratory of Transport Phenomena and Applied Thermodynamic, Department of Environmental Engineering, Technical University of Crete, Chania, Crete.

Due to the problem of lactose intolerance and also due to certain nutritive considerations, such as fat content, an array of non-dairy products has been developed in the last decades. In the place of lactose a product of starch hydrolysis, namely the maltose, is contained in those products. A thorough study of the enzymatic hydrolysis of oat starch by the combined action of α -amylase and β -amylase is required in order to optimize the performance of the enterprises dealing with the subject of consumer-friendly non-dairy products. The aim and the focus of the present work is at the understanding, in the engineering spirit, the processes of the productive line, especially the enzymatic conversion part of it. Understanding the role of the intervening parameters, it will potentially help to improve the performance of the industrial unit by proper readjustments of critical parameters, such as temperature, enzyme amount and proportions between the enzymes.

The degree of starch hydrolysis (%) was investigated versus process variables, including substrate concentration, enzyme proportions, time and viscosity. The analytical method used for the quantification of each sugar (glucose, maltose and maltotriose) was HPLC. The flow behavior and viscoelastic properties of the dispersions as well as the time dependence of viscosity changes (on a steady shear rate mode) during enzymatic hydrolysis were monitored by a rotational Physica MCR 300 rheometer. The highest maltose content was obtained by the concurrent action of the two enzymatic preparations, whereas under the action of the α -amylase alone the highest maltotriose content was observed. As for the viscosity profiles of the aqueous dispersions by the action of the three enzymatic preparations (α -amylase alone, β -amylase alone & combination of those) under continuous stirring, it is clear the reproducibility was quite good, particularly when a high concentration of the α -amylase was employed due to the effectiveness of this enzyme as a starch thinning preparation. Indeed, viscosity profiles were studied in 3 oat fractions of the starting material. The course fraction (particle size $>500\mu\text{m}$) seemed to be the more viscous, whereas the fine fraction (particle size $<250\mu\text{m}$) exhibited the lowest viscosity during the entire incubation period. Generally it has to be referred that the rheological behavior of all hydrolyzates is typical of pseudoplastic fluids. The oat flour hydrolyzates displayed the typical viscoelastic behavior of a macromolecular dispersions, where G'' was larger than G' and both moduli increased with increasing frequency, whilst in most of the cases, the behavior approached that of solid – like materials at higher frequencies, i.e. the G' was greater than the G'' .

Enzymatic modification of wheat bran arabinoxylans at reduced water content

O. Santala, E. Selinheimo, T. Suortti, P. Lehtinen and K. Poutanen

VTT Technical Research Centre of Finland
Espoo, Finland

Cereal bran, a by-product of the milling industry, is high in dietary fibre and a good source of bioactive compounds. However, due to complex and insoluble cell wall structures of bran, its applicability for foods is limited. Especially arabinoxylans (AX) have a major impact on the functionality of rye and wheat materials. Technological properties and nutritional value of bran may be improved by enzymatic treatments. Water content is an important parameter, influencing enzymatic activities, diffusion of components and rheological properties of the material. Typically enzymatic treatments are conducted at high water content. However, in industrial applications, low water content may be advantageous, especially in processes including drying as a following down-stream procedure.

The study is part of a PhD work that aims to develop techniques to utilize hydrolytic enzyme reactions at reduced water content in order to add nutritional and technological value of cereal cell-wall rich materials. The work focuses on the action of xylanases, enzymes that depolymerise and solubilise AXs.

The aim of this study was to examine the impact of water content, ranging from 20 % to 90 %, on the efficiency and kinetics of endoxylanase treatment of wheat bran. The rate of AX solubilisation and the molecular mass distribution of solubilised AX were analysed. Interestingly, AX solubilisation was highest and continued for the longest at the water content of about 40 %. This was the water content in which the bran-water mixture transformed from powder-like material to a hard, plastic mass. The effect of physical state of reaction components on enzyme activity, stability and AX depolymerisation pattern will be studied further.

Flour properties as a basis for bread and cookie quality

A. Duyvejonck, B. Lagrain, C. M. Courtin and J.A. Delcour

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

No wheat flour type is suited for all wheat based food product applications. Because of the variations in levels and properties of the cereal components, different flour types result in end products differing in quality. Today, flour quality is mainly assessed based on whether it meets standard flour specifications (*i.e.* Farinograph parameters, protein content, ash content, ...). Such flour specifications often do not take functionality parameters or the contribution of different cereal components to flour functionality into account. In practice, important differences in processing and/or end product quality can arise, even when the flour meets the predefined specifications.

The general objective of our work is to craft novel parameters predicting flour functionality and product quality. Therefore European wheat flour samples with different functionality were physico-chemically characterised. The Farinograph, Mixograph, Alveograph and Rapid Visco Analyser analysis of dough or flour slurries prepared from those European wheat flour samples provided insight in the rheological and functional characteristics.

The literature shows that the SRC test is successful in evaluating the baking quality of soft and hard non-European wheat flour. In this study, the indicative power of the water, lactic acid, sodium carbonate and sucrose SRC of European flour samples was analysed. Prior to determining the Solvent Retention Capacity (SRC) profiles of the flour samples, the SRC test was optimised. To evaluate the performance of the different flour samples, they were used as raw materials in cookie and bread production. Based on the results, the usefulness of classic flour specifications as well as of flour SRC profiles was evaluated. Overall, the results of this study showed that the SRC profile of European wheat flour can be useful in predicting cookie quality (diameter) and to a certain extent in predicting bread quality (volume).

The Debranning of Common Wheat as Innovative Tool in Milling

G. Bottega, M.A. Pagani.

Department of Food Science and Microbiology (DiSTAM), University of Milan, Italy

Debranning is an essential step for removing the hulls of covered cereals and it can be used for removing the outer bran layers in “naked” grains. On the basis of the interesting results obtained with durum wheat, we investigated the possibility to successfully apply debranning to common wheat kernels.

At first, a pilot-plant debranning machine equipped with rolls characterized by superabrasive surfaces was used. The technological parameters taken into account were: the particle size of the superabrasive elements, the processing time, the number of debranning passages and the hydration conditions of the kernels before treatment. The amount of waste (expressed as debranning level, DL) and the physical and chemical modifications of kernels associated with debranning were examined. Best results in terms of homogeneous removal of bran layers ($DL \leq 10\%$) were obtained with the addition of 3% water, keeping the hydrated grains mixed for 5 minutes and applying a short processing time (1 min). Superabrasive surfaces with fine particle size gave a quite uniform debranning process, without deep grooves in the endosperm region. A final brushing of the debranned kernels assured a more accurate removal of bran layers. Debranning levels lower than 8 - 10% allowed to minimize starch losses. The partial removal of the bran layers allowed a relevant reduction of tempering time (up to 90%) without decreasing the flour yield. Furthermore, the technological quality of flour obtained by debranned wheat kernels was comparable to that from conventional milling. Thanks to these encouraging results, debranning had been put in an industrial milling diagram obtaining similar positive results.

Comparative effects of two ozonation treatments On wheat flour technological properties

J. Saint Paul^{1,2}, F. Violleau¹, F. MacRitchie²

¹Laboratoire d'Agro-Physiologie de Purpan, Toulouse, France

²Grain Science and Industry Department, Kansas State University, Manhattan, KS, USA

Ozone, a triatomic form of oxygen with a Generally Recognized As Safe (GRAS) status from the U. S. Food and Drug Administration, is a strong antimicrobial and sanitizing agent with numerous potential applications in the food industry. One of them is the improvement of wheat flour baking qualities, by replacement of the actual chlorination treatment.

Following recent developments realized by the company Goëmar (France) which invented and patented an ozone treatment device for wheat grain and a method for making flour from ozone-treated grains, this study aims to determine the effect of ozone treatment on wheat grain and on wheat flour, and to compare them. Three different ozone concentrations with different application times rendering three quantities of absorbed ozone have been investigated. Rheological, physicochemical and baking properties of soft wheat flours stemming from both treatments were evaluated and compared to untreated flour.

Results were overall significant and showed that the treatment of flour gives more marked results than the treatment on grain for retention capacity in sucrose and volume of cakes but decreases the α -amylase activity. On the other hand, action of ozone on grain augments the maximum viscosity of the flour. Bread volume was found to be increased by both treatments in similar proportions. The treatments were also analyzed in particular and showed specific characteristics. A single treatment has not been determined to enhance all characteristics of the flour. Hence, the modification of precise features of the flour has to be related to a specific treatment.

Parboiling of rice: changes in colour characteristics

Lieve Lamberts and Jan A. Delcour

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), Katholieke Universiteit Leuven, Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

Parboiling is a three step hydrothermal treatment consisting of soaking, steaming and drying. Rice colour is a major determinant of the quality and price. It changes from white to amber during parboiling. Colour changes have mainly been ascribed to diffusion of pigments from the bran to the endosperm and non-enzymic browning of the Maillard type. However, there is little or no evidence backing or denying these hypotheses.

The objective of our study was to increase insights in the mechanisms and reactions responsible for the colour changes occurring during parboiling. To that end, different rice cultivars were processed under different soaking and steaming conditions and their colour characteristics were determined. Firstly, from the identification and quantification of the colour determining components it was clear that β -carotene, lutein and zeaxanthin are the major bran pigments of brown rice. Their trace levels in milled parboiled rice samples allowed us to conclude that bran pigments do not contribute to milled parboiled rice colour. Secondly, colour measurements of rice soaked under different atmospheres (ambient, oxygen and nitrogen) for different times in excess water, indicated that bran pigments leach out in the soaking water and some enzymic colour changes occur. However, the latter is not observable by the human eye. Finally, mildly, intermediately and severely parboiled rice samples were analysed. The impact of processing on rice colour increases with severity of parboiling and is visual observable. The decrease in brightness and increase in yellowness and redness of both brown and milled parboiled rice samples are mainly ascribed to non-enzymic browning of the Maillard type as showed by the presence of the hydrolysis product furosine (an initial stage Maillard indicator), and free 5-hydroxymethyl-2-furaldehyde (HMF; an intermediate stage Maillard indicator). Low furosine and free HMF levels are related to mild parboiling conditions. High furosine and low free HMF levels are indicative of rice samples having been subjected to intermediate processing conditions. High levels of both indicators correspond to severe hydrothermal treatments.