

## GOLDEN SPONSORS



## SILVER SPONSORS



# 13<sup>th</sup> EYCSTW

EUROPEAN YOUNG CEREAL SCIENTISTS  
AND TECHNOLOGISTS WORKSHOP



**14-16<sup>th</sup> May 2014**  
Freising, Germany

## WORKSHOP PROGRAM

### Tuesday, May 13<sup>th</sup> at Kardinal-Döpfner-Haus

17:00-21:00	Registration
19:00-21:30	Meet and Greet at entrance hall of Kardinal-Döpfner-Haus

### Wednesday, May 14<sup>th</sup> at Kardinal-Döpfner-Haus

7:30-8:30	Breakfast at dinning room
8:30-8:50	<b>Opening session</b> <b>Prof. Peter Köhler (DFA)</b> <b>Dr. Peter Weegels and Dr. Frédéric Auger (<i>Cereals &amp; Europe</i>)</b>
8:50-9:00	<b>A. Diallo:</b> Opportunities offered by AACC and C&E for cereal scientists
	<b>Session 1: Gluten</b> <b>Chair: Dr. Mario Jekle</b>
9:00-9:20	<u><b>Keynote lecture</b></u> <b>Prof. P. Köhler:</b> Structure and functionality of gluten proteins
9:20-9:40	<b>G. Gryp:</b> Rapid visco analysis for studying the impact of peptidases on wheat gluten proteins
9:40-10:00	<b>E. Babay:</b> Gluten fraction for the technological quality assessment of durum wheat varieties
<b>20 min</b>	<b>Coffee break &amp; poster set-up and viewing</b>
10:20-10:40	<b>L. Redant:</b> The role of rye proteins during dough development and baking of sugar rich model systems
10:40-11:00	<b>M. Schmid:</b> Isolation and characterization of HMW-gliadin from wheat
	<b>Session 2: Process for cereal-based food</b> <b>Chair: Dr. Frédéric Auger</b>
11:00-11:20	<u><b>Keynote lecture</b></u> <b>M. Jekle:</b> Is the protein microstructure actually determining the rheology of wheat dough?

11:20-11:40	<b>B. Bucsella:</b> Differences in mixing and pasting properties between thermally and hydrothermally treated cake and bread wheat flour
<b>1 h</b>	<b>Lunch</b>
12:40-13:00	<b>A. Dura de Miguel:</b> Functionality of enzymatically treated corn starches
13:00-13:20	<b>T. Maltry:</b> Adhesion properties of dough to different material surfaces
13:20-13:40	<b>A. Rzigue:</b> Bread-pan interace; impact of crust sturture on bread demolding
<b>5 min</b>	<b>Refreshments</b>
<b>13:45-16:30</b>	<b>Visit to Weihenstephan brewery</b>
<b>16:30–19:00</b>	<b>Guided tour of Freising</b>
<b>20:00</b>	<b>Dinner at vault of Kardinal-Döpfner-Haus</b>

### Thursday, May 15<sup>th</sup> at Kardinal-Döpfner-Haus

7:30-8:30	Breakfast at dinning room
	<b>Session 3: Health and Wellness</b> <b>Chair: Dr. Markus Brunnbauer</b>
8:40-9:00	<b>S. Ibrügger:</b> The effect of wholegrain consumption on appetite sensation and body weight
9:00- 9:20	<b>S. Perez-Quirce:</b> Effect of healthy viscous dietary fibres on the quality of gluten-free rice-based breads
9:20 – 9:40	<b>M. Villanueva:</b> Viscoelastic properties of gluten free bread dough enriched with soy and pea protein
9:40 – 10:00	<b>S. Lee :</b> Digestibility of native maize starches in animal feed
<b>20 min</b>	<b>Coffee break and poster viewing</b>
10:20-10:40	<b>X. Huang:</b> Modification of coeliac toxic peptides and proteins by non-enzymatic oxidation
10:40-11:00	<b>D. Martini:</b> Genetic, environmental and technological factors influencing the occurrence of phenolic acids in durum wheat and derived products

	<b>Session 4: Cereals, Yeast and Sourdough</b> <b>Chair: Helle Wium</b>
11:00-11:20	<b><u>Keynote lecture</u></b> <b>M. Brandt:</b> Sourdough products for convenient use in baking
11:20-11:40	<b>M. Heitmann:</b> Impact of different yeasts on wheat bread quality
1 h	<b>Lunch</b>
12:40-13:00	<b>P. Conte:</b> Adding value to wheat flour-based breadmaking matrices: impact of ancient cereals, pseudocereals and legumes
13:00-13:20	<b>A. Monteiro:</b> Application of pomegranate extract for bread conservation
13:20-13:40	<b>P. Jakobs:</b> Study on hydration properties of wheat bran.
13:40-14:00	<b>S. Hemdane:</b> Insight in the variability of wheat ( <i>Triticum aestivum</i> L.) milling co-products and their functionality in bread making
<b>20 min</b>	<b>Coffee break and poster viewing</b>
	<b>Session 5: Analytics in Cereal Science</b> <b>Chair: Edwin Moorthamer</b>
14:20-14:40	<b>L. Hajas:</b> The effects of biological variability of wheat on the accuracy of ELISA measurement
14:40-15:00	<b>M. Lambrecht:</b> Impact of extraction and elution media on separation of proteins by size exclusion high performance chromatography
15:00 -15:20	<b>J. Pico:</b> Development and validation of a method for the simultaneous determination and quantification of six carbohydrates in wheat flours by HPAEC-PAD
<b>15:20-15:30</b>	<b>Refreshment</b>
<b>15:30-19:30</b>	<b>Munich quiz</b>
<b>19:30</b>	<b>Dinner at traditional bavarian restaurant</b>

## Friday, May 16<sup>th</sup> at Kardinal-Döpfner-Haus

7:30-8:30	Breakfast at dinning room
	<b>Session 6:Enzymes</b> <b>Chair: Giorgio Mirolo</b>
8:40-9:00	<b>M. Martínez</b> : Modification of the physicochemical characteristics of native and extruded wheat flours by enzymatic amylolysis
9:00-9:20	<b>M. Schaffarczyk</b> : Analysis and baking activity of lipase reaction products in wheat breadmaking
9:20-9:40	<b>J. Figueiredo Quelhas</b> : Production and characterization of craft beers and its bioactive compounds
9:40-10:00	<b>V. Knorr</b> : Application of proteolytic malt extract for gluten degradation in beer
10:00-10:20	<b>A. Duras</b> : Ultrasonication to inactivate the proteolytic enzymes in bug-damaged wheat
<b>20 min</b>	<b>Coffee break &amp; Poster takedown</b>
	<b>Session 7: Starch</b> <b>Chair: Prof. Peter Köhler</b>
10:40-11:00	<b>A. Brøgger</b> : Rapid viscosity analysis properties for three maize starches in two solvent systems
11:00-11:20	<b>A. Doblado-Maldonado</b> : The effect of annealing on amylose leaching: a strategy to improve starch fractionation
11:20-11:40	<b>D. Dries</b> : Insights into the conversion of native to granular cold-water swelling maize starch
11:40-12:00	<b>S. Horstmann</b> : Characterisation of gluten-free starches and their evaluation in dough and model starch bread systems
<b>1 h</b>	<b>Lunch</b>
<b>13:00-13:30</b>	<b>Awards ceremony &amp; closing session</b>

# **Oral Presentations Abstracts**

# Structure and functionality of gluten proteins

Peter Köhler

German Research Centre for Food Chemistry, Leibniz Institute, Lise-Meitner-Straße 34,  
85354 Freising, Germany (peter.koehler@tum.de)

The molecular structure of the gluten proteins determines the functional properties of wheat flour. In particular the viscoelastic properties of wheat gluten are unique and most important for the quality of wheat bread. Beside viscoelasticity parameters like water absorption, water holding capacity and gas holding capacity are used to describe the functionality of the gluten. It has, however, to be emphasised that it is not sufficient to explain bread quality only by means of gluten functionality as other constituents such as starch, arabinoxylans, enzymes and (polar) lipids also have an impact on the final product.

It is generally accepted that the quantity of gluten as well as its quality are the key determinants of gluten functionality. Gluten quality is reflected by its composition with gliadins and glutenins as functional constituents. One important factor that determines gluten quality is a suitable ratio of gliadins/glutenins, which should be in the range of 1.5 to 2.3. The second factor is the content of glutenin macropolymer (gelprotein) showing concentrations of 20 to 40 mg/g of flour. These concentration-based parameters as well as the amounts and ratios of individual protein types and subunits are accessible by extraction/HPLC- or capillary electrophoretic methods and provide a good prediction of the breadmaking performance of wheat flour.

However, beside quantitative data on gluten composition, information is required on the structure of gluten, and this information is still incomplete today, in particular for the glutenins. This is due to the fact that gluten proteins are storage proteins, and no fixed structure is required to get (techno-) functionality. Therefore, various structural features und building blocks coexist. The poor solubility of gluten proteins in aqueous solvents and the high molecular mass of the polymeric glutenins are the reason that many methods used in protein characterisation fail if they are applied to gluten proteins. Furthermore, using organic solvents or disaggregating reagents renders gluten proteins soluble, but not in their native structures. This lack of structural information led to the use of other methods such as microscopy and rheology that are working in the nano- and microscopic level in order to get indirect information on the structure. From all available chemical, biochemical, microscopic and rheological data models for the structure of gluten have been developed. Modern analytical techniques are currently being used to get further insight into the molecular structure and to link it with the functionality.



# **Rapid visco analysis for studying the impact of peptidases on wheat gluten proteins**

Glenn Gryp, Ine Rombouts, Ellen Fierens, Kristof Brijs and Jan A. Delcour

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

Gluten proteins impact wheat flour dough properties and bread quality. Limited hydrolysis of gluten can impact dough handling properties, as well as crumb texture, bread color and flavor. In literature, peptidase activities are mainly determined on non-gluten substrates and at fixed temperatures. This makes it difficult to predict their effect on gluten proteins and more specifically on bread dough rheology at temperatures relevant for the bread making process. Here, a rapid visco analysis (RVA) system was used to study the impact of peptidases with different optimal working temperatures on wheat gluten. A 17% (w/v) gluten-water suspension, with or without peptidases, was subjected to a hydrothermal treatment mimicking that of bread making. The ultimate goal of our work is to relate impact of peptidases on RVA viscosity to that on dough spread, gas retaining capacity, gluten network formation and bread quality. Gas retaining capacity will be determined using a rheofermentometer and gluten network formation will be assessed based on loss of protein extractability in SDS containing media. Bread volume, crumb firmness and structure will be evaluated.

# Gluten fraction for the technological quality assessment of durum wheat varieties

Babay Elyes<sup>1,2,3</sup>, Hanana Mohsen<sup>3</sup>, Mzid Rim<sup>3</sup>, Ghorbel Abdelwahed<sup>2</sup>, Carrillo Jose Maria<sup>2</sup>, Rodriguez-Quijano Marta<sup>2</sup>, Slim-Amara Hajer<sup>1</sup>

<sup>3</sup>Institut National Agronomique de Tunisie (INAT) (Tunisia); <sup>2</sup>Escuela Técnica Superior de Ingenieros Agrónomos. Universidad Politécnica de Madrid (Spain); <sup>1</sup>Centre de Biotechnologie de Borj-Cédria (CBBC) (Tunisia)

The allelic composition at six glutenin and gliadine loci was assessed by one-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D SDS-PAGE) and acid polyacrylamide gel electrophoresis (A-PAGE) on a set of 37 varieties (from 3 Mediterranean countries). Gluten strength was determined by sodium dodecyl sulphate sedimentation (SDSS) test and mixograph parameters on samples grown under rainfed conditions. Twenty seven alleles/banding patterns were identified (9 at Glu-1, 14 at Glu-2/Glu-3 and 4 at Gli-B1 loci); 0.48 of them were only in landraces at very low frequency and 0.11 were unreported. Genetic diversity index was 0.42 for Spanish landraces, 0.31 for Tunisian landraces and 0.29 for modern varieties. The presence of *Glu-A3ax* had a positive influence on SDSS and mixograph parameters. Of all the prolamins those that have the B-LMW-GS composition *a a a* (for *Glu-A3*, *Glu-B3* and *Glu-B2* loci, respectively), when associated with the *Glu-A1c* and *Glu-B1d* gave the best semolina quality. By contrast, semolina quality is poor when this same composition is associated with the *Glu-A1c* and *Glu-B1e* and even poorer when associated with the *Glu-A1c* and *Glu-B1f*.

# **The role of rye proteins during dough development and baking of sugar rich model systems**

Lore Redant, Kristof Brijs, Joke Buggenhout, Jan A. Delcour

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

The objective of this work was to investigate the role of rye proteins during dough mixing, resting and baking of a sugar rich system. The rye protein functionality was impacted by using variable mixing temperatures and peptidases. The extractability and molecular weight distribution of rye proteins extracted with sodium phosphate buffer (50 mM; pH 6.8) containing (i) 2.0% sodium dodecyl sulfate (SDS) or (ii) 2.0% SDS and 1.0% dithiothreitol (DTT) were examined with size exclusion-high performance liquid chromatography. Whereas dough mixing had only limited impact, resting and baking drastically reduced the level of proteins extractable in SDS containing medium from 100.0% to respectively 80.6% and 51.0%. When DTT was added to the extraction medium, the level of extractable proteins increased with respectively 10 and 22 percentage points, indicating that the proteins had been cross-linked by disulfide bonds during resting and baking. Mixing at lower temperatures resulted in a decreased level of protein cross-linking during subsequent resting and baking, and a decreased height and firmness of the baked end product. Similar results were obtained when peptidases were added to the system, indicating that protein network formation is important for the end product characteristics.

# Isolation and characterization of HMW-gliadin from wheat

Markus Schmid, Herbert Wieser, Peter Köhler

German Research Centre for Food Chemistry, Leibniz Institute, Lise-Meitner-Straße 34, 85354 Freising, Germany

In gluten, gliadins act as plasticizers and play a key role for its functional properties. About 25% of the gliadin fraction consists of oligomers, which are called high-molecular-weight (HMW-) gliadin. To date there is little information about the protein subunits contributing to HMW-gliadin as well as about the linkages connecting them. Therefore, the aim of this study is to develop suitable methods to isolate HMW-gliadin, to identify the proteins present in this oligomeric fraction and to characterize the type of crosslinks between them. Firstly, wheat flour was extracted by a modified Osborne procedure, and the fraction soluble in 60% ethanol was referred to as total gliadin. Size-exclusion chromatography of total gliadin using a BioSep SEC-s3000 column yielded a monomeric and an oligomeric fraction. The latter was called HMW-gliadin. The concentration of HMW-gliadin was affected by the presence or absence of blocking reagents for free thiol groups during flour extraction. HMW-gliadin was analyzed by two-dimensional SDS-PAGE (non-reducing/reducing conditions), RP-HPLC before and after reduction, LC-MS (ESI-QTOF), N-terminal sequence analysis, quantitation of glutathione and cysteine by a stable isotope dilution assay, and by LC-MS/MS (ESI-ion trap) of enzymatic digests. Two-dimensional SDS-PAGE showed the presence of oligomers consisting of protein subunits with molecular masses of 31,000 to 50,000. LC-ESI-QTOF analysis gave precise values of the molecular masses of the proteins, which ranged from 31,713 to 54,836. RP-HPLC under non-reducing conditions yielded no observable peaks, whereas after reduction protein peaks appeared. These proteins were collected and analyzed by automated Edman degradation. The analyses showed that HMW-gliadin contained all gliadin types and, in addition, low-molecular-weight (LMW-) glutenin subunits. HMW-glutenin subunits were not present. N-terminal sequencing and RP-HPLC also provided quantitative data on the protein composition of HMW-gliadin, which consist of LMW-glutenin subunits (49%),  $\gamma$ -gliadins (22%),  $\omega$ 1,2-gliadins (13%),  $\alpha$ -gliadins (10%), and  $\omega$ 5-gliadins (6%). Based on this information it can be assumed that gliadin subunits with an odd number of cysteine residues or low-molecular-weight thiols such as glutathione or cysteine endogenously present in flour stop the polymerization of LMW-glutenin subunits by acting as so-called terminators and provide ethanol-soluble oligomers (HMW-gliadin). Initial experiments confirmed this hypothesis because (i) glutathione and cysteine were shown to be present in HMW-gliadin and (ii) individual gliadins with an odd number of cysteine residues were identified in HMW-gliadin.

# Is the protein microstructure actually determining the rheology of wheat dough?

Jekle, M.<sup>1</sup>, Becker, T.

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, Freising, Germany (<sup>1</sup>Corresponding author: mjekle@wzw.tum.de)

The relationship between structure and function is well known for some artificial materials; however, food, and wheat cereal products in particular, consists of complex biopolymer matrixes, complicating the prediction of its behavior. The knowledge about these structure-function relationships enables the engineering of specific nutritional, physical, and sensorial properties. Therefore, a number of structure-function relationships based on the molecular level were established. The main focus of these relationships was on gluten in wheat dough, since gluten proteins are of particular interest due to their unique viscoelastic characteristics. These properties predominate the mechanical behavior of wheat dough to a large extent. The connective link between the molecular level and the mechanical behavior of dough's macrostructure is the microstructure. Since the microstructure can be analyzed in a noninvasive matter, this could be a direct link to developing an enhanced structure-function relationship. So far, many studies have been performed to investigate dough and dough protein microstructure in particular. However, the evaluation and interpretation of the microstructure was mostly of qualitative character and a quantitative characterization of wheat dough's structural features has yet to be achieved. Therefore, a methodology was developed specifically for dough microstructure quantification (DoMiQ). This method enabled the acquisition of numerical structural features of micrographs and thereby delivered the basis for a correlation analysis of the structure and functionality of wheat proteins in the dough matrix. The protein microstructure of wheat dough was visualized using a non-destructive confocal laser scanning microscope and processed and analyzed with an adapted image analyzing tool. Thus, it was possible to extract highly repeatable results for structural features. With this approach, the effect of ingredients on wheat dough microstructure could be clearly and highly significantly reconstructed. The relationship between the structure and the function was first proven in wheat dough on a micro- to macrostructural scale by the correlation analysis of the structural protein features and rheological properties. Morphologies determining the functionality could be revealed and were discussed. Especially the in the study introduced branching index lead to a model which combines the microstructure and its function in a physical approach. In summary, the application of the DoMiQ methodology enabled a quantitative structure-function relationship of wheat dough proteins on a micro- and macrostructural scale during processing. The high dependency of rheology from structural elements could be verified.

# Differences in mixing and pasting properties between thermally and hydrothermally treated cake and bread wheat flour

Blanka Bucsella<sup>1</sup>, Viktória Vizer<sup>1</sup>, Urs Schwendener<sup>2</sup>, Sándor Tömösközi<sup>1</sup>

<sup>1</sup>Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Szent Gellért 4, 1111 Budapest Hungary; <sup>2</sup>Bühler AG, Department of Value Adding Processes, Gupfenstrasse 5, 9240 Uzwil, Switzerland

In order to change the biological, physical, rheological and end product properties of the flours heat treatment is considered as an effective technological process. Basically two main directions exist, thermal and hydrothermal process. In case of a simple thermal treatment, dry heat is applied on wheat flour that could result in prolonged shelf life in high fat content fractions and strengthened gluten network which makes the treated flour capable for high ratio cakes. The hydrothermal process takes place in presence of moisture with the use of steam and water. During this process, the gluten proteins denature, thus, the dough formation is modified and on the other hand, the starch granules partly gelatinized that resulted in increasing apparent viscosity. Therefore, the treated material is suitable for thickeners in sauces, soups, baby foods and coatings.

The aim of the study was to investigate the main differences in mixing and pasting properties which were caused by thermal or hydrothermal treatments using different experimental conditions. The experimental design was formed where the independent variables were the temperature, retention time or steam moisture content. In order to detect the quality based differences two types of wheat flour were selected; bread wheat flour with standard and cake flour with lower baking quality. Thermal and hydrothermal treatments were carried out in the pilot plant laboratory of Bühler AG (Uzwil, Switzerland). The mixing and pasting behavior of treated and untreated samples in dough matrices were examined with Mixolab (Chopin, France), viscosity properties were tested with rapid visco analyser (RVA, Newport Scientific, Australia) and the falling number values were measured with Falling Number System (Perten Instruments, Sweden).

The thermal treatment caused delay in dough development process, prolonged the stability in both flour types in comparison to untreated ones but the effect was larger in cake flour. Hydrothermally treated samples were not able to form proper dough structure which was manifested in the reduced stability and higher softening values. However, significant increase in peak viscosity and retrogradation was observed that showed flour type and treatment level dependency. It was concluded that product specific treatment can be achieved by well selected flour types and the application of optimized treatment parameters.

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# Functionality of enzymatically treated corn starches

A. Dura<sup>1</sup>, C. M. Rosell<sup>1</sup>

Institute of Agrochemistry and Food Technology (IATA-CSIC), Avenida Agustín Escardino, 7, Paterna 46980, Valencia, Spain (crocell@iata.csic.es; andudemi@iata.csic.es)

Starch is widely used in food products and industrial applications owing to its unique functionality. In its native form, starch does not always fulfill the requirements for certain types of processing. However, enzymatic modification can be an alternative method to meet the specific needs of industrial processes. The aim of this study was to evaluate the effect of the enzyme amyloglucosidase (AMG) on corn starch at sub-gelatinization and above gelatinization temperature, with special emphasis on biochemical features and structural analyses of treated starches. Enzymatic modification of corn starch by AMG at sub-gelatinization temperature led to porous starch granules that differed from the one treated above gelatinization temperature in both the microstructure surface and the internal morphology. Amyloglucosidase contributed on the loss of granular structural order and on changes in both amorphous and crystalline domains during sub-gelatinization temperatures. Microscopic analysis of AMG treated starch at gelatinization temperature resulted in a gel structure that showed a network matrix, highly perforated with some elongated structures from gelatinized starch. The data showed that starches pretreated with enzymes at sub-gelatinization temperature exhibited lower values of pasting properties, with the exception of pasting temperature, compared to starch samples treated at gelatinization temperature. Differences were also observed among hydration properties. Specifically, AMG activity at gelatinization temperature had stronger impact on breaking the degree of association between intermolecular bonds and more soluble compounds are leached out comparing to the treatment at sub-gelatinization temperature. Enzymatic modification of corn starch significantly affects functional properties and starch features. Results confirmed the greatest activity of AMG at starch gelatinization temperature. Nevertheless, AMG treated starches at sub-gelatinization temperature also displayed interesting modifications leading to porous starches with internal channels, which might find applications as edible absorbents.

# **Adhesion properties of dough to different material surfaces**

Teresa Maltry, Mario Jekle, Thomas Becker

Technische Universität München, Institute for Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

In the production of baked goods there often arise extra costs due to adhering dough residue on material surfaces (conveyor belts, proofing trays, dough-dividers, kneading elements). Adhesion phenomena, resulting from molecular adhesion forces between the viscoelastic dough system and the material surfaces, are responsible for the inadequate detaching of dough from contact surfaces. As a result there occurs not only unnecessary idle times of machines due to cleaning work and maintenance, but also production losses caused by sticky machinery parts and therefore an increased financial burden on the companies.

In the context of this study the stickiness, with which adhesion phenomena are often described, shall be elucidated fundamentally in a chemical and physical way. It's a question of what is causing dough stickiness at all?

First, the adhesive behavior of various materials (textiles such as mono- and multifilament fabrics, plastics and stainless steels) is studied by means of a special texture analysis, which was previously developed at the institute. The force, which is required for detaching the materials from a model dough, is measured. During this study the fundamentals of stickiness should be analyzed and defined by targeted varying of the roughness of defined material surfaces. So a influence of surface structure on the stickiness shall be investigated. Furthermore, this influence is investigated by indirect measurement of the surface energy. The surface free energy is determined by measuring the contact angle on materials of liquids, varying in polarity. Aim is to establish a correlation between the surface energy, as a surface structure characteristic, and the stickiness in order to produce targeted defined surfaces in the future. Aim of further research is also a detailed view of wetting properties of material and the analysis of remaining dough residues on the surface. By educating the adhesion mechanisms both a failure-free production can be enabled and hygienic problems, such as mould stains, can be avoided. Thus the durability of materials (predominantly proofing trays) can be improved.

A detailed analysis of a correlation between the adhesion mechanisms of the contact partners (dough and material surface) enables a specific selection of materials with low adhesion and contamination properties and thus a safer and more hygienic production.



# **Bread – pan interface; impact of crust sturture on bread demolding**

A. Rzigue<sup>a,b,c</sup>, J.Y. Monteau<sup>a,b,c</sup>, V.Jury<sup>a,b,c</sup>, A. Le-Bail<sup>a,b,c</sup>, A.Reguerre<sup>d</sup> & S. Chevallier<sup>a,b,c</sup>

<sup>a</sup> Oniris, UMR 6144 GEPEA CNRS, Nantes, F - 44307, France; <sup>b</sup> CNRS, Nantes, F - 44307, France; <sup>c</sup> LUNAM Université Nantes Angers Le Mans – France ; <sup>d</sup> UR1268 Biopolymères, Interactions, Assemblages, INRA, 44316 Nantes, France (Corresponding author: Jean-[yves.monteau@oniris-nantes.fr](mailto:yves.monteau@oniris-nantes.fr); Presenter: [asma.rzigue@oniris-nantes.fr](mailto:asma.rzigue@oniris-nantes.fr))

Processing can induce contaminants in food, especially when high temperature is reached. This occurs in the baking industry. SATIN national project ([www.satin-baking.fr](http://www.satin-baking.fr)) focuses on Pan Bread and Rusk, which are baked most of the time with Perfluorinated (PFC) coated pans. The impact of coating ageing on chemical risks and on technological issues (bread demoulding or bread decapsulation) is targeted to mitigate these issues.

The purpose of this study is to understand the growth of bubbles at the dough-pan interface during fermentation and during baking according to the roughness of the coating. In turn, the structure of the crust at the interface is expected to play a role in the risk of adhesion between the pan and the bread. A model sandwich bread has been considered for this study (750 g, 10 x 10 x 28 cm). The investigations are carried out by freezing the bread (with a cryogenic freezer) at different stages during fermentation and baking. Slices of bread and bread surface were scanned in order to study bubbles size and contact area at the pan-bread interface using image analysis software (Image-tool) and XRays Computed micro-tomography. The results showed that dough bubbles are bigger in the bottom of the pan compared to those on the flank of the pan where alveolar structure is more regular. Moreover, bubbles seem to be more regular in shape in pans with low roughness (Ra).

In addition, experiments were carried out on the impact of the waiting time elapsing between the end of baking and the time of bread decapsulation out of the pan; it was observed that a liquid phase tends to locate at the pan – bread interface with increasing waiting time. This liquid film seems to be linked to difficulties observed in bread decapsulation. Outlooks are proposed to better understand and assess the link between the composition of the liquid film and bread adhesion to the pan.

**Keywords:** pan bread, fermentation, baking, bubbles distributions, roughness

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# The effect of wholegrain consumption on appetite sensation and body weight

Sabine Ibrügger<sup>1</sup>, Pernille Lærke Bjørndal Hollænder<sup>1</sup>, Rikke Juul Gøbel<sup>2</sup> Anne Raben<sup>1</sup>, Christian Ritz<sup>1</sup>, Lotte Lauritzen<sup>1</sup>, Mette Kristensen<sup>1</sup>

<sup>1</sup> Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen - Rolighedsvej 30, DK-1958 Frederiksberg, Denmark; <sup>2</sup> The Novo Nordisk Foundation Center for Basic Metabolic Research, Section of Metabolic Genetics, Faculty of Health and Medical Sciences, University of Copenhagen - Universitetsparken 1, 2100 Copenhagen, Denmark.

Wholegrain (WG) intake has in prospective studies been associated with reduced body weight gain. This may be due to acute and long-term effects of WG consumption on regulation of food intake. In a human intervention study we investigated the effect of WG consumption for eight weeks on body weight and appetite sensation. The study was designed as a randomized, controlled cross-over trial, where 60 healthy but overweight adults were in a randomized order given WG (target WG intake >75 g/d) and refined grain (RG) products for eight weeks, separated by a six-week wash-out period. During the intervention periods, participants were provided with a variety of mixed WG or RG products, respectively, which were provided ad libitum to replace all habitually eaten cereal products. Consumption of study products was noted in a diary. Body weight and composition, determined by bio-electric impedance, as well as subjective appetite sensation, using visual analogue scales, were measured in the beginning and at the end of each period. Participants arrived in the morning after an overnight fast ( $\geq 10$  h) for fasting assessments. Subsequently, a standardized breakfast was served and appetite sensation was assessed at 30 min intervals followed by an ad libitum lunch meal after 180 min to assess energy intake. Fifty participants completed the study. Subjective appetite sensation following the standardized meal and subsequent ad libitum energy intake were not affected by treatment. However, body weight (WG:  $85.4 \pm 13.1$  kg, RG:  $86.6 \pm 13.1$  kg;  $P=0.002$ ) and body fat mass (WG:  $28.6 \pm 9.6$  kg, RG:  $29.6 \pm 9.2$  kg;  $P=0.03$ ) were significantly lower after WG consumption compared to RG, but there was no difference in body fat percentage ( $P=0.13$ ). The consumption of study products did not differ between treatments, but energy intake from WG foods was 17% lower (WG:  $2.7 \pm 0.7$  MJ/d, RG:  $3.2 \pm 0.7$  MJ/d;  $P<0.001$ ) and dietary fiber intake 51% higher (WG:  $23 \pm 5$  g/d, RG:  $11 \pm 3$  g/d;  $P<0.001$ ) compared to RG. The decreased body weight observed after WG consumption suggests an effect of WG consumption on appetite sensation that was sustained over the eight-week period, as also indicated by the lower energy intake from study products during the WG period. Interestingly, appetite sensation after meals not containing WG was not affected, suggesting that contribution of prolonged metabolic changes is limited.

# Effect of healthy viscous dietary fibres on the quality of gluten-free rice-based breads

Sandra Pérez-Quirce<sup>1</sup>, Concha Collar<sup>2</sup> and Felicidad Ronda<sup>1</sup>

<sup>1</sup> Department of Agriculture and Forestry Engineering, Food Technology, College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid, 44, 34004 Palencia, Spain; <sup>2</sup> Cereal Group, Food Science Department, Instituto de Agroquímica y Tecnología de Alimentos, IATA-CSIC, Avda. Catedrático Agustín Escardino 7, 46980 Paterna, Spain

The impact of associated viscous dietary fibres (hydroxypropylmethylcellulose semi-firm – SFE- and weak – NE- gel forming, and barley  $\beta$ -glucan, BBG) incorporated at different amounts (1.6–7.5%, flour basis) into gluten-free rice-based dough formulations on the breadmaking performance and staling behaviour of hydrated (70–110%, flour basis) fibre-flour composite blends has been investigated. Single BBG addition fails to mimic gluten viscoelasticity properly, but simultaneous incorporation of either SFE or NE contributes to bread improvement in terms of bigger volume and smoother crumb. 3.3 g of BBG (70% purity) and 104 mL of water addition to 100 g rice flour provided sensorially accepted breads (7.6/10) with a theoretical  $\beta$ -glucan content of 1.24 g per 100 g GF bread that would allow a daily  $\beta$ -glucan intake of 3 g provided a bread consumption of 240 g day<sup>-1</sup>. Complementary tests should be carried out to know the amount and molecular weight of  $\beta$ -glucan in the final bread before assuring the nutritional benefit of this addition.

# Viscoelastic properties of gluten free bread dough enriched with soy and pea protein

Marina Villanueva<sup>1</sup>, Raúl R. Mauro<sup>1</sup>, Concha Collar<sup>2</sup> and Felicidad Ronda<sup>1</sup>

<sup>1</sup> Escuela Técnica Superior de Ingenierías Agrarias. Universidad de Valladolid. Avda. Madrid 57, 34004, Palencia, Spain; <sup>2</sup> Instituto de Agroquímica y Tecnología de Alimentos, IATA. CSIC. Avda. Catedrático Agustín Escardino, 7. 46980 Paterna, Spain

Celiac disease (CD) is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible persons. The main solution available so far for CD sufferers is to completely adhere to good quality gluten-free foods. Despite several gluten free (GF) products are nowadays available on the market, baked products from gluten-free ingredients are generally of poor physicochemical and sensory quality, and lack fibre, vitamins and nutrients, which results in a worsening effect on the already nutritionally unbalanced diet of CD sufferers. Therefore, incorporation of non-gluten proteins is being considered as the means for addressing some of these problems. Therefore, this study is intended evaluate the impact of legume proteins (pea protein and soy protein isolates) incorporation at different doses (5% and 10%) into GF rice starch-based dough matrices and breads. Oscillatory tests (stress sweep and frequency sweep) and creep-recovery test were employed to study the viscoelastic behavior of the doughs. Stress sweep tests allowed quantifying the linear viscoelastic region which is the maximum stress ( $\tau_{max}$ ) that the dough matrices can tolerate. Changes in bread volume and textural properties were also evaluated. The results obtained indicate that protein addition affected dough viscoelasticity and the extent of the effect was dependent both protein source and dose. Effect of soy protein on both the elastic and viscous components was significantly ( $p < 0.01$ ) higher than pea protein. In addition, increasing the dose from 5 to 10 % increased the values of the elastic and viscous moduli. Incorporation of legume protein led to significantly lower instantaneous ( $J_0$ ) and retarded ( $J_1$ ) elastic compliance in both creep and recovery phases and it is associated with a lower dough deformation subjected to a constant stress, and a higher recovery when stress is removed, respectively. A significant interdependence between elastic moduli with bread volume and hardness was found. Furthermore dough steady viscosity was also significantly dependent on bread hardness.

# **Digestibility of native maize starches in animal feed**

Sophie Lee<sup>1</sup>, Julian Wiseman<sup>2</sup>, Helen Masey O'Neill<sup>3</sup>, Sandra Hill<sup>1</sup>

<sup>1</sup> Division of Food Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK; <sup>2</sup> Division of Animal Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, UK; <sup>3</sup> AB Vista, Marlborough SN8 4AN, UK

Cereals form an important part of diets for both humans and animals. Approximately 80 % of Maize produced is used in animal feed as the primary source of available energy. However, maize quality and composition can also vary noticeably with environmental factors, growth region, agronomic input and variety. Consequently the nutritional value of the maize and subsequent animal performance has been shown to differ, making quality a key concern for formulators and producers. However, it is not known how to accurately predict quality in vitro. There is a lack of integration between the techniques and expertise employed in the human food sector and that which is commonly applied in the animal feed industry. The current study investigated quality in terms of starch granule digestibility in vivo in chicks. The integrity of the maize diet was assessed in different sections of the digestive tract at 10-70 min following feed intake. Unlike humans, birds are fed native, un-cooked starches that are held within plant cell wall and protein matrixes, thereby making the starches somewhat resistant to digestion. The current study investigated how the bird copes with the digestion of such starches. Blood glucose and digesta pH measurements of fed and non-fed birds generally support previous findings. Scanning electron microscope (SEM) images and measurements of starch, dextrin and glucose content of digesta taken from different sections of the tract reveal the progression of starch digestion in broilers. Interestingly, we found starch granules throughout the digestive tract of birds even after 17 hours of starvation, demonstrating the important issue of incomplete starch digestion in birds. Whether prevention of granule digestion is caused by the surrounding cell wall material or protein matrix, or a combination of both, is still yet to be elucidated.

# **Modification of coeliac toxic peptides and proteins by non-enzymatic oxidation**

Xin Huang, Hannu Salovaara, Tuula Sontag-Strohm

University of Helsinki, Agnes Sjöberginkatu 2, PL 66, FI-00014, Helsinki, Finland

A 33-mer peptide from alpha2-gliadin 56-88 residues was used a model peptide in coeliac disease study. Because of its high proline content, 33-mer peptide is highly resistance to luminal proteases and intestinal brush-border enzymes. C-hordein, a monomeric protein from barley prolamins, is almost entirely composed of repeats of octapeptide PQQPFPQQ. Peptide 33-mer and C-hordein protein were treated by non-enzymatic oxidation, or metal-catalysed oxidation, where hydroxyl radicals were formed in the presence of transition metal and oxidising agent. The aim of our study was to examine coeliac toxic peptide and protein in different metal-catalysed oxidation conditions, and the activity of oxidation products in immunological detection. Our results showed 33-mer peptide and C-hordein protein were modified through metal-catalysed oxidation. Certain level of degradation was observed in different oxidising conditions. The activity of oxidation products was decreased in competitive enzyme-linked immunosorbent assay, using antibody against pentapeptide QQPFP. Carbonyl groups and dityrosine cross-links were readily formed. Oxidative treatment can be applied for modification of cereal prolamins, since it appears to be a potential alternative for reduction in coeliac immunological activity detection.

# Genetic, environmental and technological factors influencing the occurrence of phenolic acids in durum wheat and derived products

Daniela Martini<sup>1,2</sup>, Federica Taddei<sup>1</sup>, Roberto Ciccoritti<sup>1</sup>, Isabella Nicoletti<sup>3</sup>, Danilo Corradini<sup>3</sup>  
and Maria Grazia D'Egidio<sup>1</sup>

<sup>1</sup>C.R.A.-Research Unit for Cereal Quality (QCE), via Cassia 176, 00191 Rome, Italy; <sup>2</sup>Campus Bio-Medico University of Rome, via Alvaro del Portillo 21, 00128 Rome, Italy; <sup>3</sup>CNR- Institute of Chemical Methodologies (IMC), Via Salaria km 29.300, 00015 Montelibretti (RM), Italy

Phenolic acids (PAs) represent the most common form of phenolic compounds occurring in whole grain cereals as soluble free acids, soluble conjugates and as insoluble bound forms and mainly concentrated in the outermost layers of the kernels. These bioactive compounds are important for their antioxidant properties, in particular it has been demonstrated that they can act as scavengers of free radicals, which can lead to aging processes and many chronic diseases.

Studies in literature have shown that the content of these compounds may have a wide range of variability, conceivably due to several factors which can influence the occurrence of grain antioxidants. Despite this, few studies focused on durum wheat. This communication reports the results of a study performed to determine the effects of genetic, environmental and technological factors on the content of free, conjugated and bound PAs in durum wheat and derived products (milling fractions and pasta).

The impact of genetic effects on the profile and the content of PAs was evaluated analyzing the content in 10 different genotypes of durum wheat grown in an experimental field across 3 crop years. In order to elucidate effects of growing locations, 3 of the selected genotype, grown in 2 additional locations in the same crop years, were analyzed for their PAs content.

Finally, impact of technological factors was evaluated by the studying effects of milling and pasta-making process on PAs occurrence, comparing products obtained by the traditional process with the ones made by wholemeal. For all samples, free, conjugated and bound PAs were extracted following a method developed by Li et al (1) with some modifications (2,3) and determined by HPLC method.

Results show that genotype, growing location but most of all crop year affect the content of the three PAs forms. As regards the technological aspects, traditional milling appears the process mainly influencing the occurrence of PAs, causing a drastic reduction of PAs content in semolina and pasta with respect to the intact kernel.

On the contrary, products obtained using wholemeal preserve most of these antioxidants and therefore it results a more interesting process to obtain durum wheat derived products with high nutritional value. In conclusion, our results suggest that genetic, environmental and technological factors affect the occurrence of PAs in durum wheat and derived products; moreover they support the importance of knowledge of their impact on the occurrence of bioactives for the production of durum wheat derived food rich in bioactive compounds.

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# **Sourdough products for convenient use in baking**

Markus. J. Brandt<sup>1</sup>

<sup>1</sup>Ernst Böcker GmbH & Co. KG, Ringstrasse 55-57, 32427 Minden, Germany

Sourdough fermentation in bakery requires specific knowledge on the effects of process parameters, raw materials and microorganisms in order to obtain a specific, reproducible quality of the resulting baked goods. Sourdough starter cultures supporting these processes are now for about one century on the market. As fermentation is a labour-intensive and time consuming process, a growing demand for convenient products arised early. First steps were the direct use of organic acids as dough acidifiers, but flavour quality of the resulting breads was unsatisfactory. Based on optimization and modification of the traditional sourdough processes, dried, liquid and pasty sourdoughs with a long shelf life were developed. They allow the convenient, direct production of baked goods with constant quality combined with all advantages of the biological fermentation process, e.g. flavour, reduced staling rate or prolonged microbial shelf life. In order to obtain stable sourdoughs it is – in contrast to starter cultures – a necessity to inactivate physiological activities of sourdough microbiota by e.g. pasteurization, drying or autosterilisation. The use of such sourdough products allow the baker beside its convenience, in addition the use of a broad variety of fermented cereals.



# Impact of different yeasts on wheat bread quality

Mareile Heitmann<sup>1</sup>, Emanuele Zannini<sup>1</sup>, Elke K. Arendt<sup>1</sup>

<sup>1</sup> University College Cork, School of Food and Nutritional Science, College Road, Cork,  
Ireland

Yeast as an ingredient for the production of bread, beer and wine has a very long tradition. Usually dough fermentation is performed by specifically selected baker's yeast. During this study six different yeast strains, such as baker's (used as a control), lager, ale (2x), champagne and white wine yeast, from two different species (*Saccharomyces cerevisia* and *Saccharomyces bayanus*) were investigated for the fermentation of dough. The objective of the study was to evaluate the impact of a range of yeast on the quality parameters of dough and bread. The influence of the six yeasts on dough quality parameters such as dough development, gas holding capacity, extensibility and dough stickiness were determined. Their impacts on bread quality parameters such as volume, texture, colour and crumb structure were also determined. The results of the trials revealed, that the yeast strains showed large differences both in dough and bread quality characteristics. The evaluation of dough in the Rheofermentometer revealed that ale\* yeast had the lowest dough development height of 40.5 mm followed by white wine yeast of 45.9 mm. The best dough development height was measured for ale yeast of 71.2 mm. Gas holding capacity was also measured and it was found that white wine yeast, had the lowest gas production of just 663 mL whereas ale yeast showed the highest total gas production of 2081 mL. These results correlated well with the specific volume of the bread. The smallest bread volume was measured for white wine yeast of 2.21 mL/g followed in ascending order by champagne, ale\*, baker's, lager, and ale yeast with the highest volume of 4.45 mL/g. The type of yeast also showed a significant change in a wide range of quality characteristics such as extensibility and dough stickiness. In conclusion yeast strains can have a large impact on a wide range of quality and sensory parameters of bread. They can be used as a tool to modify bread characteristics and flavour profiles.

# **Adding value to wheat flour-based breadmaking matrices: impact of ancient cereals, pseudocereals and legumes**

Paola Conte<sup>2</sup>, Costantino Fadda<sup>2</sup>, Antonio Piga<sup>2</sup>, Concha Collar<sup>1</sup>

<sup>1</sup> IATA-CSIC Avda. Catedrático Agustín Escardino 7, 46980 Paterna, Spain; <sup>2</sup> Dipartimento di Agraria. Università degli Studi di Sassari. Viale Italia 39, 07100 Sassari, Italy

Cereals are basic, ubiquitous and healthy raw materials, providing excellent vectors for diversity and innovation. It raises a great deal of recent interest that minor cereals, ancient crops, pseudocereals and legumes, besides wheat, constitute nutrient-dense and healthy grains with potential breadmaking applications. The current proposal is aimed at exploring the competences and exploiting the suitability of non-breadmaking whole grains (teff, buckwheat and green pea flours) with unique nutritional components, to be simultaneously included in mixed wheat matrices, to obtain novel and healthy fermented baked goods meeting the functional and sensory restrictions of viscoelastic breadmaking systems. Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff, buckwheat and green pea flours provided dough systems with variable mechanical, viscometric and viscoelastic profiles with no significant hindrance of either dough machinability, or gassing power ability during fermentation. After baking, multigrain breads exhibited acceptable physico-chemical, technological and sensory features, and superior nutritional value compared to the 100% wheat flour counterparts, in terms of larger amounts of bioaccessible polyphenols, lower and slower starch hydrolysis, higher anti-radical activity and superior nutritional composition. All multigrain breads can be labeled as source of dietary fibre ( $\geq 3$  g dietary fibre/100 g bread). The formulation based on wheat:teff:green pea:buckwheat flours, 55:15:15:15 fulfilled from 28% (men) to 43% (women) of dietary fibre, and from 54% (men) to 66 % (women) of protein daily requirements, when a daily consumption of 250 g of bread (WHO bread intake recommendation) is accomplished. This fact encompasses a substantial nutritional benefit with respect to the refined wheat flour bread that delivers when eaten daily at an amount of 250 g, from 9% to 14% of dietary fibre, and from 50% to 60% of protein daily requirements for men and women, respectively.

## **Application of pomegranate extract for bread conservation**

Livia Benossi<sup>1</sup>, Mariane Menconi Chinellato<sup>1</sup>, Antonio Roberto Giriboni Monteiro<sup>1\*</sup>

Food Engineering Department – State University of Maringa – Av Colombo, 5790 bloco 13 –  
Brazil (\*argmonteiro@uem.br)

The objectives of applying natural extracts in the conservation of breads and pasta involve the selection of fruits and other vegetables, the aqueous, alcoholic or supercritical extraction of bioactive compounds for characterization and application in the conservation of products in several concentrations in order to replace synthetic preservatives used in industry. After the extracts are applied, the products have their shelf life monitored to assess the efficiency in controlling fungi development, texture alteration through texturometer and sensory analysis by a group of trained tasters. This study conducted extractions and characterization of pomegranate to apply it in breads conservation. After extraction, the fruit was pulverized through a compressor (120psi) 4 different concentrations of aqueous extract, 4 different concentrations of alcoholic extract, as well as 2 commercial preservatives for breads and one control sample. The breads were packed in polyethylene packages and kept at 25C to be assessed every 48 hours for fungi development and texture. The sensory analysis was carried out strictly for the initial time. The pomegranate alcoholic extract was identified with higher efficiency in controlling fungi development in breads than standard and commercial synthetic preservatives. Regarding texture and the sensory analysis, no meaningful difference for the variation among treatments was observed thus indicating that the extract did not alter the product sensory characteristics. It was possible to conclude that the application of pomegranate extract can represent a natural alternative to preserve food in industry able to replace partially or totally synthetic preservatives.

## **Study on hydration properties of wheat bran**

Pieter Jacobs, Emmie Dornez, Jan A. Delcour, Christophe M. Courtin

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition  
Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20 - box 2463, 3001 Leuven,  
Belgium

A commercial coarse wheat bran sample was milled with a Foss Tecator Cyclotec 1093 Sample Mill to obtain six samples with average particle sizes ranging between 1687  $\mu\text{m}$  and 77  $\mu\text{m}$ . Their hydration properties were evaluated by determining swelling capacity, Enslin-Neff water absorption, Farinograph water absorption and water retention capacity, the latter according to the AACC method. The results showed that the largest bran particles displayed up to three times the water uptake that the smallest did, suggesting that destruction of the bran matrix decreases its water uptake potential. However, no significant differences were observed in Farinograph water absorption and water retention capacity as determined by an alternative water retention capacity assay which allowed water to be drained from the bran. This observation relates to the fact that the latter impose mechanical stress on bran causing larger particles to release the excess water absorbed in conditions free of mechanical stress. The inconsistent trend seen in both water retention capacity analyses was ascribed to bias caused by particle stacking and reabsorption of water which might occur in the conventional AACC method.

# **Insight in the variability of wheat (*triticum aestivum* L.) milling co-products and their functionality in bread making**

Sami Hemdane, Sofie Leys, Emmie Dornez, Jan A. Delcour, Christophe M. Courtin

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Kasteelpark Arenberg 20 - box 2463, 3001 Leuven, Belgium

The aim of present study was to investigate the impact of different wheat milling co-products (coarse bran, coarse weatings, fine weatings and low grade flour) on bread making. To this end, the chemical composition, enzyme activity levels and physical properties of the co-products were analyzed. An increase in total starch, damaged starch and lipids and a decrease in dietary fiber and ash could be observed when the co-product was of lower average particle size. No significant differences in protein levels could be observed. Interestingly, a high variability in enzyme activity levels was observed among the samples, which could not be related to other bran properties. Particle size and hydration properties were positively related with ash and total dietary fiber levels. Finally, their impact on bread making was investigated. To that end, meals were composed in two different ways. In a first approach, an equivalent co-product level was used, and in a second one, an equivalent starch level, as starch was considered to be a good marker for the endosperm contamination of the co-products under study. The results suggest that the non-endosperm material from fine weatings and low grade flour are more detrimental to bread volume than non-endosperm material from coarse bran and coarse weatings. This decrease in bread volume may be due to the smaller particles or to higher levels of deleterious reactive components in fine weatings and low grade flour. However, further research is needed to interpret these detrimental effects caused by the latter co-products.

# **The effects of biological variability of wheat on the accuracy of ELISA measurement**

Lívía Hajas, Kitti Török, Zsuzsanna Bugyi, Sándor Tömösközi

Budapest University of Technology and Economics (BUTE) Szent Gellért tér 4, Budapest,  
1111, Hungary

Certain food commodities can trigger hypersensitivity reactions in the susceptible individuals. Currently there is no cure for these disorders; therefore the only effective treatment is to avoid the provoking proteins. The Regulation (EU) No 1169/2011 lays down the rules concerning the labeling of the substances causing allergies or intolerances, their presence must be labeled for all food; the patients can choose the foods suitable for their diet accordingly. The cereals can cause several adverse reactions with different pathomechanisms, e.g. wheat allergy and celiac disease. These disorders can be triggered by a number of proteins which can be found in all protein fractions. According to the regulation, information on cereals containing gluten such as wheat, rye, barley, oat, spelt and kamut should always be provided to the consumer. For monitoring the gluten content in food, the analytical methods based on immunochemical binding (ELISA= enzyme-linked immunosorbent assay and LFD= lateral flow device) are currently preferred because of their specificity and simplicity. However the gluten determination by ELISA method is limited by several factors up to now. The available ELISA kits have used different target epitopes indicating the presence of gluten containing cereals. The qualitative and quantitative variations in protein composition among cereals have embarrassed the selection of appropriate target molecule and antibody. The sample preparation is also a problematic issue. The composition of extraction buffer, the conditions of extraction procedure and the food matrix have effect on the accuracy of measurement. The food processing may also affect the extraction and detection of the given proteins, so it probably has an influence on the accurate estimation of the gluten amount in a sample. For lack of a suitable reference material for gluten quantification the improvement and validation of ELISA methods have been hindered. The aims of present study were to investigate the impact of the biological variability of wheat on the immunoanalytical results and to estimate the analytical uncertainty of the ELISA measurement. According to the goals, different sources of wheat protein/gluten/gliadin were used to produce powder mixtures and heat-treated cookies. Recovery values and other parameters of analytical performances were determined using several commercially available gliadin/gluten ELISA kits. Considerable variations in recoveries were found among different source materials. Same trend could be observed in powder mixtures and heat treated cookies, but in latter samples overall lower recovery values could be detected.

This research is related to the scientific goals of MoniQA Association and the national project “Development of quality orientated, harmonized educational and R+D+I strategy and operational model at the Budapest University of Technology and Economics” (ÚMFT TÁMOP-4.2.1/B-09/1/KMR-2010-0002).

# **Impact of extraction and elution media on separation of proteins by size exclusion high performance chromatography**

Marlies A. Lambrecht, Ine Rombouts, Jan A. Delcour

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

Size exclusion chromatography is a popular tool to study heat-induced changes in molecular weight distribution of food proteins. Ideally, it separates proteins based on hydrodynamic volume. However, ionic and hydrophobic interactions between resins and proteins can lead to non-ideal separation. Such non-size effects can be minimized by adding co-solvents (salts, organic modifiers, amino acids, detergents) to the elution medium. In contrast, the impact of co-solvents added to the extraction medium on the separation of different proteins remains to be studied. Against this background, model proteins coexisting in some food systems, including wheat and egg proteins, were extracted with extraction media containing various co-solvents [salt, urea, sodium dodecyl sulfate (SDS), dithiothreitol] and separated using various elution media. When using 50% acetonitrile with 0.05% trifluoroacetic acid as elution medium, SDS increased while urea decreased non-size effects both under non-reducing and reducing conditions. In contrast, the addition of salt, urea or SDS to the extraction medium did not substantially impact the separation when using a sodium phosphate buffer (0.05 M, pH 6.8) with 2.0% SDS as elution medium. These results demonstrate the need of selecting an appropriate combination of extraction and elution media to minimize non-size effects during size exclusion chromatography.

# **Development and validation of a method for the simultaneous determination and quantification of six carbohydrates in wheat flours by HPAEC-PAD.**

Joana Pico<sup>a</sup>, Mario M. Martínez<sup>b</sup>, Manuel Gómez<sup>b</sup>

<sup>a</sup> I.U.Cinquima, Analytical Chemistry Group, University of Valladolid, Valladolid, Spain. <sup>b</sup> Food Technology Area, E.T.S. Ingenierías Agrarias, University of Valladolid, Palencia, Spain.

Among all sugars, those deriving from starch hydrolysis occurring in cereals are very important in the baking and brewing industry. Starch is the main component of cereals, therefore their transformation processes can lead to starch hydrolysis sugars like glucose, maltose, and other glucose oligosaccharides. In terms of the latter, there are several reasons for examining these sugars: they are fermentation substrates in the cereal industry such as that of bread and they participate in Maillard and Caramelization thermal reactions that occur during baking. In pastry-making, sugars of flours act as a bulking agent in whipped doughs, in addition to contributing to the sweet taste. Finally, in the sugar industry, flour is the raw material obtaining sugars like glucose syrup. Thus, the saccharification reaction yield is key to controlling the process; this yield increases with the use of extruded flours. Consequently, an analysis of glucose oligosaccharides is of great importance in cereal products like flours. An HPAEC-PAD method has been developed and validated for the simultaneous determination and quantification of six sugars (glucose, isomaltose, maltose, maltotriose, maltotetraose and maltopentaose) in wheat flours, by extraction with water and precipitation of proteins with Carrez II. Analyses were carried out on a Hamilton RCX-30 column with a gradient elution of NaOH 50mM (A) and NaOH 50 mM + NaAcO 500 mM (B). Total run time was 38 minutes. Detector conditions were as follows: E1, + 100 mV; E2, + 550 mV; E3, - 100 mV. The method was validated, with LODs ranging between 0.03 – 0.21 mg L<sup>-1</sup> and LOQs between 0.10 – 0.71 mg L<sup>-1</sup>, R2 between 0.9941 and 0.9983; recoveries were from 74.16 to 110.86 % and RSDs for intraday repeatability, interday repeatability and reproducibility between 0.35 – 8.34 %, 2.34 – 6.64 % and 1.90 – 5.68%, respectively. The method was successfully applied to quantification of these sugars in extruded and non-extruded, enzymatically and non-enzymatically treated wheat flours.



# Modification of the physicochemical characteristics of native and extruded wheat flours by enzymatic amylolysis

Mario M. Martínez<sup>a</sup>, Joana Pico<sup>b</sup>, Manuel Gómez<sup>a</sup>

<sup>a</sup> Food Technology Area, E.T.S. Ingenierías Agrarias, University of Valladolid, Palencia, Spain; <sup>b</sup> I.U.Cinquima, Analytical Chemistry Group, University of Valladolid, Valladolid, Spain.

Native starches and flours are widely used as raw materials, due to their particular polymeric characteristics, which make them suitable for numerous food applications. However, the new quality requirements of foodstuff are forcing the manufacturers of starchy ingredients to find out new functionalities. Flour modification by enzymatic hydrolysis could be an alternative to modify its functionality. However, the starch molecules compacted inside the granules cannot be readily accessible by enzymes, thus the previous disruption of the starch granular structure of flours by extrusion could enhance its susceptibility to enzymatic hydrolysis. The effect of two different enzymes,  $\alpha$ -amylase, amyloglucosidase and their combination on microstructure, oligosaccharide content, crystalline order, pasting, gel hydration and colour properties of native and extruded wheat flours was investigated. Micrographs showed how native flour particles treated by amylase appeared disaggregated, disrupted and pasted to each other. However, extruded flour particles showed a more amassed structure, with a melt component joining the granules. Native flour particles treated by amyloglucosidase showed only a superficial corrosion promoted by the enzyme whereas the particles of extruded wheat flour ended up almost completely disrupted with a melt component joining the granules. This different susceptibility to enzymatic hydrolysis gave rise to different XRD patterns, pasting, hydration and colour properties, and oligosaccharides profile, achieving a 300% and a 500% more of glucose and maltose contents respectively in extruded flours compared to their native counterparts. In general, the enzymatic hydrolysis of wheat flour (already extruded) offers an interesting way to achieve flours with different functionalities which could result interesting for different applications in the food industry, in which later studies should be developed.

# **Analysis and baking activity of lipase reaction products in wheat breadmaking**

Monika Schaffarczyk<sup>1</sup>, Henrik Østdal<sup>3</sup>, and Peter Köhler<sup>1,2</sup>

<sup>1</sup> German Research Centre for Food Chemistry, Leibniz Institute, Lise-Meitner-Straße 34, 85354 Freising, Germany; <sup>2</sup> Hans-Dieter-Belitz-Institute for Cereal Grain Research, Lise-Meitner-Straße 34, 85354 Freising, Germany; <sup>3</sup> Novozymes A/S, Krogshøjvej 36, 2880 Bagsvaerd, Denmark

Beside synthetic emulsifiers such as diacetyl tartaric esters of monoglycerides (DATEM) lipases along with other enzymes are being used to improve the baking quality of wheat flours. Currently available lipases for baking applications hydrolyze a number of different lipid structures in flour and lead to improved surface activity of endogenous lipids thus resulting in the significant increase in bread oven rise and specific volume. The aim of this study was to quantitate lipase reaction products of lipids from wheat dough after addition of two different lipases and to determine the baking performance of lipase-treated lipid mixtures and fractions by means of micro-scale reconstitution baking tests. An improved high-performance liquid-chromatography method with evaporative light scattering detection (HPLC-ELSD) using a monolithic column was established, which was able to separate and quantitate all wheat lipid classes and lipase reaction products in one single run. It was clearly shown that water-saturated n-butanol (WSB; 20°C) was the preferred solvent for lipid isolation compared to 2-propanol (75°C). Specific lipid classes were hydrolyzed and the concentration of the corresponding reaction products increased. A micro-scale reconstitution baking test using 2-propanol defatted flour (20°C) was established to determine the baking performance of different lipase-treated lipid fractions. Proper selection of solvent and extraction temperature was of major importance to sustain the functionality of defatted flour. Dough and gluten from flour defatted with WSB (20°C) and 2-propanol (75°C) had inferior extensibility and loaf volume as compared to the control flour extracted with 2-propanol (20°C). In particular products from galacto- and phospholipids were shown to improve the baking performance, whereas others such as free fatty acids were detrimental.

## **PRODUCTION AND CHARACTERIZATION OF CRAFT BEERS AND ITS BIOACTIVE COMPOUNDS**

Diego Rodrigues Marques<sup>1</sup>, João Olavo Figueiredo Quelhas<sup>1\*</sup>, Antonio Roberto Giriboni Monteiro<sup>1</sup>.

Food Engineering Department – State University of Maringa – Av Colombo, 5790 bloco 13 –  
Brazil \*olavofq@gmail.com

According to Beer Judge Certification Program - BJCP , there are 26 styles of beer with its unique characteristics (color, flavor and alcohol content). During brewing large-scale process, it is done the clarification process by using polyvinylpolypyrrolidone (PVPP), removing the phenolic compounds, in order to prevent the polyphenols to interact with the protein that causes the cold turbidity in the product. After this process the beer needs to be stabilized with exogenous antioxidants to improve its flavor stability, however the consumers are looking to purchase products with reduced amount of additives. Beverages account for a high proportion of antioxidants in Mediterranean diet, as: coffee, red wine , fruit juice and beer. Among the antioxidants present in food, phenolic compounds are by far the most abundant. This study aimed to produce four types of beer American Pale Ale, Brown Porter, Classic American Pilsner and Irish Red Ale in a nano-brewery, without using additives and to characterize these beers in their physico-chemical parameters and bioactive compounds. The experiments were performed in a nano-brewery in the Department of Food Engineering, State University of Maringa - UEM. The manufacture of beers was done by following proposed process of (LINKO et al , 2008.), with modifications to nano-brewery reality: weighing the ingredients of beer, sanitization, grinding the barley malt, mashing, filtration, boiling, cooling, fermentation, maturation, filling, carbonation and storage. The analysis performed were original extract, apparent extract, apparent attenuation, real attenuation, alcohol, calories , color, bitterness, vicinal diketones, dissolved CO<sub>2</sub>, turbidity, pH, Nibem, total phenolic compounds, DPPH and phenolic acids. The beers had different original extract varying from 12.01 to 13.9 ° E, this fact is due to the use of more roasted malt in the Brown Porter and irish Red Ale, this roasting process provides a dark color to the malt and reduces its enzyme activity, consequently obtains a wort with less sugars. Color, bitterness, alcohol, CO<sub>2</sub> and Nibem conform to the parameters requested by the BJCP, the acid pH in beer helps in microbiological control. The vicinal diketones is an attribute with values found above in comparison to the commercial beers, because the beer should not come in contact with oxygen in the stages of maturation and bottling, however in a nano-brewery it is difficult to control it. The total phenolic compounds showed concentrations ranging from 448.57 to 531.30 mg GAE L<sup>-1</sup>, highlighting the Brown Porter beer that also showed the highest antioxidant capacity 48.9% due to the high concentration of caffeic acid, it was followed by the American Pale Ale, the Irish American Red Ale and the Pilsner. It was concluded that by not having the filtration process the craft beers, it offered quite a high quantity of bioactive composition, in particular phenolic compounds, therefore these products present potential as functional products.

Keywords: craft beer, nano-brewery, phenolic acid, DPPH and physicochemical analysis.

# Application of proteolytic malt extract for gluten degradation in beer

V. Knorr<sup>1</sup>, H. Wieser<sup>1</sup>, P. Köhler<sup>1,2</sup>

<sup>1</sup>German Research Centre for Food Chemistry, Leibniz Institute, Lise-Meitner-Straße 34, 85354 Freising, Germany; <sup>2</sup> Hans-Dieter-Belitz-Institut für Mehl- und Eiweißforschung, Lise-Meitner-Straße 34, 85354 Freising, Germany

Celiac disease is a small intestinal disorder triggered by storage proteins from wheat (gliadins, glutenins), rye (secalins) and barley (hordeins). Celiac patients adhering to a gluten-free diet are allowed to drink only beer-surrogates, which differ from barley-based beers in terms of aroma and taste. As gluten is massively degraded by endogenous peptidases during germination, malt with optimized peptidase activity might be used to degrade gluten during beer production to yield a product with a gluten content below 20 mg/kg and quality parameters comparable to conventional beer. Therefore, barley grain was germinated under systematically altered conditions, the grain was kiln-dried, and peptidases were extracted with water. Gluten-specific peptidase activity in the extracts was determined by RP-HPLC using two celiac-active peptides as substrates (PQPQLPYQPQLPY = P1 from  $\alpha$ -gliadin and SQQQFPQPQQPFPQQP = P2 from  $\gamma$ -hordein), and generated fragments were identified by LC-MS2. Additionally, the influence of kiln-drying and heat-treatment of aqueous enzyme extracts on the peptidase activity were examined. The extract was concentrated by removing water under reduced pressure at 50 °C. The concentrated extract was used to study gluten degradation in wort as affected by pH, incubation-time, temperature, and degree of concentration. The results were transferred to a large-scale experiment and the resulting beers were tested for gluten content and sensory attributes. The experiments showed that extracts from germinated cereals were able to degrade both celiac-toxic peptides by cleaving peptide bonds involving proline residues. Fragment peptides with less than nine amino acids, which are known to be no longer celiac-active, were detected. The peptidase activity of the malt was significantly higher than of non-germinated grains and by the variation of the conditions of germination the activity was optimized. The enzyme activity was not affected by the high temperatures up to 80 °C during kiln drying. In aqueous solution temperatures up to 50 °C were tolerated without loss of activity. The concentrated malt extract had a higher peptidase activity than the raw extract and by raising the water-to-grist-ratio the activity during brewing was further increased. During the concentration process both peptidase activity and gluten content of the extract increased simultaneously. After adding the extract to cold wort, gluten was most efficiently degraded at higher temperatures and higher pH, however the enzymes were active for a longer time at lower temperatures. In a large-scale experiment, 10 % malt extract (water-to-grist-ratio 1:2.5, 40 % brix) was added to wort, the mixture was incubated at 50 °C for 24 h, fermented, and filtered. The final beer was gluten-free, however, it had a honey- and malt-like off-flavor.

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# Ultrasonication to inactivate the proteolytic enzymes in bug-damaged wheat

Atike Nur Durak, Mustafa Erbaş and Sultan Arslan

Dept. of Food Engineering, Faculty of Engineering, Akdeniz University, 07058 Antalya,  
Turkey

Wheat, one of the most essential food sources of mankind, is produced approximately 700 million tons per year in the world. In some regions of the world, serious yield and quality loss in cereals and particularly in wheat caused by sunn pests are seen. The pests belonging to the genera of *Eurygaster* spp. and *Aelia* spp. cause damage in the area including Eastern Europe, Northern Africa and Middle East regions while *Nysius* spp. cause damage in New Zealand. Sunn pest feeds on wheat and causes damage by injecting its saliva including proteolytic enzymes to the kernel. Despite the pest management, million tons of wheat is still harvested as bug-damaged every year as sunn pest could not be eliminated. Compared to the sound kernels, the bug-damaged wheat kernel has no significant difference in case of physical characteristics so it could not be separated from the sound wheat kernels during cleaning of wheat prior to milling. Thus, it causes undesirable effect on the quality of end product. Baking quality of the flour contaminated with bug-damaged kernel reduces due to bug originated proteolytic enzymes. There are many studies aiming to ensure a certain quality of bread by diminishing the effect of bug damage. Even though there are partial achievements they are not satisfying. In this research, the possibility of using ultrasonic waves at different power levels and time periods to inactivate proteolytic enzymes originating from bug-damaged wheat kernels were investigated. For this aim, sound wheat as the control sample and 3% bug-damaged wheat containing wheat samples were sonicated in water (1:2) at different power levels (25%, 50%, 75% and 100%) and time periods (0, 4, 8 and 12 min) with 10-second-on-5-second-off pulses. There was a significant ( $p < 0.01$ ,  $p < 0.05$ ) difference among the samples depending on the bug-damaged kernel content and ultrasonic treatment with respect to wet gluten content, sedimentation value and free amino acid content. Depending on the increase in time period of ultrasonic treatment, a significant difference was determined in wet gluten content and sedimentation value of bug-damaged wheat samples and the mean values of increase rates were 7.6% and 7.5%, respectively. A significant decrease was also determined in total free amino acid content and prolin and glutamic acid contents of bug-damaged wheat samples and the mean values of decrease rates were 20%, 21% and 29%, respectively. It is considered that these results sourced from inactivation of proteolytic enzymes injected to wheat by sunn pest. Wet gluten contents and sedimentation values increase, while free amino acid contents decrease because proteins could not be hydrolyzed further. Consequently, ultrasonic treatment has a statistically significant effect on decreasing the proteolytic activity of bug-damaged wheat while it does not affect the quality of the sound wheat.

**Keywords:** *Eurygaster* spp., *Aelia* spp., wheat, bug-damage, proteolytic activity, protease, ultrasound

## **Rapid viscosity analysis properties for three maize starches in two solvent systems.**

A. F. Brøgger<sup>1</sup> P. Hume<sup>2</sup>, M. Rose<sup>3</sup> and S. E. Hill<sup>1</sup>

<sup>1</sup>Division of Food Science, School of Biosciences, University of Nottingham, Sutton Bonington Campus, LE12 5RD, Leicestershire, United Kingdom, <sup>2</sup>McCain Foods, Scarborough, North Yorkshire, YO11 3BS, United Kingdom, <sup>3</sup>Newly Weds Foods, Banbury, Oxfordshire, OX16 4TJ, United

The importance of starches, especially those from cereals, continues to be a highly discussed topic as the Food Industry seeks to understand the functional structure relationship across different starch types in various applications. Maize starches are very commonly used as a thickening agent in food applications and their functionality will depend on the hydrodynamic volumes of the macromolecules and the association of the glucans to form larger structures, such as swollen hydrated granules. This study focuses on the properties of three maize starches with differing amylose levels; HA (high amylose maize starch with an amylose content of approximately 70%), MA (amylose 21%) and WM (waxy maize, amylose <1%). The rheological properties of the starches were examined during and after a thermal treatment. Rapid viscosity analyses were used to characterize the pasting curves for these samples at different concentrations (2.5-14%) in water. Pasting and solubility profiles for these starches were then undertaken using N-methylmorpholine N-oxide (NMMO) instead of water as the solvent/dispersant. For the WM and MA starches in water the pasting profiles were as expected with the characteristic peak viscosities followed by a lower viscosity of pasting at 95°C. The viscosities increased with increasing concentration for all time points along the pasting curve. However, it was found that the viscosities and shapes of the curves are highly dependent on the concentrations. These characteristic curves are formed due to the gelatinisation of the starch granules and the concomitant swelling and hydration of granule structure, followed by dissolution of the macromolecules and breaking down of the swollen granule. The pasting curves in water for maize starch and waxy maize are similar, whereas for high amylose maize the profile is very different due to the gelatinisation temperatures not being exceeded and therefore little hydration of the granules. When heated and stirred in NMMO the starches are expected to solubilise from the outside of the granule, and the viscosities reflect the volumes of the glucans in the solution. The concentrations of starches used in this solvent are low as the material becomes very elastic and difficult to stir as the starches go into solution. It is often assumed that at the end of a pasting cycle in water all the polymers are in solution and the end viscosity reflects the average molecular weight of the starch macromolecules. However, the end viscosities in NMMO are greater than that of the starches in water (at the same concentration), which could be due to more polymers in solution or the conformation of the polymer being different in the NMMO. When using NMMO as the dispersant, the impact of the amylose: amylopectin ratios was different from that observed when pasting in water. The profile for HA in NMMO is similar to MA, whereas WA had a higher peak and end viscosity, perhaps indicating that the amylopectin has more hydrodynamic volume in this solvent than comparable weights of amylose. The relative importance of granule and macromolecular assemblies on the properties of starches compared to the molecular weight and shape of the amylose and amylopectin has always been a problem when investigating starches as they undergo processing. This work, using different solvents, may aid in a better understanding of the critical phenomena that relates the behaviour of starch to its molecular characteristics.

# **The effect of annealing on amylose leaching: a strategy to improve starch fractionation**

Andrés F. Doblado-Maldonado, Sara V. Gomand, Bart Goderis, Jan A. Delcour

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Leuven, Belgium (dorien.dries@biw.kuleuven.be)

Two techniques can be used for separating amylose from amylopectin. In the first, after achieving complete dispersion of starch, amylose is precipitated by adding a ligand or complexation agent. The second technique is based on aqueous leaching of amylose during starch pasting. One of the disadvantages of the latter is that leaching amylose at high temperatures can also lead to amylopectin solubilization. In view of this, annealing of starch prior to leaching has been proposed as a way to improve purity of the amylose extract. In this study, we aimed to unravel the specific effect of annealing of wheat, maize and high amylose maize starches on amylose leaching. Polarized light microscopy detected no changes in morphology and birefringence of the granules as a result of annealing. Pasting behavior and thermal transitions were investigated by using Rapid Visco Analysis (RVA) and differential scanning calorimetry (DSC), respectively. Peak and final viscosity were significantly altered after annealing and to a degree depending on the starch source. Apart from this, annealing narrowed the range of DSC melting events and increased the peak melting temperature, while it slightly increased the gelatinization enthalpy. Structural changes after annealing were studied by means of small and wide angle X-ray diffraction (SAXS/WAXD). X-ray data elucidated slight changes in crystalline attributes but not in the overall degree of crystallinity. Thus, changes in melting events could be related to a structural reorganization. Depending on the type of starch, annealing may have impacted the surface free energy or crystal thickness. When amylose leaching was performed using annealed starch rather than the starting material, size exclusion chromatography elucidated a significant increase in the purity of the extract. Therefore, we can conclude that the crystalline structure and degree of organization of such crystals influence the amylose leaching and may contribute on a more efficient fractionation of starch. Hence, annealing affects the crystalline structure in such extent that less but more pure amylose leaches out.

# **Insights into the conversion of native to granular cold-water swelling maize starch**

Dorien M. Dries, Sara V. Gomand, Bart Goderis, Jan A. Delcour

Laboratory of Food Chemistry and Biochemistry and Leuven Food Science and Nutrition Research Centre (LFoRCe), KU Leuven, Leuven, Belgium (dorien.dries@biw.kuleuven.be)

Starches of enhanced cold-water swelling capacity can be used in instant and convenience foods like puddings, pie fillings, gravies, soups and sauces. Since traditionally produced pre-gelatinized starches have poor properties due to loss of granular integrity, efforts have been made to develop granular cold-water swelling starches. This can generally be achieved by heating starch in the presence of aqueous alcohol, during which the native double helical order is transformed into single helical order. Presumably, this happens by complex formation between both amylose and amylopectin with the alcohol. Amylose is essential for retaining granular integrity. Removal of the residual alcohol from the single helix cavity is alleged to render cold-water swelling starch.

This study aimed at further understanding the conversion of native into granular cold-water swelling starch. First, a laboratory scale aqueous ethanol procedure was successfully used to gradually convert native maize starch to granular cold-water swelling starch by varying ethanol concentration and treatment temperature. Swelling power increased concomitant with starches showing an increased level of VH-type crystallinity. A waxy maize starch was included as well to study the role of amylopectin in the formation of V-type crystals. Removal of alcohol by high temperature drying resulted in a VA-type X-ray diffraction pattern. However, this transition was reversible and it was shown that high temperature drying is not necessary to confer upon starch its cold-water swelling characteristics. Finally, beyond-state-of-the-art time-temperature resolved X-ray measurements during the conversion of native to cold-water swelling starch provided insights in the structural transitions taking place.



# **Characterisation of gluten-free starches and their evaluation in dough and model starch bread systems**

S. Horstmann, M. Belz, E. Zannini, E. K. Arendt

University College Cork, School of Food and Nutritional Sciences, College Road, Cork,  
Ireland

Celiac disease (CD) is an immune-mediated disease, triggered in genetically susceptible individuals by ingested gluten from wheat, rye, barley, and other closely related cereal grains. The only available treatment for celiac patients until now is a gluten free diet, which enables to normalize the clinical and histological manifestation and allows the patients to live an otherwise normal life. The replacement of gluten presents a significant technological challenge, as it is an essential structure-building protein, which is essential for high quality baked goods. This work aimed to gain a deeper knowledge of the interaction of starches in a gluten-free dough and bread system. The morphological characterisation of the different starches (potato starch, tapioca starch, corn starch, rice starch, wheat starch) was performed using scanning electron microscopy and laser scanning microscopy. The composition of the different starches were analysed (contents of: moisture, damaged starch, total starch, amylose, amylopectin, protein, and also the enzyme activity of  $\alpha$ - and  $\beta$ -amylase) by using a range of different assay kits. The pasting properties of the different starches were determined with a RVA and rheological characterisations were performed with a fundamental Rheometer. The quality characteristics of The best results both for the bread as well as dough analysis were achieved with wheat starch followed by potato starch. This study not only provides a detailed analysis of commonly used gluten free starches but also characterised the impact of these starches on a model bread quality.

# **Poster Abstracts**

# Gelling properties of three tef [*Eragrostis tef* (Zucc.) Trotter] varieties

Workineh Abebe<sup>1</sup>, Felicidad Ronda<sup>1</sup>

<sup>1</sup> College of Agricultural and Forestry Engineering, University of Valladolid, Av. Madrid 57, 34004 Palencia, Spain

Tef [*Eragrostis tef* (Zucc.) Trotter] is recently been receiving global attention particularly because of its promising potential in nutrient-rich food product formulation, especially in gluten-free and functional foods. With the aim of assessing the suitability of tef for gel type food products gelling properties of three Ethiopian tef varieties (DZ-01-99 (brown tef), DZ-Cr-37 (white tef), and DZ-Cr-387 (white tef)) were evaluated. In this study viscoelastic properties at 6, 8, 10, 12 & 14% w/w concentrations and 25 °C and 90 °C were measured. In addition, evolution of texture and color for 16% (w/w) gels were evaluated. Rice, refined and whole wheat flours were analyzed as reference. The minimum flour concentration required for gel formation from the three tef varieties was 6-8%, similar to wheat flour. All tef flour suspensions pre-heated to 95°C led to gels with a solid-like behavior ( $G' > G''$ ), both at 25 °C and 90 °C, with higher consistency than wheat gels at the same concentration. The dependence of viscoelastic moduli with concentration fulfilled the power law. The Avrami model was successfully fitted to the textural evolution of tef gels. Important differences were observed within the tef varieties and with rice and wheat flours, which is probably contributed by their differences in protein, starch, lipid and fiber constituents. Gelling properties characterized suggest that tef flours would be suitable ingredients in gel food formulations.

# **A visualizing method for microstructural analysis of dough - the confocal laser scanning microscopy**

Isabelle Bernklau, Mario Jekle, Thomas Becker

Technische Universität München, Institute of Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

The characteristics and properties of each material depend on its structural composition because of a relation between its structure and function. So, for the elucidation of dough and bread properties studying dough microstructure is an important component. By visualizing dough microstructures knowledge about the structure-function relationship and interaction of various dough ingredients can be expanded. This allows conclusions to be drawn on dough rheology and end product quality, such as stability, texture, taste and appearance.

A suitable method for studying the microstructure is the confocal laser scanning microscopy (CSLM). The principle of CLSM is based on a fluorescence measurement. For this purpose, the sample has to be stained with fluorescent dyes. A major advantage of the CLSM is the possibility of multiple staining of the matrix components by using several lasers with different excitation wavelength. Because of the confocal system no out of focus light reaches the detector. That increases the resolution of the images in comparison to the light microscopy. Furthermore, it is possible to generate three-dimensional pictures.

One of the research aims is to optimize the specific staining of starch and proteins. Typically, dyes like Rhodamine B, Nile Blue, Nile Red, Acid Fuchsin or Light Green are used. For the elucidation of influences and interactions of dyes on the starch-protein matrix different staining methods are examined and compared in order to obtain a real and accurate representation of dough microstructure.

With optimizing the staining method a more precise visualization of the whole manufacturing process would be enabled. Thereby, dough structural changes can be analysed during the process, for example during the fermentation time. This permits a targeted adjustment of quality characteristics or a development of new structural designs.

# Measurement of the dielectric properties of starch

Erin Crossland

University of Leeds

Since its creation in 1945, the microwave has become a popular method of cooking food on both a domestic and commercial scale. As microwave heating primarily relies on dielectric heating, it is important to understand the interaction of different food materials within the microwave field, as this will enable future product development in areas such as improved textural properties and uniformity of cook amongst others.

Here we present new methods for measuring accurately the dielectric properties of starch powders at different moisture contents. As starch is a complex material it is anticipated that different methods will have to be applied across the moisture range due to increasing conductivity of the starch within the microwave field. Previous research in this area has looked at using a co-axial probe to determine the dielectric properties of starches. However, co-axial probes have poor definition of the penetrating microwave field and it can be difficult to rule out container effects. Due to issues with packing, it has also been suggested that the co-axial probe technique is unsuited to the measurement of powders. Here we present the results of resonator methods for the accurate measurement of the dielectric properties of starch powders.

# Improvement of gluten-free dough aeration through adapted processing conditions and ingredients

Dana Elgeti, Mario Jekle, Thomas Becker

Technische Universität München, Institute for Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

Gluten is responsible for the formation of a stable sponge-like network with homogeneously distributed gas cells in the final crumb. Thus, it is challenging to produce gluten-free bread, which is able to meet the expectations of consumers. The goal of the present study is the reduction of the bread density to a value comparable to the one of traditional wheat pan bread with fine gas cells. For this purpose, two different strategies have been pursued.

Similar to the aeration of cake batter the mixing process has been optimized in order to entrap a maximum amount of gas. The gas volume fraction after mixing was determined by measuring volume and weight of a dough sample with gas and comparing these values to the dough density after (ultra-) centrifugation. As it turned out, mixing at 420 rpm for 480 s with a wire whip inside a planetary mixer (Baer Varimixer, RN10) resulted in the highest gas volume fraction of 12.7 % as compared to the 8.0 % when using a kneader at 200 rpm for 260 s.

However, previous trials have demonstrated that a 2:1 mixture of the typical gluten-free flours from rice and corn are not suitable for the stabilization of higher gas amounts (Elgeti et al. 2014). Confirmative, the increased gas fraction in the dough was not maintained throughout the baking process. Therefore, in the second approach, the effects of different ingredients have been compared. The use of quinoa white flour without bran instead of the traditional flours led to considerably more gas entrapment of up to 25 %. Likewise, the proportions of water, hydrocolloids and lipids were found to influence the gas content not only after mixing but also throughout proofing and baking. Notably, the best results were obtained when the new aeration method was combined with the right choice of functional ingredients.

In summary, several techniques are presented to improve the aeration and foam stability of gluten-free bread. Future trials will be directed to elucidate the underlying mechanisms of foam stabilization in the gluten-free dough matrix.

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# **Protein extraction from gluten-free milling fractions for food applications**

Maike Föste, Dana Elgeti, Anna-Katharina Brunner, Mario Jekle, Thomas Becker

Technische Universität München, Institute for Brewing and Beverage Technology, Research Group Cereal Process Engineering, 85354 Freising, Germany

With regard to people suffering from celiac disease, pseudocereals such as quinoa gain in importance due to their high protein and mineral content and their lack in gluten. Furthermore, plant based protein sources are of great economic and dietetic relevance. The extraction process requires a starch-less milling product, which is a technological challenge in view of the small kernel size. As a solution, in previous trials the separation of protein and starch components by fractionation through milling was conducted. The aim of this study is the development and optimization of process parameters for protein extraction strategies in quinoa. Through roller milling in lab scale, the protein content was concentrated in quinoa bran up to 24 % (dry base). In order to ensure the application in foods, water was used as a solvent for the extraction process, whereby pH 10 resulted in protein solubilisation of about 60%. Depending on extraction time (30 min to 4 h) and temperature (20 to 50 °C) no significant differences were observed. For protein purification the extract was precipitated at pH 4, resulting in a protein yield of 25 %. In summary, the optimized extraction parameters are a promising tool for further food applications, in particular with regard to the protein enrichment.

# Enzyme-mediated improvement of the baking performance of rye dough

Isabel Großmann<sup>1</sup>, Peter Köhler<sup>1,2</sup>

<sup>1</sup>Hans-Dieter-Belitz-Institute for Cereal Grain Research, Lise-Meitner-Str. 34, 85354 Freising, Germany; <sup>2</sup>German Research Centre for Food Chemistry, Lise-Meitner-Str. 34, 85354 Freising, Germany

Despite of a protein subunit composition similar to wheat, rye proteins do not form a continuous, viscoelastic gluten network. This may be, on one hand, due to the number and location of cysteine residues in rye protein subunits, which only allow a limited formation of high molecular weight protein aggregates. On the other hand arabinoxylans cover the protein with a slimy layer when water is added to the flour and prevent aggregation and formation of a gluten-like continuous mass. Therefore, the aim of this study was to improve the aggregation of rye proteins in dough by adding xylanase and transglutaminase to partially degrade arabinoxylans and subsequently crosslink the proteins. The altered dough structure should finally improve the baking performance of rye flour. Doughs with xylanase and varying amounts of transglutaminase were analysed via Osborne fractionation of the proteins. With this method the proteins were separated into the salt-soluble albumins and globulins, the prolamins soluble in 60% ethanol and the glutelins soluble under reducing conditions. Quantitation was carried out by reversed-phase (RP-) HPLC/UV detection, and reference gliadin from the Prolamin Working Group (PWG) was used for calibration. With increasing transglutaminase activity the concentration of prolamins decreased and increased, respectively, and the prolamins to glutelin ratio was considerably lowered. The overall amount of extractable protein decreased with increasing transglutaminase activity showing that crosslinking by transglutaminase provided high molecular weight protein aggregates that were not soluble any more even after reduction of disulphide bonds. The effects caused by xylanase were monitored via gel-permeation (GP-) HPLC/RI detection of the water-extractable arabinoxylans. The decrease of the high molecular weight arabinoxylan fraction and the concurrent increase of the medium molecular weight fraction confirmed the degradation of arabinoxylans by the xylanase.



# **Influence of particle size distribution and particle form of starch on the foamability and stability of sponge cake batter**

Inga Huisman<sup>1</sup>, Uwe Grupa<sup>1</sup>

<sup>1</sup>Hochschule Fulda – University of Applied Sciences, Marquardstr. 35, 36039 Fulda, Germany

Edible foams have become increasingly important for the food industry over the past years. Traditional food foams include all kinds of bakery products; modern applications are e.g. foamed chocolate or spreads (margarine, creamcheese). All food foams come with the challenge that they have to be stable during processing and storage. This problem can be handled very well with additives, but in the modern society many of these have become unpopular and the food industry strives to find alternatives. One way to control the stability of foams is the addition of solid particle systems. The properties of the particles determine their effectiveness in stabilizing gas bubbles in a given medium. Important factors include size of the particles in relation to gas bubbles, particle size distribution, hydrophobicity (contact angle) and particle form. This study investigates the influence of starches from different botanical sources as added solid particle system on foamability, gas bubble size distribution and stability in egg-sucrose-foams (sponge batter). Methods comprise of resistance to drainage, determination of gas bubble size distribution and Ostwald-ripening and rheological measurements to investigate resistance to strain/stress. Preliminary experiments show distinguishable differences in drainage and bubble size distributions of foams made up with different starches. Further experiments will also take a closer look on the influence of the form of the starch particles.

# Studies on the effects of redox-reagents and enzymes in gluten-free baking

Iris Lovric<sup>1</sup>, Peter Köhler<sup>1,2</sup>

<sup>1</sup>German Research Centre for Food Chemistry, Lise-Meitner-Str. 34, 85354 Freising, Germany; <sup>2</sup>Hans-Dieter-Belitz-Institute for Cereal Grain Research, Lise-Meitner-Str. 34, 85354 Freising, Germany

In wheat bread making it is well known that the techno-functional properties of the dough and the baking quality can substantially be affected by using redox reagents or enzymes. However, only little information is available on the effects of redox reagents and enzymes in gluten-free baking. Therefore, the aim of this study is to investigate the impact of several redox agents and enzymes on the properties of gluten-free doughs and breads. Rice and buckwheat flours were used in this study, and wheat flour served as the control. L-ascorbic acid, potassium bromate, L-cysteine, reduced and oxidised glutathione, glucose oxidase, transglutaminase, sulfhydryl oxidase and trypsin were used as additives. First, the protein composition of the flours was quantitated using an extraction/HPLC-method on the basis of UV-absorption at 210 nm. The concentration of free thiol groups in flours was determined with 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent). Glutathione (GSH), oxidized glutathione (GSSG), cysteine (CSH) and cystine (CSSC) were quantitated in flour and dough via stable isotope dilution assays with synthetic internal standards. The effects of additives were demonstrated by means of rheological and baking experiments using doughs from rice-buckwheat mixtures. Due to poor extractability of a large part of the rice and buckwheat proteins the established extraction/HPLC method had to be optimised to get meaningful results for the protein distribution. In comparison to wheat, where the prolamins make up the largest part of wheat proteins, rice proteins mainly consist of SDS-soluble glutelins and buckwheat proteins of albumins and globulins. The quantitation of free thiol groups showed a four and five times higher content in buckwheat and rice flour, respectively, as compared to wheat. Quantitative analysis of GSH and related compounds showed that buckwheat flour had the highest amount of GSH (thirty times higher than in wheat flour and fourteen times higher than in rice flour), whereas the GSSG contents were fairly similar. Micro-scale baking experiments and rheological measurements using the aforementioned additives showed varying effects of the additives on the crumb structure and on the viscoelastic properties of the batters, however, the effects on the loaf volume were limited.

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# **Study of the nutritional changes and evolution of microbiota during sourdough like fermentation of wheat bran.**

Federica Manini<sup>1</sup>, Milena Brasca<sup>2</sup>, Carme Plumed-Ferrer<sup>3</sup>, D. Erba<sup>1</sup>, M.C. Casiraghi<sup>1</sup>

<sup>1</sup>Dept. of Food, Environmental and Nutritional Science, University of Milan, Milan, Italy;

<sup>2</sup>Institute of Sciences of Food Production - Italian National Research Council, Milan, Italy;

<sup>3</sup>Institute of Public Health and Clinical Nutrition, University of Eastern Finland, Kuopio, Finland

Several studies have emphasized the possibility to enhance nutritional properties of cereal by-products through biotechnological processes. Bran fermentation positively affects the bioavailability of several functional compounds and in particular it could increase water-extractable arabinoxylans (WEAX), compounds with positive effects on glucose metabolism and prebiotic properties. This study was aimed to increase the amount of bran's bioactive compounds through sourdough like fermentation process. Wheat bran fermentations were conducted through continuous propagation by back-slopping of fermented bran (10% inoculum) until a stable microbiota was established, reaching high counts of lactic acid bacteria and yeasts (10<sup>9</sup> and 10<sup>7</sup> CFU g<sup>-1</sup> respectively). After fermentation, levels of soluble fiber increased (+ 30%), WEAX and free ferulic acid were respectively fourfold and tenfold higher than in raw bran, results probably related to microbial xylan-degrading activity, while phytic acid was completely degraded. At each refreshment step, bacterial strains were isolated, clustered, molecularly analysed by Randomly Amplified Polymorphic DNA and identified at the species level by 16S rRNA gene sequencing. *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactobacillus plantarum*, *Pediococcus pentosaceus* and *Pichia fermentans* were dominating the stable sourdough ecosystem. These strains were characterized by their metabolic and enzymatic activities, such as xylan- and phytate-degrading activities. These preliminary data suggest that fermented bran could be considered as an interesting functional ingredient for nutritional enhancement. The characterization of the bacteria involved in sourdough like fermentation process represents the first step toward selecting starter cultures, according to their functional aspects, in order to conduct "tailored" bran fermentation process aimed at improving its functional and nutritional properties.

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**Peter Weegels**, Chair of *Cereals&Europe*, Sonneveld, Netherlands

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**Isabel Großmann**, PhD Student, German Research Centre for Food Chemistry, Germany

**Iris Lovric**, PhD Student, German Research Centre for Food Chemistry, Germany

**Prof. Peter Köhler**, Vice Director of the German Research Centre for Food Chemistry, Germany

**Aboubakry Diallo**, Student board Member of *Cereals&Europe*, University of Nottingham, UK

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