Proceedings
of the
2\textsuperscript{nd} International ImpARAS Conference

\textbf{Warsaw, Poland}

\textbf{20\textsuperscript{th} – 22\textsuperscript{nd} September 2016}

\textit{This conference is organized by COST Action FA1402 ImpARAS}\n\textit{Improving Allergy Risk Assessment Strategy for new food proteins}

www.imparas.eu
Scope and welcome address

As chair of COST Action ImpARAS FA1402, it is a real pleasure to welcome you at our second ImpARAS conference in Warsaw in Poland. ImpARAS, Improving Allergenicity Risk Assessment Strategy (for novel and modified proteins), is an European network that aims to build an interdisciplinary European network of scientists with a broad range of expertise to discuss, with an out-of-the-box view, new ideas and more predictive models and approaches to improve the current allergenicity risk assessment strategy. ImpARAS will help to develop an improved allergenicity risk assessment strategy for novel proteins by adding more predictive tools to the current risk assessment strategy, and accelerate the introduction of novel protein (sources) onto the market, to mitigate the concern of consumers around novel or genetically modified protein (products) and to advice policy makers on the safety of novel protein (products).

The networks focusses on different topics:

- Physical/chemical properties of proteins impacting allergenicity, including effect of processing, matrix effect, glycosylation, lipid binding, digestion, bioinformatics, protein purifications and analysis and others.
- In vitro methods to predict sensitization to food allergy, including epithelial transport of proteins, DC-T cell interactions, activation innate and adaptive immune system and others.
- In vivo methods to predict sensitization to food allergens, including mouse, rat or other models to measure effect on the immune system and others.
- Allergenicity Risk assessment, including current status, examples and applications, visions and others.

ImpARAS is a COST Action that is supported for 4 years (December 2014 – December 2018) by COST (European Cooperation in Science and Technology). More than 210 scientist from Industry, Universities, knowledge centers and regulatory bodies from 30 countries are united in ImpARAS. The network is active through a range of networking tools, such as meetings, workshops, conferences, training schools, and exchange of staff between partners also called short-term scientific missions (STSMs). ImpARAS is open to researchers from universities, public and private research institutions, as well as to NGOs, industry and SMEs. So you are welcome to join our network! You can find more information on our ImpARAS website; www.ImpARAS.eu or become member of our LinkedIn group.

I would like to take this opportunity to thank the local organizers for all their efforts to make this conference a success and to welcome you in the wonderful atmosphere of Warsaw. Also thanks to the scientific committee that made its best to offer you such an exciting and interesting program. I hope that you will enjoy the conference, make new contacts and start new opportunities with colleagues from other countries. Of course I also hope to meet you again on one of our future meetings.
Summary

Scientific and organizing committees ................................................................. 4
Scientific Program ................................................................................................ 6
CVs invited speakers ............................................................................................. 10
List of Oral presentations ..................................................................................... 15
List of Poster presentations ............................................................................... 17
List of Flash presentations ................................................................................... 19
Abstracts Oral, Poster & Flash presentations ..................................................... 20
Participants ........................................................................................................... 64
Local Information .................................................................................................. 66
Sponsors & organizers .......................................................................................... 69
Scientific and organizing committees
Scientific committee

Kitty Verhoeckx  
TNO, The Netherlands,  
(Chair)

René Crevel  
Unilever, UK,  
(Vice Chair)

Karin Hoffmann-Sommergruber  
Medical University of Vienna,  
Austria

Gabriel Mazzucchelli  
University of Liege, Belgium

Erwin Roggen  
3rsmc, Denmark

Edyta Sienkiewcz-Szlapka  
University of Warmia and Mazury,  
Poland

Liam O’Mahony  
Swiss Institute of Allergy and  
Asthma Research, Switzerland

Katrine Lindholm-Bøgh  
National Food Institute,  
Technical University of Denmark  
Denmark

Anne Constable  
Nestlé Research Centre,  
Switzerland

Ben Remington  
TNO, The Netherlands

Paola Roncada  
Istituto Sperimentale Italiano  
Lazzaro Spallanzani, Italy
Organizing committee

Barbara Wroblewska
Pan Olsztyn, Poland

Agata Szymkiewicz
Pan Olsztyn, Poland

Lidia Markiewicz
Pan Olsztyn, Poland

Joanna Fotschki
Pan Olsztyn, Poland

Anna Szyć
Pan Olsztyn, Poland

Kitty Verhoeckx
TNO, The Netherlands

Astrid Kruizinga
TNO, The Netherlands

Marloes van der Waal-Bellaart
TNO, The Netherlands
## Scientific Program

### Day 1 - Tuesday September 20\textsuperscript{th}

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:30-14:00</td>
<td>Registration</td>
</tr>
<tr>
<td>14:10-14:20</td>
<td>Opening welcome from Olsztyn Pan</td>
</tr>
<tr>
<td>14:20-14:30</td>
<td>Presentation of COST Action FA1402 ImpARAS</td>
</tr>
<tr>
<td></td>
<td>Kitty Verhoeckx, Chair of the Action, TNO, The Netherlands</td>
</tr>
</tbody>
</table>

**Chair:** Barbara Wróblewska & Eva Untersmayr

<table>
<thead>
<tr>
<th>14:30-15:15</th>
<th><strong>Keynote lecture:</strong> The role of intestine microbiota in allergy development. [Bożena Cukrowska, Hospital - Institute &quot;Memorial - Children's Health Center&quot;, Warsaw, Poland]</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:15-15:35</td>
<td><strong>Identification of Allergens in the Mediterranean fish species gilthead seabream and European seabass.</strong> [Denise Schrama, Centre of Marine Science, University of Algarve, Portugal]</td>
</tr>
</tbody>
</table>

**Chair:** Gabriel Mazzucchelli & Thomas Holzhauser

<table>
<thead>
<tr>
<th>16:05-16:25</th>
<th><strong>Effect of processing and protein material on the performance of a normalised real-time PCR approach to quantify soybean as a potential hidden allergen in foods.</strong> [Isabel Mafra, University of Porto, Portugal]</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:25-16:45</td>
<td><strong>IgE cross-reactivity between the major peanut allergens Ara h 2 and Ara h 6.</strong> [Stéphane Hazebrouck, CEA-INRA, France]</td>
</tr>
<tr>
<td>16:45-17:05</td>
<td><strong>Assessing hazelnut allergens in model chocolates by sandwich elisa as affected by matrix.</strong> [Joana Costa, University of Porto, Portugal]</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>17:15-19:05</th>
<th><strong>Working group 1-2-3-4 meeting for ImpARAS members</strong></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>19:00-23:00</th>
<th><strong>Conference Dinner</strong></th>
</tr>
</thead>
</table>
Day 2 - Wednesday September 21st

Chair: Erwin Roggen & Sandra Denery
09:00-09:45  Keynote lecture: The role of gastric digestion in food allergy.
  Eva Untersmayr, Medical University Vienna, Austria
09:45-10:05  Relationship between deamidation intensity and allergenicity of acid Hydrolyzed
  Wheat Proteins preparations: from France to Japan.
  Olivier Tranquet, INRA, France
10:05-10:25  Receptor mediated uptake of peanut proteins in dendritic cells.
  Joost Smit, Institute for Risk Assessment Sciences, Utrecht University, Netherlands
10:25-10:55  Coffee Break

Chair: Edyta Sienkiewicz & Katrine Lindholm Bøgh
10:55-11:15  Establishing methods to evaluate intestinal uptake of food proteins.
  Katrine Graversen, DTU National Food Institute, Denmark
11:15-11:35  Impact of processing on physicochemical characteristics and digestive proteolysis of
  insect flours.
  Uri Lesmes, Technion, Israel
11:35-11:55  An in vivo safety approach in mice to determine adjuvanticity of genetically modified
  (GM) novel foods.
  Michelle Epstein, Medical University of Vienna, Austria
11:55-13:00  Lunch
13:00-14:30  Poster session

Chair: Araceli Diaz Perales
14:30-15:15  Keynote lecture: The effect of house dust mite allergen on airway epithelial barrier
  function.
  Irene Heijink, University Medical Center Groningen, The Netherlands

Chair: Kitty Verhoeckx & Michelle Epstein
15:15-16:15  Flash presentations of ESRs (5 min + 1 min questions)
  1) Andrijana Nesic; Molecular characterization of recombinant Mus a 5 allergen from banana fruit.
  2) Daniel Lozano Ojalvo; Assessment of the allergenicity of egg protein hydrolysates.
  3) Nuria Cubells-Baeza; Assessment of CD1d presentation of food lipid allergens.
  4) Mathilde Claude; Degranulation ability of native and aggregated ovalbumin after in vitro simulated
  digestion.
  5) Simona Lucia Bavaro; Investigation on protein profiles of raw and roasted peanuts submitted to
  two different in vitro digestion models.
  6) Marija Perusko; Effects of Maillard reaction on immunogenicity of β-lactoglobulin.
16:15-16:45  Coffee Break

Chair: Charlotte Bernhard Madsen & Joost Smit
16:45-17:05  Lower gut microbiota diversity is associated with higher susceptibility of BALB/c mice
  to allergic sensitization.
  Aicha Maiga, CEA-INRA, France
17:05-17:25  Oral immunotherapy in combination with a non-digestible oligosaccharide supplemented diet in a peanut allergy mouse model. 
Laura Wagenaar, Institute for Risk Assessment Sciences, Utrecht University, Netherlands

17:25-17:45 Differences in gut microbiota composition in non-responders and responders with regard to allergen sensitization and anaphylaxis in a food allergy model in mice.
Unni Nygaard, Norwegian Institute of Public Health, Norway

17:45-18:15 MC-meeting

19:00-23:00 Conference Dinner
Day 3 - Thursday September 22nd

Chair: Rocio Fernandez Canton

09:00-09.45  Keynote lecture: Bioinformatic Screening and Detection of Allergen Cross-Reactive IgE-binding Epitopes
Scott McClain, Syngenta, USA

09:45-10:00  Summary of the Warsaw Training school 2016
Ben Remington, TNO, Netherlands

10:00-10:30  Coffee Break

10:30-11:30  Working group 1-2-3-4 meeting for ImpARAS members

Chair: Ann Constable & René Crevel

11:30-11:50  Allergenicity assessment of genetically modified plants –need to know vs nice to know.
Rocio Fernandez Canton, Monsanto Company, Belgium

11:50-12:10  Development of a tiered risk assessment approach for unintended allergen presence.
Astrid Kruizinga, TNO, Netherlands

12:10-12:30  Individual and population based food allergen thresholds: Update of current knowledge and possible ImpARAS applications.
Ben Remington, TNO, Netherlands

12:30-12:40  Conclusions and perspectives

12:40-13:30  Lunch
CVs invited speakers
Bożena Cukrowska

Hospital - Institute "Memorial - Children's Health Center", Warsaw, Poland

Professor Bożena Cukrowska was graduated from the faculty of medicine, Medical University of Bialystok where she received MD title. Since 1985 she worked at the Department of Children's Diseases in Bialystok, and then in 1991-1999 at the Department of Immunology of the Czech Academy of Sciences in Prague. In 1997 she received PhD in immunology from Charles University, Prague. Since 2000 she has worked at the Department of Pathology the Children's Memorial Health Institute in Warsaw, where she has headed the Laboratory of Immunology. In 2012, at the hands of the President of the Polish Republic she received the title of the professor of medical sciences.

Prof. Bożena Cukrowska is an expert in the field of mucosal immunology, intestinal microbiota and immunological mechanism of mucosa associated diseases including allergy, celiac disease and inflammatory bowel diseases. She is also experienced in population-based genome wide association studies, which were running as part of international projects. She is the author and co-author of 158 full-text research papers with a total "impact factor" 176.3 as well as the co-inventor of the patent describing specific Lactobacillus strains activating anti-allergic processes. The invention is being used for the production of probiotic formulation intended for children with atopic dermatitis.
Eva Untersmayr studied Medicine at the Medical School of the University of Vienna, Austria and the University of Florence, Italy. In 2001 she started her research carrier focusing on risk factors and mechanisms of food allergy in the research group of Prof. Erika Jensen-Jarolim at the Department of Pathophysiology in Vienna. Performing a clinical study in fish allergic patients Eva Untersmayr was a clinical research fellow in the laboratory of Prof. Lars K. Poulsen at the University of Copenhagen in 2003 and 2004. Since 2005 she is independent leader of numerous third-party funded research grants focusing on risk factors for food allergic reactions as well as the characterization of novel biomarkers and therapeutic targets for allergy treatment. In 2007 she established her independent research group at the Department of Pathophysiology and Allergy Research of the Medical University of Vienna, where she is currently working as an Associated Professor. Eva Untersmayr is author of 46 highly cited publications in peer reviewed journals and winner of several national and international prizes. Being a medical specialist in Immunology she is additionally affiliated with the private allergy clinic AllergyCare focusing on component resolved diagnosis of allergic patients.
Irene Heijink studied Biology at the University of Groningen, and graduated in 1998 with a specialization in Medical Biology, after which she started her PhD studies on the regulation of T cells in asthma at the departments of Hematology, Allergology and Pulmonology, University Medical Center in Groningen. After receiving her PhD degree in 2004, she continued her line of research as post-doctoral researcher within the same departments, studying the interaction between T cells and the airway epithelium in asthma. After two years, she received a personal grant from the Netherlands Lung Foundation (previously Asthma Foundation), which enabled her to perform post-doctoral research in the St. Michael’s Hospital, University of Toronto, Canada. Here, she studied plasticity and repair responses of the airway epithelium in asthma. After 1,5 year, she moved back to Groningen, where she was appointed as a post-doc on a KNAW fellowship from Prof. Dr. Dirkje Postma, which enabled Heijink to further elaborate her line of research on the epithelial repair and immunological barrier function in asthma and COPD. In 2011, Heijink entered the Tenure Track of the UMCG. In 2013, she was appointed as Assistant Professor, heading the Experimental Pulmonology and Inflammation Research (EXPIRE) lab. In 2015, she was appointed Tenure Track Associate Professor. Heijink is currently staff member of the Medical Biology, board member of the Groningen Research Institute for Asthma and COPD (GRIAC), member of the Netherlands Respiratory Society (NRS), European Respiratory Society (ERS) and American Thoracic Society (ATS). The main focus of her current research is the repair and immunological barrier function of respiratory epithelium in asthma and COPD. Specifically, her research centers on i) the interaction of airway epithelium with the environment, including environmental exposures (e.g. allergens), other structural cells (e.g. fibroblasts) and inflammatory cells (e.g. lymphocytes) using 3D and co-culture models, ii) the consequences of epithelial damage for airway inflammation and airway remodeling, including the use of a murine conditional E-cadherin knock-out model, iii) strategies to improve lung epithelial regeneration and barrier function, including mechanisms to improve corticosteroid sensitivity and the use of mesenchymal stem/stromal cells.
Scott McClain’s academic background is in physiology and environmental toxicology having received his M.S. and Ph.D. degrees in the Center for Environmental Toxicology and Statistics at Miami University, in Oxford, OH. Dr. McClain’s professional background is in the biotechnology sector with training primarily in immunology, clinical allergy diagnostics, product efficacy and safety testing. He started his professional career supporting research programs in immunology and biochemistry with a focus on developing bioactive food ingredients. The last 10 years have been in the regulatory divisions of the agricultural biotechnology sector with an emphasis on conducting and communicating risk assessments in allergy and toxicology which support the safe introduction of novel food and feed crop products. Dr. McClain has worked in Syngenta Product Safety for 7 years. He represents Syngenta as co-chair of the ILSI/HESI Protein Allergenicity Technical Committee and Chair of the Crop Life International Allergy Expert Team.
List of Oral presentations

O01 The role of intestine microbiota in allergy development
Bożena Cukrowska, Hospital - Institute "Memorial - Children's Health Center", Poland

O02 Identification of Allergens in the Mediterranean fish species gilthead seabream and European seabass.
Denise Schrama, Centre of Marine Science (CCMAR), University of Algarve, Portugal

O03 Effect of processing and protein material on the performance of a normalised real-time PCR approach to quantify soybean as a potential hidden allergen in foods.
Isabel Mafra, REQUIMTE-LAQV, Faculty of Pharmacy, University of Porto, Portugal

O04 IgE cross-reactivity between the major peanut allergens Ara h 2 and Ara h 6.
Stéphane Hazebrouck, CEA-INRA, Service de Pharmacologie et d’Immunanalyse, Laboratoire INRA d’Immuno-Allergie Alimentaire, France

O05 Assessing hazelnut allergens in model chocolates by sandwich elisa as affected by matrix.
Joana Costa, REQUIMTE-LAQV/Faculty of Pharmacy University of Porto, Portugal

O06 The role of intestine microbiota in allergy development
Eva Untersmayr, Medical University Vienna, Austria

O07 Relationship between deamidation intensity and allergenicity of acid Hydrolyzed Wheat Proteins preparations: from France to Japan.
Olivier Tranquet, INRA - Institut National de la Recherche Agronomique, France

O08 Receptor mediated uptake of peanut proteins in dendritic cells.
Joost Smit, Institute for Risk Assessment Sciences, Utrecht University, The Netherlands

O09 Establishing methods to evaluate intestinal uptake of food proteins.
Katrine Graversen, DTU National Food Institute, Denmark

O10 Impact of processing on physicochemical characteristics and digestive proteolysis of insect flours
Uri Lesmes, Technion, Israel

O11 An in vivo safety approach in mice to determine adjuvanticity of genetically modified (GM) novel foods
Michelle Epstein, Medical University of Vienna, Austria

O12 The effect of house dust mite allergen on airway epithelial barrier function
Irene Heijink, UMCG, The Netherlands

O13 Lower gut microbiota diversity is associated with higher susceptibility of BALB/c mice to allergic sensitization.
Aicha Maiga, CEA-INRA, Service de Pharmacologie et d’Immunanalyse, Laboratoire INRA d’Immuno-Allergie Alimentaire, France

O14 Oral immunotherapy in combination with a non-digestible oligosaccharide supplemented diet in a peanut allergy mouse model.
Laura Wagenaar, Institute for Risk Assessment Sciences - Utrecht University, The Netherlands

O15 Differences in gut microbiota composition in non-responders and responders with regard to allergen sensitization and anaphylaxis in a food allergy model in mice
Unni Nygaard, Norwegian Institute of Public Health, Denmark
O16  Bioinformatic Screening and Detection of Allergen Cross-Reactive IgE-binding Epitopes
Scott McClain, Syngenta, U.S.A.

O17  Allergenicity assessment of genetically modified plants – need to know vs nice to know
Rocio Fernandez Canton, Monsanto Company, Belgium

O18  Development of a tiered risk assessment approach for unintended allergen presence
Astrid Kruizinga, TNO, The Netherlands

O19  Individual and population based food allergen thresholds: Update of current knowledge and possible ImpARAS applications
Ben Remington, TNO, The Netherlands
List of Poster presentations

P01 Effect of EDTA enriched diets on farmed fish allergenicity; a proteomics approach
Cláudia Raposo, Universidade do Algarve, Portugal

P02 Investigation of allergen protein modifications induced by food processing.
Eszter Schall, Budapest University of Technology and Economics, Hungary

P03 Metabolic fate of allergenic proteins in whole raw and roasted peanuts.
Gianfranco Mamone, Institute of Food Sciences – CNR, Italy

P04 Production and characterization of quinoa (Chenopodium quinoa Willd.) protein isolate for production of hypoallergenic foods.
Gianluca Picariello, Institute of Food Science and Technology, National Research Council, Italy

P05 New allergens from anise and caraway from Apiaceae family.
Iwona Majak, Lodz University of Technology, Faculty of Biotechnology and Food Sciences, Institute of General Food Chemistry, Poland

P06 Production, characterization and evaluation of the digestibility of hemp protein isolates (Cannabis sativa L.).
Pasquale Ferranti, University of Naples Federico II, Italy

P07 The relationship between food processing and infantile food allergies.
Sibel Karakaya, Ege University, Turkey

P08 The Impact of food processing and food matrix on the allergenic properties of foods.
Sibel Karakaya, Ege University, Turkey

P09 Quantification of whey protein after in vitro digestion: from mouth to colon.
Teresa Sánchez Moya, UNIVERSITY OF MURCIA, Spain

P10 Effect of orally administrated hydrolysed OVA on mice mucosal immune system response.
Barbara Wroblewska/ Dagmara Złotkowska, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland

P11 The effect of transglutaminase linking on mare’s milk in aspect of crosslinking with cow’s milk proteins.
Joanna Fotschki-Dabkowska, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland

P12 Hydrolysates of proteins from rice and oat milks modulate physiological status of intestinal barrier.
Lidia H. Markiewicz, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Poland

P13 Lactobacillus casei LOCK0919: promising probiotic bacteria.
Petra Hermanova, Institute of Microbiology of the Czech Academy of Sciences, Laboratory of Gnotobiology, Czech Republic

P14 Component resolved diagnosis (CRD) in patients with food allergy.
Sedef Nehir, Ege University, Turkey

P15 Greetings from the plant side – transcriptomic characteristics of pollen allergens in the region of Ukraine.
Jana Žiarovská, Slovak University of Agriculture in Nitra, Slovak Republic
Serum diamine oxidase activity as a diagnostic test for pseudoallergy in pediatric population-the pilot study.
Joanna Kacik, Paediatric, Nephrology and Allergology Clinic, Military Institute of Medicine, Poland

Is mealworm allergy indicative for allergy to other insects?
Henrike Broekman, UMCU, The Netherlands

The nature of wheat gliadins modifies the immune response in a mice model of food allergy
Gregory Bouchaud, INRA, France
List of Flash presentations

F01 Molecular characterization of recombinant Mus a 5 allergen from banana fruit
Andrijana Nesic, Faculty of Chemistry, University of Belgrade, Serbia

F02 Assessment of the allergenicity of egg protein hydrolysates.
Daniel Lozano Ojalvo, Instituto de Investigación en Ciencias de la Alimentación (CIAL, UAMCSIC), Spain

F03 Assessment of CD1d presentation of food lipid allergens.
Nuria Cubells-Baeza, Universidad Politécnica de Madrid, Spain

F04 Degranulation ability of native and aggregated ovalbumin after in vitro simulated digestion.
Mathilde Claude, INRA, France

F05 Investigation on protein profiles of raw and roasted peanuts submitted to two different in vitro digestion models.
Simona Lucia Bavaro, Institute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR), Italy

F06 Allergenic potential of Maillard products of β-lactoglobulin.
Marija Perusko, Faculty of Chemistry, University of Belgrade, Serbia
Abstracts Oral, Poster & Flash presentations
The role of intestine microbiota in allergy development

Bożena Cukrowska

Microbiota (formerly called microflora) is a group of microorganisms that inhabit the skin and mucous membranes of the human body. The largest microbiota (10^{14} cells), mainly bacteria, is located in the gastrointestinal tract. The gut microbiota is an integral part of the human body forming an organ essential for life, which affects metabolism, production of vitamins, digestion and absorption of nutrients, organ development, with particular emphasis on the barriers of the mucous membranes and the immune system.

Microbial hypothesis assumes that the microbiota inhabiting the digestive tract programs the organism affecting the health in later years. The pregnancy and infantile period is especially important step for health programming. It is believed that the disruption of the gut microbiota homeostasis observed from the first hours after birth has a direct impact on the reduction of functional biodiversity of microbiome and the inappropriate activation of the immune system. It is assumed that the optimal composition of microbiota have healthy breast-fed newborns of mothers giving natural vaginal birth at home. The consequence of dysbiosis in early infancy may be a number of life-style chronic diseases, including allergy.

In recent years, a growing interest in activities affecting the microbial programming that are aimed at early prevention of allergy by modulation of the gut microbiota using probiotics, i.e. live microorganisms which were administered in adequate amounts confer a health benefit on the host. Probiotics are also used for treatment of allergic diseases, particularly atopic dermatitis. i.e. the most often the first disorder staring so called allergic march. The most widely used probiotics are lactic acid bacteria, specifically Lactobacillus and Bifidobacterium species. The mechanisms of action of probiotic strains in allergic diseases may include modulation of the intestinal microbiota, maturation of the gut barrier, and immunomodulation. However, the effects of probiotics described in experimental models need to be confirmed for human use through randomized controlled trials. Unfortunately, clinical trials provide various contradictory findings that do not allow probiotic supplementation to be included in the guidelines for the management of allergic diseases. Thus, modulation of intestinal microbiota is promising but requires further studies to optimize the ingredients used, as well as the dose and duration, and to identify when in the life cycle they should be introduced.
Identification of Allergens in the Mediterranean fish species gilthead seabream and European seabass.

Denise Schrama¹, Cláudia Raposo¹, Annette Kuehn², Pedro Rodrigues¹

¹ Centre of Marine Sciences, CCMAR, Universidade do Algarve, Campus de Gambelas, Edifício 7, 8005-139 Faro, Portugal
² Luxembourg Institute of Health, Department of Infection and Immunity, 29, rue Henri Koch, L-4354 Esch-sur-Alzette, Luxembourg

Background: Fish is an important food product. In the Mediterranean area gilthead seabream and European seabass are the two main farmed fish species. Fish allergy, which is mostly IgE-mediated, affects about 1-3% of the general population. Parvalbumin is the main fish allergen, being a small and highly stable muscle protein. Other allergens are fish enolases, aldolases or gelatin. While cod allergens have been characterized in detail, the identification of gilthead seabream and European seabass allergens has not yet been addressed. The aim of the present study was to identify IgE-reactive proteins from these two Mediterranean species.

Methods: Protein extracts were prepared from cod, gilthead seabream and European seabass. IgE-reactivity using sera from fish-allergic patients was analyzed by IgE line blots. Fish proteins were labeled with fluorescence dyes and separated by 2-dimensional (2D) SDS-PAGE using a multiplex flatbed system. 2D immunoblots were performed using patient sera. IgE-reactive proteins were identified using MALDI-TOF/TOF.

Results: The three fish extracts showed variable protein patterns at 6 to 70 kDa by SDS-PAGE. Commercial antibodies detected parvalbumin in these samples. Five out of 14 patient sera showed IgE-reactivity to parvalbumin-like bands in all samples. These sera were chosen for pooling and use in subsequent 2D IgE-immunoblot. The three fish extracts separated with various proteins spots between pH 3 and pH 9 in 2D SDS-PAGE. Main IgE-reactive proteins from cod were identified to be parvalbumin, enolase and aldolase. Gilthead seabream and European seabass proteins with positive IgE detection signals reaction seemed to be similar to cod allergens but are still subject of ongoing MS analysis.

Conclusion: The allergenicity of different fish species might be variable for sensitized patients. The characterization of allergens from Gilthead seabream and European seabass will need to be addressed in future studies.
**Effect of processing and protein material on the performance of a normalised real-time PCR approach to quantify soybean as a potential hidden allergen in foods**

*Joana Costa¹, Joana S. Amaral¹², Liliana Grazina, M. Beatriz P. P. Oliveira¹ and Isabel Mafra¹* *

¹REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.  
²ESTIG, Instituto Politécnico de Bragança, Bragança, Portugal. *E-mail: isabel.mafra@ff.up.pt

**Background**: Soybean is a food ingredient with both technological and biofunctional properties, whose use has been increasing considerably in the past decades [1]. Among its numerous applications, soybean is widely used by the food industry in processed meat products, such as sausages, hamburgers or cooked hams due to its emulsifier properties, gelling capability, texture improving and water-binding capacity. For that reason, soybean derived products can be present in a wide range of highly processed foods as a hidden allergen. Thus, in order to ensure consumer’s safety, verify labelling compliance and help industry managing food allergens, the development of proper and highly specific/sensitive analytical methodologies is of utmost importance. It is also critical to assess their applicability on the traceability of different forms of soybean materials as affected by processing.

**Methods**: In this work, a normalised real-time polymerase chain reaction (PCR) system was proposed for the quantitative analysis of soybean as a potential hidden allergen in meat products. Different binary model mixtures of pork meat spiked with known amounts of soybean protein isolate (SPI) or soybean protein concentrate (SPC), with and without thermal treatment, were prepared. A calibration model was developed based on real-time PCR using primers and hydrolysis probes specifically designed to target eukaryotic reference (universal) and lectin (specific for soybean) genes.

**Results**: The method achieved a limit of detection of 9.8 ng of soybean DNA (8.6 DNA copies), with adequate real-time PCR performance parameters, regardless the soybean protein material (concentrate or isolate) and after thermal treatments. A normalised real-time PCR approach was also proposed in the range of 0.001%-10% (w/w) of soybean protein material in pork meat, which was successfully validated and applied to processed meat products. From the tested samples of cooked hams and mortadellas, soybean was identified in more than 40% of the products in the range of 0.1-4% (w/w), being 3 (12%) of them in incompliance with labelling because of undeclared soybean.

**Conclusion**: A reliable and accurate tool for soybean detection and quantification at trace amounts in processed foods was proposed, highlighting the need of regulatory authorities to ensure labelling compliance of foods.

**References**

**Acknowledgments**: This work was supported by FCT (Fundação para a Ciência e Tecnologia) through project UID/QBI/50006/2013 – POCI/01/0145/FEDER/007265 with financial support from FCT/MEC through national funds and co-financed by FEDER, under the Partnership Agreement PT2020. J. Costa is grateful to FCT grant SFRH/BPD/102404/2014, financed by POPH-QREN (subsidised by FSE and MCTES).
IgE cross-reactivity between the major peanut allergens Ara h 2 and Ara h 6

Stéphane Hazebrouck¹, Blanche Guillon¹, Evelyne Paty², Karine Adel-Patient¹ and Hervé Bernard¹

¹ UMR CEA-INRA Service de Pharmacologie et d’Immunoanalyse, Université Paris-Saclay, 91991 Gif-sur-Yvette, France and
²Université Paris Descartes, APHP, Hôpital Necker Enfants Malades, 75743 Paris, France.

Background: 2S-albumins Ara h 2 and Ara h 6 are the most potent allergens and IgE responses toward these proteins are now considered to be good predictors of clinical reactivity in sensitized patients. According to a sequence identity of 59% and to similar tertiary structures, IgE-binding capacity of Ara h 6 has been suggested to be partially due to cross-reactivity with Ara h 2. Here, we investigated the cross-reactivity between the 2S-albumins and its potential impact on component-resolved diagnostics (CRD).

Methods: Ara h 2 and Ara h 6 proteins were purified from raw and roasted peanuts. Recombinant allergens were produced in order to ensure the absence of any cross-contamination. Cross-reactivity between Ara h 2 and Ara h 6 was evaluated by competitive inhibition of IgE-binding with sera from 17 peanut-allergic patients and by degranulation test of humanized rat basophilic leukemia (RBL) cells.

Results: Competitive inhibition assay using native or recombinant allergens provided comparable results. The level of cross-reactivity observed between Ara h 2 and Ara h 6 was then variable among peanut-allergic patients. A cross-reactivity higher than 5% was observed in only four patients. In two other patients, the IgE response to Ara h 6 was only due to cross-reactivity with Ara h 2 and Ara h 6 displayed then a weak capacity to induce RBL cell degranulation. In contrast, no significant or low-affinity cross-reactivity (lower than 0.5%) was observed in the other tested sera.

Conclusions: Cross-reactivity was actually observed between Ara h 2 and Ara h 6 but IgE binding to both 2S-albumins was mostly mediated by non-cross-reactive epitopes, which is indicative of co-sensitization to both allergens. Our results thus confirmed the importance of measuring specific IgE to the two peanut components Ara h 2 and Ara h 6 in order to get the best predictive value from CRD.
Assessing hazelnut allergens in model chocolates by sandwich ELISA as affected by matrix

Joana Costa¹², Parisa Ansari¹, M. Beatriz P.P. Oliveira², Isabel Mafra²*, Sabine Baumgartner¹ *

¹Christian Doppler Laboratory for Rapid Test Systems for Allergenic Food Contaminants, Center for Analytical Chemistry, Department of IFA-Tulln, University of Natural Resources and Life Sciences, Vienna, Austria. ²REQUIMTE-LAQV, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal. Email: jbcosta@ff.up.pt;

Background: Chocolates encompass a multiplicity of product formulations that often include one or more allergenic ingredients (hazelnut and other nuts). They are among the most appreciated candies, being widely consumed all over the world, equally by children and adults. However, for a significant part of the world’s population, namely the sensitised/allergic individuals, the selection of a simple chocolate can represent a major challenge. If by one side, the correct labelling of processed foods is mandatory and the only proficient means of preventing accidental allergic reactions, by the other side, the excessive use of precautionary labelling restricts choices for the sensitised/allergic patients. In this sense, adequate methodology that would allow defining quantitative amounts for the unintended presence of allergenic foods, such as hazelnut, is of great importance [1].

Method: The aim of this study was to develop a highly specific and sensitive sandwich ELISA for the detection and quantification of hazelnut in complex food matrices, such as chocolates. Due to the high content of polyphenols, carbohydrates and aromatic compounds, the high interference of chocolate matrix makes difficult its analysis both by protein- or DNA-based approaches [2-4]. Using anti-hazelnut poly- and monoclonal antibodies in-house developed, a sandwich ELISA was proposed to target hazelnut proteins from hazelnut model chocolates. Results: The results from the application of ELISA enable the detection of hazelnut proteins down to 1 mg/kg. Owing to the complexity of the matrix (chocolate), accurate quantification of hazelnut was possible down to 50 mg/kg in model chocolates. Further immunoblotting and LC-MS/MS analysis allowed confirming the detection of specific hazelnut proteins and to evaluate matrix effects on the performance of the proposed ELISA system.

Conclusion: These results highlight and reinforce the value of ELISA as rapid, reliable and cost-effective tools for the detection of allergenic ingredients in foods [2, 3].

References

Acknowledgements: The ELISA and MS systems were developed and performed in the Christian Doppler Laboratory “Rapid Test Systems for Allergenic Food Contaminants”, which is funded by the Christian Doppler Research Association and RomerLabs. The authors also thank Marcela Hermann, Institute of Medical Biochemistry, Department of Molecular Genetics, University Vienna, and Thomas Kolbe, University of Veterinary Medicine Vienna, for the immunisation of rabbits and mice, respectively. J. Costa is grateful to FCT grant SFRH/BPD/102404/2014, financed by POPH-QREN (subsidised by FSE and MCTES).
The role of intestine microbiota in allergy development

Eva Untersmayr

Institute of Pathophysiology and Allergy Research, Medical University of Vienna, Austria

Food adverse reactions represent an increasing health concern worldwide and substantially reduce the quality of life of affected patients. Moreover, food allergy is associated with an enormous economic burden by increasing overall health care costs influencing the budget of social insurances and allergic patients. Thus, characterization of underlying mechanisms, development of novel therapeutic strategies and safety evaluations for novel food proteins are of importance to enhance patients’ safety and to prevent development of food sensitizations.

For a long time only food allergens being resistant to digestive enzymes were accepted to harbor sensitizing capacity via the oral route. Protein digestion experiments are considered to be of importance in safety evaluations and characterizations of novel food proteins. However, over the past years several studies reported that even potent food allergens might be readily degraded by digestive enzymes. The enzymatic degradation of proteins is initiated in the gastric lumen. Food proteins are exposed to gastric acidity resulting in protein denaturation. Moreover, only at low pH levels gastric proteases are activated and protein cleavage takes place. After this initial step of protein degradation, the acidic chyme containing peptide fragments is released into the duodenum and stimulates secretin release by S-cells inducing the secretion of pancreatic enzymes. Interestingly, a number of in vitro experiments confirmed that impairment of physiological gastric digestion by elevated gastric pH levels was associated with protein resistance. Additionally, pharmacological gastric acid suppression was found to be a risk factor for food allergy induction in murine as well as human studies. The impact of gastric digestion on food allergy was additionally confirmed for situation with reduced gastric digestion capacity due to surgical interventions. Gastric bypass surgery was found to be associated with increasing sensitization rates. In contrast, posttranslational protein modifications such as tyrosine nitration resulting in higher susceptibility to digestive enzymes were reported to decrease the sensitization capacity via the oral route.

These data underline the essential gate keeping function of gastric digestion. Thus, protein digestion in the gastrointestinal tract might contribute to the network of diverse factors substantially influencing food allergy.

Supported by the Austrian science fund (FWF) grants KLI284 and WKP039.
Relationship between deamidation intensity and allergenicity of acid Hydrolyzed Wheat Proteins preparations: from France to Japan

Olivier Tranquet, Florence Pineau, Roberta Lupi, Colette Larré, Sandra Denery

Acid hydrolysis combined with heating has been applied to gluten proteins to give them new functionalities. These hydrolyzed wheat proteins (HWP) were used as ingredients in food and cosmetics. From the 2000’s cases of severe food allergy to HWP have been reported in individuals elsewhere tolerant to native wheat proteins. Denery et al. demonstrated that deamidation of wheat proteins, a consequence of acid hydrolysis, generate essential neo-epitopes in this particular allergy to wheat. More recently in Japan, a HWP preparation (named GluPearl 19S) elicited severe skin reactions and food allergy in more than 1800 individuals and was likely to contain deamidated gluten proteins.

Level of deamidation depends on treatment intensity; HWP preparations with either low or high level of deamidation can be found as ingredients. This study aimed at exploring the impact of deamidation level of wheat proteins on the degranulation of basophile sensitized with IgE from patient allergic to deamidated gluten (DG).

Impact of deamidation level of gliadins and glutens on IgE reactivity from 8 patients allergic to DG was determined by ELISA. Impact of deamidation on basophile degranulation was also explored with the RBL SX38 cell line passively sensitized with IgE from patients and subjected to crosslinking with a set of deamidated samples. Finally IgE Repertoire specific to deamidated wheat protein was then explored by inhibition with INRA-DG1, a mouse monoclonal antibody specific for deamidated gliadins.

Intensity of binding of patient IgE onto deamidated samples and the degranulation potency were correlated with level of deamidation. Pre-incubation of deamidated gluten with INRA-DG1 mAb inhibited half of its degranulation capacity with patient IgE. These results suggested that the patient IgE repertoire specific for deamidated gluten proteins is likely to be limited to a very few specificities. GluPearl 19S, involved in the Japanese cases, was determined as highly deamidated. It was the most recognized sample among the 5 deamidated glutens tested in this study. Although differences exist between French and Japanese cases (such as the tolerance of native wheat proteins), this result suggested that Japanese and French cases suffered from the same unconventional allergy to wheat.

Receptor mediated uptake of peanut proteins in dendritic cells

Joost Smit, Friederike Sonnet, Raymond Pieters

Dept. of Immunotoxicology, Institute for Risk Assessment Sciences, Utrecht University, the Netherlands.

Degradation of proteins has been shown to determine allergenicity. Most, but not all allergens are usually stable to digestion, or proteins become allergenic when digestion is inhibited. However, it is unclear how allergens are degraded inside pivotal immune cells such as dendritic cells (DC), and how this affects the subsequent immune response to these allergens. In our studies, we determined whether we are able to measure uptake and degradation of allergens, including peanut proteins Ara h 1, 2, 3, and 6, inside DC and whether differences in these factors exist between allergens.

First, using mouse bone marrow-derived DC and fluorescent-labeled proteins, we observed that DC uptake of Ara h1 was much higher than Ara h2, 3 and 6. Using blocking reagents and receptor binding assays for various uptake routes in DC, we observed that the principal route of uptake for Ara h 1 was endocytosis via the mannose receptor. However, other routes of uptake and the route of uptake for the other peanut proteins are currently under investigation.

Second, using proteins coupled to beads we observed that intracellular protein degradation was higher for Ara h1 and Ara h3 than for Ara h2 and 6.

Final, when Ara h 1, 2, 3 or 6 enriched CD4+T cells were added to matching allergen-pulsed DC, we observed that while Ara h1, 3 and to lesser extend Ara h6 elicited strong Th2 type responses, Ara h2 did not elicit any T cell responses. Here, we show that allergenicity of (peanut) proteins may be determined at the level of allergen uptake and breakdown inside the antigen presenting cell.

These findings may be relevant to the risk of existing allergens but also to the risk of novel or modified proteins. This also illustrates the usefulness of in vitro, cell based assays to examine initial responses to potential allergens at the beginning of the allergic cascade.
Establishing methods to evaluate intestinal uptake of food proteins

Katrine Graversen¹, Katrine L. Bøgh, Joost J. Smit²

¹National Food Institute, Technical University of Denmark, Søborg, Denmark
²Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands

Background: What makes a food protein an allergen remains unknown. However, it has been suggested that the route of uptake in the intestine may impact on the sensitising capacity of proteins, and that the route may be influenced by protein chemical characteristics. The aim of this study was to establish methods to evaluate intestinal uptake of food proteins in order to correlate uptake to protein characteristics.

Methods: As model proteins heat-treated whey, consisting of partly denatured and aggregated proteins, was compared to native whey. The intestinal transport was investigated 1) in vivo in BALB/c mice i.g. dosed with the products and sacrificed after 5-45 min to determine BLG levels in serum, 2) ex vivo in bone marrow derived dendritic cells (BMDCs), by incubation with FITC conjugated proteins for 5-120 min before fluorescence intensity of the cells was determined by flow cytometry, and 3) in vitro in Caco-2 cells by determining basolateral BLG levels 1-24 hrs after adding the products apically. In addition, BLG, M cells and CDs were visualised in sections of the murine small intestine.

Results: This study indicates that the in vivo intestinal uptake rate differs between the two products, with the native readily being taken up and detectable 5 min after dosing. This is in line with in vitro results, indicating that the epithelial transport rate is faster for the native protein compared to the heat-treated. It could not be determined from intestinal sections if this was due to different uptake routes. However, ex vivo results indicate that DC uptake of the heat-treated proteins is greater than the native.

Conclusion: We succeeded in establishing methods that could distinguish intestinal uptake of two products with different chemical properties. These methods can be used in future studies of the interplay between uptake and sensitising capacity of food proteins to establish new ways to predict allergenicity in assessment of novel proteins.
Impact of processing on physicochemical characteristics and digestive proteolysis of insect flours

Tatyana David-Birman and Uri Lesmes

Technion – Israel Institute of Technology, Haifa, Israel

Edible insects are gaining increasing interest as a highly sustainable food source and rich source of dietary protein. Various processes are applied to produce commercial insect-based food products, e.g. brownies and crisps, Yet, very little is known on the implications of processing on insect protein digestibility and subsequent allergenicity. This study sought to characterise insect flours in terms of physio-chemical properties, assess the ramifications of thermal and high-pressure homogenization (100MPa for 1, 3 and 5 cycles) and subsequently elude possible implications to insect protein breakdown in the human gastro-intestine.

First, cricket, locust and silk moth flours (CF, LOF and SMF respectively) were characterized before and after varying high-pressure homogenization cycles. In turn, samples were subjected to an in vitro dynamic gastro-intestinal digestion system. SDS-PAGE analysis of digesta reveal homogenization had no appreciable effect on proteolytic breakdown profiles of CF and LOF. Contrary, homogenization was found to increase the breakdown of SMF compared to unprocessed SMF, which is attributed to aggregate disruption and protein solubilisation.

Second, protein-rich CF samples were subjected to thermal treatments, namely cooking (up to 240 minutes at 70°C) and baking (30 minutes at 180°C) in the presence and absence of fructose (aimed to study the possible impact of the Maillard reaction). SDS-PAGE analyses of samples collected from simulated gastro-intestinal digestion indicate differences in the proteolytic breakdown profiles of the cricket proteins. Altogether, these findings emphasize that different common processing operations might have various effects on the physiochemical characteristics and the digestive fate of edible insect proteins. This mandates further and through exploration of such changes and their ramifications to bioaccessibility allergenicity.
An in vivo safety approach in mice to determine adjuvanticity of genetically modified (GM) novel foods

Reiner, D.¹, Lee, R.Y.¹, Higgins, T. J. V.², Epstein, M.M.¹

¹Division of Immunology, Allergy and Infectious Diseases, Experimental Allergy, Department of Dermatology, Medical University of Vienna
²Commonwealth Scientific and Industrial Research Organization Plant Industry, Camberra, ACT, Australia

Background: Genetically modified (GM) foods are evaluated for allergenicity, but few studies have tested adjuvanticity, i.e. influencing allergic responses to other unrelated allergens at sensitization or at elicitation of allergic responses.

Methods: We sought to evaluate the effect of feeding of Bacillus thuringiensis (Bt)-maize and alpha-amylase inhibitor peas (GM peas) on ovalbumin (OVA)-induced experimental allergic asthma in mice. Adult BALB/c mice were provided diets for 4 consecutive weeks that either contained 33% GM or non-GM maize or suspensions of GM and nGM peas administered by gavage twice a week before either 1) sensitization or 2) elicitation of allergic asthma in mice with pre-existing allergy. The mice were then evaluated for OVA specific antibodies, allergic lung inflammation and mucus production.

Results: We observed that feeding GM peas or maize did not affect OVA-induced eosinophilic airway and lung inflammation, mucus hypersecretion or OVA-specific antibody production at initiation or relapse of allergic asthma. There was no adjuvant effect upon GM-maize and GM pea consumption on the onset or severity of allergic asthma.

Conclusions: Here, we show that neither GM-maize nor GM-pea consumption influenced allergic responses to disease induced by an unrelated, non-crossreactive protein. Although there is no adjuvant effect observed in our study, a positive control with high adjuvanticity would be necessary to validate this model.
The effect of house dust mite allergen on airway epithelial barrier function

Irene H. Heijink

University of Groningen, University Medical Center Groningen, Department of Pathology and Medical Biology, Groningen, The Netherlands

Allergic asthma is characterized by wheezing, sputum production, variable airflow limitation and airway hyper-responsiveness. The development of asthma involves environmental as well as genetic factors. Early-life sensitization to aeroallergens is an important environmental risk factor. Allergen-specific T helper cells secrete type-2 cytokines to attract and activate eosinophils and mast cells, giving rise to the clinical symptoms of asthma. Although allergy is known as the strongest identifiable predispositions of asthma, it cannot explain the prevalence of asthma alone. Of interest, many of the identified asthma susceptibility genes are expressed in the airway epithelium, including GSDMB, PCDH1, CDHR3 and IL33. The airway epithelium forms the first continuous line of defense against inhaled environmental insults. A number of observations, in vivo and in vitro, suggest that the airway epithelial barrier is disrupted in asthma, with loss of epithelial junctions and cell-cell contact molecules such as E-cadherin. This may have important consequences for the disease, increasing epithelial permeability and the access of allergens to the submucosa. Moreover, epithelial damage and disruption of barrier function may lead to the release of pro-inflammatory mediators, specifically of cytokines that drive type-2-mediated immunity. Many aeroallergens, including house dust mite (HDM), fungi, cat, pollen and cockroach contain proteolytic activities, which can both directly and indirectly cause disruption of epithelial junctions, thus compromising barrier function. Indeed, we observed that HDM exposure causes a transient decrease in airway epithelial barrier function, with a greater loss of barrier function in epithelial cells from asthma patients than from healthy controls. Our mouse model of asthma showed that the ability of HDM extract to disturb epithelial barrier function is related to allergic sensitization. A direct link between compromised barrier function and allergy has been also provided by findings in atopic dermatitis. Mutations in the epidermal barrier gene FLG lead to a defect in epithelial barrier function, resulting in increased epithelial permeability and penetration of exogenous substances (e.g. allergens, bacteria). Furthermore, in eosinophilic esophagitis, a food allergy-related disorder, recent GWAS studies have identified the gene encoding junction protein Desmoglein 1 as susceptibility gene for the disease. Therefore, it is of great interest to understand the mechanisms of disturbed epithelial barrier function in allergic diseases.
Lower gut microbiota diversity is associated with higher susceptibility of BALB/c mice to allergic sensitization

M.A. Maiga¹, P. LePage², N.G. Cortez-Perez², P. Gerard², K. Adel-Patient¹, and S. Hazebrouck¹

¹UMR CEA-INRA Laboratoire d’Immu-No-Allergie Alimentaire, CEA de Saclay, 91191 Gif-sur-Yvette, France
²Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

Background: The hygiene hypothesis suggests that neonatal alterations of microbial exposure promote the development of allergic diseases by affecting the maturation of the host immune system. In previous works, we observed that BALB/c mice experimentally sensitized to cow’s milk (CM) could exhibit a rather high inter-individual variability for the production of CM-specific IgE antibodies. Here, we aimed to determine whether the level of sensitization could be correlated to the early gut microbiota composition.

Methods: Six-week-old BALB/c mice (n=92) were orally sensitized to CM proteins. Fecal samples were collected one week before sensitization to analyze the composition of the gut microbiota (454 pyrosequencing targeting the 16S ribosomal RNA gene V3–V4 region) and the production of short-chain fatty acids (SCFA). Mice received six intra-gastric administrations of CM with cholera toxin, an adjuvant required to induce oral sensitization. Specific IgE and IgG1 responses against β-lactoglobulin (BLG), a major cow’s milk allergen were measured in sera.

Results: As expected, a great variability of the BLG-specific antibody responses was observed among sensitized mice. 13 high-IgE responder (HR) mice and 13 low-IgE responders (LR) mice were then selected for further analysis of the gut microbiota composition. A lower gut microbiota diversity was observed in HR mice compared to LR mice. Even though the dominant microbiota was not significantly different, the relative abundance of the genus Lachnospiraceae incerta sedis was significantly higher in LR mice than in HR mice. No significant difference was observed for SCFA production.

Conclusion: Our results suggest that the gut microbiota could actually influence the host susceptibility toward an allergic sensitization. This is in agreement with previous human studies reporting a lower gut microbiota diversity in children developing food-sensitization. Further studies are required to characterize the impact of the gut microbiota composition at earlier time points (before and after weaning).
Oral immunotherapy in combination with a non-digestible oligosaccharide supplemented diet in a peanut allergy mouse model

L. Wagenaar¹, M.M. Vonk², M. van Roest¹, L.J.W. Kruijssen¹, B.C.A.M. van Esch², L.M.J. Knippels², J. Garssen², R.H.H. Pieters¹ and J.J. Smit³, NUTRALL consortium.

1 Department of Immunotoxicology, Institute for Risk Assessment Sciences, Faculty of Veterinary Medicine, Utrecht University, The Netherlands
2 Department of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Faculty of Science, Utrecht University, The Netherlands
3 Department of Immunology, Nutricia Research, Utrecht, The Netherlands

Background Improving oral immunotherapy (OIT) for food allergy is necessary to reduce side effects and achieve tolerance. Non-digestible oligosaccharides, like scFOS/lcFOS (FF), have shown to reduce allergic symptoms in allergy models. This study aims to evaluate the capacity of FF to support OIT in an established peanut allergy mouse model.

Methods After sensitization using peanut extract (PE) and CT, mice received a 1% FF (9:1) or control diet and were treated with PE or PBS intra-gastric (5 times/wk) for three weeks. Hereafter, mice were exposed to PE via an intradermal (d64), intra-gastric (d70) and intraperitoneal (d77, i.p.) challenge to determine clinical efficacy.

Furthermore, antibody levels, cytokine production and number of various immune cells were measured during the study (d0, d35, d50, d63, d71 and d78).

Results OIT on its own, was able to reduce allergic symptoms upon PE challenges, in addition, serum levels of IgE, IgG1 and IgG2a, were raised after OIT. OIT+FF was able to lower the acute allergic skin response and mast cell degranulation after peanut exposure. FF did not show an additive effect on antibody levels. On d63 (after therapy) and d78, the production of IL-5 and IL-10 by splenocytes, and on d63 by MLN cells was elevated in the OIT+FF group compared to the OIT group. On d63, percentage of B cells in the spleen was lower in the OIT+FF group compared to the OIT group. On d78, Th1 cells were higher in the MLN and Th2 cells were lower in the spleen in the OIT+FF group vs the OIT group. Also, activated CD8+ T cells were lower in the MLN and CD103+CD11b+ DCs were higher in the spleen.

During therapy, the short-chain fatty acid (SCFA) content in the caecum showed a shift by the FF diet, to a higher butyrate, lower acetate ratio. Conclusion These data show that in a mouse model for peanut allergy, OIT+FF protect against allergic responses upon peanut exposure.

Furthermore, cellular parameters suggest Th2 suppression after OIT+FF, compared to only OIT. FF also altered the cytokine production and influenced SCFA content in the caecum.
O15

Differences in gut microbiota composition in non-responders and responders with regard to allergen sensitization and anaphylaxis in a food allergy model in mice.

Knut Rudi¹, Monica Andreassen², Hubert Dirven² and Unni C. Nygaard²

¹Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås, Norway.
²Domain for infection control and environmental medicine, Norwegian Institute of Public Health, Oslo, Norway

Associations between altered gut microbiota diversity and composition in relation to asthma, allergic rhinitis and eczema have been reported both in humans and animal models, but the role in food allergy has been less studied. In the present study we aimed to determine putative associations between the gut microbiota and sensitization and anaphylaxis in a lupine food allergy model in mice, including assessment of the immunized but non-responding animals. Secondly, we investigated the microbiota variations throughout the gut.

After repeated oral immunizations with an extract of the food allergen lupine with the adjuvant cholera toxin, the animals were i.p. challenged with a high dose of the allergen. The negative control animals received buffer both during immunization and challenge. Anaphylactic responses were monitored (anaphylactic score and rectal temperature) and sensitization assessed by allergen-specific IgG1 and total IgE levels. Intestinal content was collected at four locations, the small intestines, cecum, colon and fecal pellets, and the microbiota diversity and composition was determined by use of deep sequencing of the 16S rRNA-gene.

While most immunized animals became allergic, a few non-responders demonstrated low anaphylaxis accompanied by an intermediate level of allergen-specific IgG1. Both the gut segment location and the sensitization had major effects on the microbiota diversity and composition. The differences between the microbiota composition in sensitized and control animals were consistent across the gut segments, and ten bacterial species were identified to differ significantly for at least one of the segments. All ten species were significantly different in the colon sample, suggesting that colon may be the most sensitive gut segment to detect microbiota changes due to allergen immunization. Animals immunized but not sensitized (the non-responders) displayed the overall same microbiota response as the responders. Interestingly, however, a species belonging to the Lacnospiraceae showed an intermediate level. The results suggest that the susceptibility for acquiring changes in the microbiota and/or responding with allergic sensitization after oral allergen immunization are linked features in this food allergy model in mice. This may indicate that the gut microbiome represent a potential interventional target in establishing more reproducible animal models for food allergy.
O16

Bioinformatic Screening and Detection of Allergen Cross-Reactive IgE-binding Epitopes

Scott McClain

Syngenta, U.S.A.

Allergens are related at the sequence level and this is the core of understanding how bioinformatics can help assess cross-reactivity potential. Shared sequence can be the basis of using the interpretation of shared structure to identify unique aspects of allergens. Cross-reactivity among allergens is based on shared IgE-binding which is indicative of shared B-cell epitope sequence(s). Localized sequence similarity is a component of modeling how epitopes may be used to establish criteria that identify relevant levels of structural relatedness among allergens. Representative allergens were examined at the level of their IgE binding epitopes to determine how the FASTA bioinformatics algorithm could be used to set a threshold for similarity.

A statistical measure of sequence similarity (E-value) was used to set a threshold. The allergens peanut Ara h 1 and Ara h 2, shrimp tropomyosin Pen a 1, and Birch tree pollen allergen, Bet v 1 were sources of known epitope sequence. Each epitope or set of epitopes was inserted into random sequence to create hypothetical proteins. Hypothetical proteins were examined for alignments with allergens and each was noted for the ability to identify the epitope’s source allergen as well as match any cross-reactive or other homologous allergens.

A minimum FASTA E-value range was identified that could identify the presence homologous allergens and which could be used to screen proteins that may share biologically relevant sequence.
Allergenicity assessment of genetically modified plants – need to know vs nice to know.

R. Fernández-Cantón*, K. Glenn†, A. Salamini‡

*Monsanto Europe S.A., B-1150 Brussels, Belgium; †Monsanto Company, St. Louis, MO 63167, US

*[rocio.fernandez.canton@monsanto.com]

Prior to the commercialization of genetically modified (GM) plants, a comprehensive safety assessment is done to evaluate its risks, including an evaluation of their potential allergenicity derived from either the introduced protein and or any food or feed derived from the GM plants. International safety assessment guidelines for GM plants include two elements focused on food allergies: 1) to ensure that any introduced protein is unlikely to be allergenic in the diet and 2) that the process of introducing a gene into a plant has not inadvertently increased the levels of endogenous allergens, especially in crops like soybean that are considered commonly allergenic foods.

For the introduced protein, several studies are needed for a comprehensive allergenicity assessment such that a weight of evidence approach is recommended: (a) bioinformatics comparison of the protein amino acid sequence to known allergens, (b) susceptibility of the protein to pepsin digestion, (c) source organism of the gene for the protein, (d) history of safe use of the protein, etc. For the endogenous allergens in plants like soybean, one aspect that has drawn a lot of attention is understanding the natural variability of these levels relative to the possibility that plant transformation inadvertently affected their levels. Several publications have shown that both environment and breeding genetics have large effects on allergen levels in soybeans, a well-studied crop. However, new EU regulations that require quantitative comparison of endogenous allergen levels of the new GM plant to conventional comparators has caused some controversy.

With the large natural variability seen in conventional varieties, the value for extensive measurements of these allergens in GM crops is questionable. As summarized in this presentation, all studies to date have shown that the effect of plant transformation on allergen levels is small and typically not statistically significantly different from levels in the conventional comparators.

Conversely, without any regulatory requirements for measurement of allergens in conventionally cultivated soybeans, there is a history of safe consumption of soybean-derived foods in spite of the large natural variability of the allergens in the food supply. Overall, this presentation reviews how the allergenicity assessments of GM plants are done, presents the difference in interpretations of the international safety assessment guidance by regulators globally, the data that has been generated to meet regulatory requirements, and how the use of inappropriate or unvalidated methods could lead to the generation of results with limited or no additional added value to regulators and risk assessors.

Key words: genetically modified (GM) plants, allergenicity assessment, endogenous allergens, weight of evidence
Development of a tiered risk assessment approach for unintended allergen presence

Astrid Kruizinga

TNO, Zeist, Netherlands

Quantitative risk assessment methodologies were previously developed for scenarios concerning the unintentional consumption of major food allergens. These risk assessment methodologies are further developed into a 2-stage, user-friendly approach within the EU project iFAAM. The tiered approach consists of:

1) The Tier 1 risk assessment provide an initial risk screening with a degree of built-in conservatism. The risk assessment is based on a comparison of the potential exposure to the unintended allergen with the reference dose for that allergen, to provide a binary outcome. The goal of the development of the Tier 1 risk assessment is to develop a tool which can be readily applied by food companies of all sizes, without the need for expert knowledge.

2) The Tier 2 risk assessment is a more sophisticated model which takes into account a broader range of factors, their associated variability & uncertainties and results in a quantitative risk estimate.

This line of thinking may also be applied to the risk assessment of new or modified proteins which will be further elaborated within ImpARAS.
O19

Individual and population based food allergen thresholds: Update of current knowledge and possible ImpARAS applications

Ben Remington

TNO, Zeist, The Netherlands

In early 2011, the VITAL® (Voluntary Incidental Trace Allergen Labeling) Scientific Expert Panel of The Allergen Bureau of Australia & New Zealand (ABA) reviewed individual NOAELs and LOAELs obtained from over 1800 clinical food challenges in an effort to guide advisory labeling decisions for use on food labels. Reference Doses were established for 11 allergenic foods including peanut, cow’s milk, egg, hazelnut, soybean, wheat, cashew, shrimp, sesame seed, mustard and lupine (in terms of mg of total protein). Reference Doses were not established for fish, celery or other tree nuts due to a lack of sufficient quality data at the time. As part of the VITAL® update process, the threshold database was supplemented with additional data collected from 2011-2016. The results of that update are presented here.

Individual NOAELs and LOAELs from published clinical literature were reviewed and verified by scientists at the Food Allergy Research & Resource Program (FARRP) at the University of Nebraska and at TNO in the Netherlands. Selected publications focused on low-dose oral food challenges and additional unpublished clinical records from TNO and FARRP collaborators were also used to supplement the published data.

Over 1300 data points were added from data made available during 2011-2016. The TNO-FARRP allergen threshold database now contains over 3100 individual NOAELs and LOAELs from clinical food challenges for 32 allergens, including 15 priority allergens from different international labelling regulations. However, limited data restricted distribution calculations for a number of non-priority foods. In general, the newly calculated population threshold distributions did not significantly vary for a majority of allergens between the 2011 and 2016 datasets.

The results from this study indicate that the population thresholds generated for most VITAL® allergens are stable and were not significantly altered with the addition of new data. If a novel food is shown to have IgE-sensitizing capabilities, these results should contribute to the development of a safe clinical testing strategy for investigating the elicitation capabilities of the novel food in question.
Effect of data of EDTA enriched diets on farmed fish allergenicity; a proteomics approach

Cláudia Raposo\textsuperscript{1}, Denise Schrama\textsuperscript{1}, Annette Kuehn\textsuperscript{2}, Martine Morisset\textsuperscript{3}, Pedro Rodrigues\textsuperscript{1}

\textsuperscript{1} Centre of Marine Sciences, CCMAR, Universidade do Algarve, Campus de Gambelas, Edificio 7, 8005-139 Faro, Portugal
\textsuperscript{2} Luxembourg Institute of Health, Department of Infection and Immunity, 29, rue Henri Koch, L-4354 Esch-sur-Alzette, Luxembourg
\textsuperscript{3} National Unit of Immunology and Allergology, Centre Hospitalier de Luxembourg, Luxembourg.

Background
Fish is a common elicitor of food-allergic reactions. The panallergen in fish, parvalbumin (PV), is a stable, calcium-binding muscle protein. PVs are known to be highly IgE cross-reactive molecules. Conserved IgE epitopes are located in the calcium-binding domains where the IgE binding is reduced upon calcium depletion. This study aimed at analyzing the modulation of fish allergenicity in targeted aquaculture with specifically designed diets to induce the expression of the calcium-free, potentially less allergenic PV in fish muscle.

Methods
Gilthead seabream (\textit{Sparus aurata}) was reared in conventional aquaculture as well as using fish diets supplemented with different concentrations of EDTA, a calcium chelator. Fish were slaughtered after 98 days of trial and protein extracts were prepared from fish muscles. Proteins were separated by 2D-DIGE followed by spot identification using mass (MS) spectrometry (MALDI-TOF/TOF). Fish from the different conditions were compared by organoleptic testing and their allergenicity evaluated by skin testing with fish-allergic patients.

Results
Protein extracts from all fish showed a high number of spots corresponding to isoelectric points ranging from pH 3 to 7 and molecular weights ranging from 10 to 70 kDa in 2D-gels. 2D-gels of all samples were mostly superimposable, with some exceptions in distinct molecular masses and pH range that are currently being identified by MS analysis. Fish from the different conditions were found to show no differences in the organoleptic testing. Skin tests are currently being performed.

Conclusions
EDTA-diet is expected to induce the calcium-free, less allergenic PV allergen in fish muscle. Based on this pilot study, further analysis will be required to understand the effect of the new fish feed on the stability and IgE-binding capacity of seabream PV.
Investigation of allergen protein modifications induced by food processing

Eszter Schall, Lívia Hajas, Zsuzsanna Bugyi, Kitti Török, Sándor Tömösközi

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

Natural food components -mainly proteins- are able to cause hypersensitive reactions for susceptible individuals. During food processing different environmental effects have an influence on food components. The most typical processes are heat treatment, pH or ion strength change, high pressure or enzymatic treatments. These effects can cause protein modifications (denaturation, aggregation, intra- or intermolecular interactions between proteins) and induce interactions with other food components. During different processes, the immune dominant epitopes can be modified, which can potentially affect the parts of proteins inducing adverse physiological effects. But the physical (i.g. solubility) and chemical (i. g. immuno-reactivity) changes in the target proteins can affect the detection of the harmful proteins, too.

There are several analytical possibilities to monitor the presence of allergens or other protein components that can cause hypersensitivity reactions and to determine their quantity. Today the method of choice is the immune-analytical based ELISA method. This method can be highly specific and sensitive; however, there are certain issues that limit its reliability, e.g. the lack of reference methods and materials, the variability of results due to differences among assays and target proteins, and the problem of protein analysis in processed food matrices.

In last years our research group worked on the evaluation of food model matrices incurred with different allergen protein sources. Till now, four allergens -soy, milk, egg and gluten/gliadin- were used individually and together in the model matrices. The food models were applied for identification and investigation of main effects responsible for analytical errors in ELISA-based allergen methods. Our work include the investigation of protein modifications during food processing and also the interaction between proteins and proteins with other matrix components. The protein profile and subunit composition of model products were examined using SDS-PAGE, Lab-on-a-chip and SE-HPLC. We think that the methods we used for these experiments can be applied for the new allergen sources, also.
Metabolic fate of allergenic proteins in whole raw and roasted peanuts

Luigia di Stasio1,2, Mariantonietta Mongiello1, Linda Monaci3, Gianluca Picariello1, Roberto Berni Canani4, Rita Nocerino5, Pasquale Ferranti1,2, Gianfranco Mamone1.

1Institute of Food Science and Technology (ISA) CNR, Avellino, Italy
2Department of Agricultural Sciences, University of Naples ‘Federico II’, Portici, Italy
3Institute of Sciences of Food Production (ISPA) CNR. Bari, Italy
4Department of Pediatrics, University of Naples ‘Federico II’, Naples, Italy

Peanut allergy is one of the most common, persistent and severe food allergies, with an estimated prevalence in the general population of 0.8% in children and 0.6% in adults. Prevalence of peanut allergy is steadily increasing during the last few decades for reasons that are currently unclear. Among the nearly 47 potential allergens reported so far, Ara h1, Ara h2, Ara h3 and Ara h6 have been characterized in depth, as they exhibit the highest affinity to IgE-antibody from the majority of peanut allergic patients. Stability to proteolytic degradation in the digestive tract is considered a general feature shared by most of the food allergens. To this purpose, several studies have attempted to assess the stability of food proteins by mimicking the physiological steps of digestion. As far peanut is concerning, in vitro digestion trials have demonstrated that Ara h2 and Ara h6 are considerably more stable to gastric digestion than Ara h1 and Ara h3. Accordingly, Ara h2 and Ara h6 seem the allergens more correlated to the injuring effects in humans. The digestion stability has been assayed exclusively for purified peanut allergens so far, neglecting the relevant matrix effects that occur for real food systems. Food matrix is expected to dramatically affect the accessibility of proteases to allergens, thereby contributing to the determination of the bioaccessibility and hence the bioavailability of allergenic determinants (epitopes).

The aim of present research was to determine the stability of peanut allergens to gastrointestinal digestion in the raw and roasted real peanut, by using in vitro static digestion models followed by proteomic and immunological characterization of the digests.

Peanut was digested by using gastrointestinal enzymes as well as porcine brush border membrane enzymes (BBM) to simulate jejunal digestion. Digestion was monitored by SDS-PAGE, and allergens were specifically analyzed by Western-Blot using sera of peanuts allergic patience’s. Among the allergens, Ara h2, Ara h6 and fragment of Ara h3 (at 35kDa) were resistant to hydrolysis. LC-MS/MS (OrbitrapTM instrument) analysis showed that a large number of peptides survived to digestion, including several Ara h3 and Ara h1 encrypting IgE binding epitopes. RP-HPLC purification of digested peanut followed by DOT-BLOT analysis and mass spectrometry characterization, confirmed the IgE-binding assay of resistant peptides.

These results provide new insights into the persistence of peanuts allergen during digestion.
**P04**

**Production and characterization of quinoa (Chenopodium quinoa Willd.) protein isolate for production of hypoallergenic foods**

Gianfranco Mamone¹, Gianluca Picariello¹, Annachiara Bracciale², Alessandra Aiello², Luigia Di Stasio³, Maria Adalgisa Nicolai², Pasquale Ferranti¹,²

¹Institute of Food Science and Technology (ISA), Avellino, Italy  
²Department of Agricultural Sciences, University of Naples ‘Federico II’, Portici, Italy

Quinoa (Chenopodium quinoa Willd.) is a dicotyledonous annual plant cultivated in the Andes since 5000 BC, challenging highly different environmental conditions. Its grains are starchy and also rich in protein. Quinoa has remarkable nutritional properties and this supports its use as new ingredient for novel food products (baked foods, meat substitutes and snacks). While quinoa is still traditionally grown in South America, today it is also cultivated in the USA (Colorado and California), China, Europe, Canada, and India, and on limited scale in Europe (Finland and UK). Today, technological efforts are directed to improve product sensory properties by removing vegetal after-taste and bitterness due to the presence of saponins. A more relevant question is related to the possible still unexplored allergenic effects.

We have investigated chemical composition and nutritional properties of quinoa flour. Quinoa flour showed an excellent nutritional profile, especially a high protein (about 14%), lipid (about 7%) and ash (about 2%) content. A procedure has been developed to efficiently remove saponins and to obtain a high purity quinoa protein isolate, which was characterized by MS-based proteomic techniques. Electrophoretic analysis highlighted that quinoa protein isolate is composed two main fractions, the albumins with a molecular weight nearly 15kDa, and the globulins, constituted by an acidic (30-40kDa) and a basic (20-25kDa) subunit. Proteomic and R5 ELISA analyses showed absence of gluten, confirming quinoa as a naturally gluten-free crop. Furthermore, as the resistance to gastrointestinal digestion is a major prerequisite for allergenicity, we studied in vitro digestibility of quinoa proteins using a static in vitro model of protein gastrointestinal digestion. MS/MS analysis of the products of protein digestion showed a high degree of digestibility and survival of only few resistant peptides, none of which was recognized by Western blotting with sera of individuals allergic to cereals nor by in silico screening on allergenic sequence databases.

With these premises, we have also developed dough and bread exclusively based on quinoa flour, with valid nutritional, sensorial and texture properties. Bread technological characteristics were optimized with the use of food-grade enzymes, among which transglutaminase and proteolytic enzymes. Data collected indicated that quinoa proteins, as a flour or isolate, showed a high degree of digestibility under an in vitro digestion model, supporting their excellent nutritional value and the use of quinoa as ingredient in substitutive dough formulations.
New allergens from anise and caraway from Apiaceae family

M. Słowianek, I. Majak, J. Leszczyćńska

Allergies caused by the ingestion of herbs and spices are still relatively rare and have not been studied extensively. Thus, there are few literature data pertaining to this problem. Most of the detected allergens from the family Apiaceae are analogues of Bet v 1 and profilins. The identified anise allergens, homologues of Bet v 1 and profilins with a molecular mass of 12–17 kDa have been named Pim a 1 and Pim a 2. Similar allergenic proteins have been discovered in cumin (Cum c 1 and Cum c 2), fennel (Foe v 1 and Foe v 2), coriander (Cor s 1 and Cor s 2), parsley (Pet c 1 and Pet c 2). The caraway analogs of Bet v 1 and profilin were not found yet.

The aim of this work was to find new allergens of anise and caraway. The electrophoresis and immunoblotting was used. Extracts from anise and caraway were investigated by immunoblotting with the use of serum of patients sensitive to spices and antibodies against Bet v 1 and profilin. The selected proteins were analysed using LC-MS/MS conducted in the biochemical laboratory of the Biocentrum company.

The presence of Bet v 1 analogues and profilins in anise was confirmed and a new allergen, elongation factor α, was identified. New caraway allergens were found: a Bet v 1 analogue, profilin analog, and glyceraldehyde 3-phosphate dehydrogenase. According to the Allergome database, examples of allergenic plant proteins similar to elongation factor α include a latex allergen from Siphonia brasiliensis, Hev b 1. Elongation factor 1 β is also a fungal allergen found in Penicillium citrinum, Pen c 24. Allergenic proteins similar to glyceraldehyde 3-phosphate dehydrogenase mostly include airborne insect allergens and airborne allergens from fungi in the families Alternaria (Alt a 10), Beauveria (Bea b Ald), and Cladosporium (Cla h 10). Finally, the Alergen.org database lists Tri a 34, an airborne allergen from wheat (Triticum aestivum), as a plant allergen of this type.
Production, characterization and evaluation of the digestibility of hemp protein isolates (*Cannabis sativa* L.)

Gianluca Picariello¹, Gianfranco Mamone¹, Alessia Ramondo², Luigia di Stasio², Maria Adalgisa Nicolai², Pasquale Ferranti¹,²

¹Institute of Food Science and Technology (ISA), Avellino, Italy
²Department of Agricultural Sciences, University of Naples ‘Federico II’, Portici, Italy

Hemp (*Cannabis sativa* L.) is cultivated for industrial use and harvested for fibers, seeds, oil and meal. The plant tolerates a variety of growing conditions and is resistant to pests and disease. Recently hemp has attracted much interest as a sustainable cultivation for recovering marginal soil. Hemp is grown in many parts of the world: its major producers include Canada, France, and China. The environmental advantages and the nutritional benefits of industrial hemp seem to be worth lifting its restrictions in countries like the USA, mainly due to concerns for its phylogenetic proximity with the psychotropic *Cannabis indica*. Hemp has many industrial uses, from paper and textiles to plastic and fuel. Hemp seeds are also high in nutritional value, being rich in phytosterols, omega-3 and omega-6 essential fatty acids and proteins, which contain all the essential amino acids. For these reasons, hemp seeds have started to be used in a variety of food productions, including pasta and baked products. Importantly, hemp seed-based food products are considered less allergenic than those based on the most used edible seeds, although this statement has never been verified from the scientific point of view.

The objective of this study was to set up an efficient and scalable method for production of a hemp protein isolate (HPI) and for its proteomic characterization. The first step was production of hemp seed flour, which showed an excellent nutritional profile, also characterized by a high fiber amount (about 49,2%). A procedure was then developed to obtain the enriched HPI, whose chemical composition and nutritional characteristics were also evaluated. A high purity grade HPI was obtained by a fast and cheap process, to be used either for nutritional studies or for perspective use as food ingredient. In particular, in vitro digestibility of the HPI was determined using a static model of gastrointestinal digestion, which also included a final step with use of purified brush border membrane enzyme preparations, in order to evaluate the survival of bioactive and/or allergenic peptide sequences. The product of the HPI digestion were structurally characterized by LC-ESI-MS/MS using an OrbitrapTM instrument. The HPI showed a high degree of digestibility confirmed also by Western blotting. Only a limited number of peptide sequences were found to survive the whole process of digestion. Among them, fragments of protein Z4, a serpin, were identified. This finding deserve further investigation, as protein Z4 from other seed species is considered an allergen, while no information is available on hemp yet. On the contrary the main HPI protein component, edestin, belonging to the class of albumins, was shown to be completely digested. In conclusion, data collected support the use of hemp and HPI as ingredient for production of hypoallergenic foods.
P07

The relationship between food processing and infantile food allergies

Beste Ozsezen\textsuperscript{a} and Sibel Karakaya\textsuperscript{b}

\textsuperscript{a} Nafiz Korez Sincan State Hospital, Ankara Turkey
\textsuperscript{b} Ege Univ., Fac. Engn., Dept. Food Engn., Izmir, Turkey

Since the turn of this century it is well known that every food contains potential allergens, and can therefore trigger allergic reactions in sensitized children. In the first years of life cow milk proteins are essential due to their high PROTEIN quality. The major allergens in cow’s milk are caseins (Bos d 8, 20-30 kDa) and whey proteins β- lactoglobulin (Bos d 5, 18 kDa), α-lactalbumin (Bos d 4, 14 kDa), and serum albumin (Bos d 6, 67 kDa). β- lactoglobulin is a member of the lipocalin superfamily, to which a number of other inhalant allergens belong. It is highly resistant to proteolysis and is taken up in an intact form by the gut in experimental systems, a factor which is thought to contribute to its allergenicity. The IgE epitopes have been identified in β- lactoglobulin with four main IgE binding regions being located on the more mobile surface loops of β-lactoglobulin.

Infants who are allergic to cow’s milk, milk proteins should be substituted for nutritionally adequate complements in their diets. The choice of an adequate cow’s milk substitute among several hypoallergenic formulas is mandatory for infants with cow’s milk allergy.

Food processing has the potential to reduce the allergenicity of proteins by several ways including removal of the proteins, proteolysis of proteins, modification of allergens, removal of epitopes and masking of epitopes. Enzymatic hydrolysis is the most efficient process for disrupting sequential and conformational epitopes, and therefore allergenicity reduction is best achieved by this method. The proteolysis depends not only on the amino acid sequence of the protein but also on its secondary structure (e.g., presence of disulfide bonds) and posttranslational modifications.

Some examples of enzymatic hydrolysis discussed include the reduction of the allergenic potential of rice with actinase, proteases that reduce the allergenic potential of soybean, and the use of trypsin and chymotrypsin for producing hypoallergenic formulas. Ultrafiltration of hypoallergenic infant formulas based on the enzymatic treatment of milk with proteases permits the removal of the remaining traces of intact proteins, and suppresses the allergenicity of milk.

However, proteolytic treatments are not always able to destroy all epitopes due to incomplete hydrolysis (e.g., peanut or peach). Peptides may also re-associate to form aggregates that may increase the allergenic potential of foods or such treatments may also potentially unmask existing allergens.

In conclusion, hypoallergenic formulas, like all formulas intended for infant feeding, must demonstrate nutritional suitability to support infant growth and development. To be labeled as hypoallergenic, these formulas, after appropriate preclinical testing, must demonstrate in clinical studies that they do not provoke reactions in 90% of infants or children with confirmed cow’s milk allergy with 95% confidence when given in prospective randomized, double-blind, placebo-controlled trials.
The Impact of food processing and food matrix on the allergenic properties of foods

Birgül Hızlar and Sibel Karakaya

Ege Univ., Fac. Engn., Dept. Food Engn., Izmir, Turkey

Recently studies on allergenic reactions to foods and prevention of allergenic reactions have been intensified. The most important reason is that a certain proportion of the population is sufferer from adverse health consequences as a result of the consumption of particular foods or food ingredients. In addition to this reason, introducing new protein sources such as insects and algae to human consumption requires studies on adverse effects of these new protein sources.

Previously the most commonly allergenic foods were considered to be cows’, milk, hens’ eggs, peanuts, tree nuts, soy, wheat, shellfish and fish (the ‘big 8’). More recently in Europe, that list has been expanded in number to 14: cereals containing gluten, crustaceans, mollusks, eggs, fish, peanuts, tree nuts, soybeans, milk, celery, mustard, sesame, lupine and Sulphur dioxide.

Over the last few decades the prevalence of food allergy has increased presenting an important challenge to both clinical allergology and the food industry. The causes of the increase and spread of allergic reactions are still unclear and may be due to a combination of different factors like genetic, nutrition and environmental factors. Nowadays, it is assumed that around 6–8% of children and up to 3% of adults are affected by allergic reactions to food.

Several studies indicate that 75 % of allergic reactions among children are due to a limited number of foods, namely egg, peanut, cows’ milk, fish and various nuts. It is known to 1 in 13 children in the U.S. has a food allergy.

Hypersensitivity reactions have been classified into two groups including mediated by IgE antibodies or other immunological pathways (non IgE-mediated), and non-immunological responses (food intolerance). Adverse reaction to peanut is an example of IgE-mediated allergic reaction. Food intolerance encompass disorders such as lactose intolerance (due to lack or absence of lactase) can arise other disorders of digestive-absorptive processes, toxic and pharmacological reactions due to the release of histamine or tyramine after consumption of specific foods. The principle of the management of food allergy is based on allergen avoidance and immunological approach. Generally proteins in foods can cause allergenic reactions. Common proteins that are present in large quantities in a food will have a greater probability of becoming allergens than proteins that are present in small quantities. Storage proteins of many nuts and seeds are an example. Processing and preparing of food may increase or decrease its allergenicity. Also, the food matrix may influence the likelihood of inducing an allergic reaction, its severity, and/or the time of the reaction after food ingestion.

The allergenic activity of a food may decrease, remain unchanged, or even increase by food processing. Considering the multiplicity of the allergenic proteins contained in a whole food and that different proteins may be differently affected by the same treatment, the impact of food processing on the structural and allergenic properties of allergen foods/ingredients is difficult to predict. In addition, the extent to which allergenic proteins are modified during food processing depends on the type of process and its conditions, the structure of the proteins, and the composition of the matrix. Although the effects of different (technological and cooking) treatments on the IgE-binding capacity of several allergens have been investigated, less information is available on the effects of processing on clinical reactivity.
P09

Quantification of whey protein after in vitro digestion: from mouth to colon


Department of Food Science and Nutrition, Faculty of Veterinary Sciences, Regional Campus of International Excellence “Campus Mare Nostrum” (Murcia, Spain).
* email: tsm09382@um.es

Obesity has increased dramatically during past decades, reaching epidemic proportions in both developed and developing countries. Due to the obesogenic environment in most of the actual societies, it is necessary to develop new foods capable of increasing satiety. With this regard, protein is known as the most satiating macronutrient and specifically whey proteins due to its content in glycomacropeptide and amino acids such as tryptophan and lysine, and branched chain amino acids (leucine BCAA). The aim of the study was to evaluate the profile of protein after its degradation after in vitro gastrointestinal digestion and batch culture fermentation of whey protein from milk of several domestic mammalian species (Friesian cow, Segureña sheep, Murciano-Granadina goat and a mixture of the above (60:20:20)). Simulated gastrointestinal digestion was proposed as a general standardized static in vitro digestion method suitable for foods by COST Infogest network. Following it, batch culture fermentation was made with digested samples using fecal inoculums from a healthy donor. Protein quantification of raw, digested and fermented samples was carried out by means of the colorimetric method Bradford (Coomassie (Bradford) Protein Assay Kit), using 96 well plate.

Results: As it was expected, protein degradation occurred through whole simulated digestion, from mouth to colon. Peptides formation in intestinal phase was mainly due to hydrolysis by gastrointestinal digestion enzymes, meanwhile in colon the proteolytic system of lactic acid bacteria (LAB) was the most active. We found a different trend between analysed samples.

Conclusion: The present data shed light on protein degradation through the in vitro digestion and subsequent fermentation of several types of whey protein. The used methods could be a useful tool to determine the protein digestion and final degraded products.
P10

Effect of orally administrated hydrolysed OVA on mice mucosal immune system response

Dagmara Złotkowska, Barbara Wróblewska

Institute of Animal Reproduction and Food Research of PAS
Department of Food Immunology and Microbiology
ul. Tuwima 10, 10-748 Olsztyn, Poland
b.wroblewska@pan.olsztyn.pl

Background: Protein hydrolysis is used to be the most effective process to change protein immunoreactivity. Protein hydrolysates are used to produce hypo-allergenic formulas directed to kids with allergy. Pepsin hydrolysis is first step of protein degradation of human digest system. The aim of this study was to check how OVA hydrolysis, in stimulated gastric fluid conditions (SGF), affect immunoreactivity of OVA (very common food allergen) and mucosal immune system response to it.

Results: Balb/C mice were orally fed with OVA and SGF hydrolysed OVA (OVA-H). After 21 days specific serum IgG level was 213.7±0.4, 211.5±0.2, serum IGA 27.2±0.7, 25.5±0.2 and fecal IGA 23.6±0.4 and 23.4±0.4 respectively for OVA and OVA-H. Level of specific serum IgG2b was significantly lower in OVA-H mice (p≤0.001). B cells analysis confirmed that SGF hydrolysis significantly decreased number of antigen forming cells in intestinal lamina propria (iLP), spleen (SPL) and mesenteric lymph nodes (MLN) compare to OVA group.

Flow cytometry analysis showed that OVA-H immunization result in significant expression CD4+ T cells in ILN- 53.62% and MLN – 59.10% compare to OVA group, 25.36% and 43.30% respectively. Lymphocytes from SPL’s, MLN’s, Payer Patche’s (PP) and iliack lymph nodes (ILN) were cultured with antigen stimulation (OVA and OVA-H). Modified OVA significant stimulate CD4+TGFβ+ in SPL, PP and iLP. Antigen stimulation cultures confirm changes in cytokine production between groups IL-17, IL-10 and INF-γ.

Conclusion: SGF hydrolysed OVA stimulates mucosal immune system response differently as compared to native form. Expression of T cells populations CD4+, CD25+, CD4+IFNγ+, CD4+TGFβ+, CD4+IL-10+and regulatory CD4+CD25+ FoxP3+ were changed.
The effect of transglutaminase linking on mare’s milk in aspect of crosslinking with cow’s milk proteins

Joanna Fotschki, Anna Maria Szyc, Barbara Wróblewska

Department of Immunology and Food Microbiology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, 10-748 Olsztyn, Poland, j.fotschki@pan.olsztyn.pl

Background: Mare's milk, known for reduced allergenic properties, is considered as a substitute for cow’s milk, directed to people who suffer from cow’s milk allergy. However, our previous study showed that mare's milk proteins have the similar immune-reactive epitopes to cow's milk proteins. Therefore, the aim of the project is to explore the possibility of reducing the immunoreactivity of mare's milk proteins, namely beta-lactoglobulin and alpha-casein, by microbial transglutaminase linking.

Material: The immunoreactivity level of mare's and cow's milk with different amounts of enzyme (1U, 10U, 100U/ per 1g of protein in milk) was compared with use of antibodies directed to proteins of cow's milk: beta-lactoglobulin and alpha-casein (competitive ELISA). The immunoreactivity was calculated from the standard curves of the standard antigens. All the analyses were carried out in triplicate and the average values were converted to concentration equivalents in μg per μl. The antigenicity of proteins was estimated as the 50% inhibition of antigen binding towards a standard protein.

Results and Conclusion: The transglutaminase linking modulates the level of immunoreactivity of studied mare’s milk proteins depending on the quantity of the enzyme. However future research in this area should focus on mare’s milk processing resulting in the destruction of most allergenic epitopes with use of combine techniques e.g. cross-linking and lactic acid fermentation.

*The authors would like to thank the Genactiv Company for the mare’s milk. This study was supported by the National Science Center (Poland, No. UMO-2015/17/N/NZ9/03666) and the grant of KNOW Consortium “Healthy Animal - Safety Food” (MS&HE; Decision No. 05-1/KNOW2/2015)
Hydrolysates of proteins from rice and oat milks modulate physiological status of intestinal barrier

Dominika Świątecka, Lidia H. Markiewicz, Barbara Wróblewska

Department of Immunology and Food Microbiology, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, ul. Tuwima 10, 10-748 Olsztyn, Poland, b.wroblewska@pan.olsztyn.pl

Background: Many people use in daily diet milk substitutes such as rice or oat milks, which undergo hydrolysis in the GI tract. Protein hydrolysates may significantly modulate the condition and activity of intestinal barrier, influencing its integrity, permeability and function and is therefore relevant to the gut homeostasis, especially in terms of people suffering from food allergies. Therefore this study aimed at determining the impact of hydrolysates from proteins from rice milk on the physiological status of epithelial cells.

Methods: The proteins from the rice and oak milks were subjected to the enzymatic (pepsin-pancreatin) hydrolysis imitating the processes occurring in vivo. Such prepared hydrolysates were used to study their impact on the proliferation, metabolic activity and IL-8 secretion by enterocytes (represented by Caco-2 cells) by BrdU test, WST-1 test and ELISA method respectively. In addition, fecal bacterial isolates, obtained from people allergic to milk, were used to build an intestinal barrier constructed of enterocytes and microorganisms.

Results: Hydrolysates from oat milk lowered the proliferation rate and dehydrogenase activity of enterocytes, whereas hydrolysates from rice milk had no effect on those parameters. Interestingly, the response of enterocytes to analyzed hydrolysates was altered in the presence of adhered particular bacterial isolates belonging to genera Lactobacillus, Escherichia and Enterococcus. The observed effect was also changed when the intestinal barrier was built with heterogeneous population of these bacteria immobilized to enterocytes.

Conclusions: Hydrolysates from rice and oat milks significantly influenced the metabolism of enterocytes, hence are potential modulators of the physiological status of the intestinal barrier. Studies conducted within the project no: N N312 305940
**P13**

*Lactobacillus casei* LOCK0919: promising probiotic bacteria

Petra Hermanova¹, Martin Schwarzer¹, Dagmar Sruytova¹, Hana Kozakova¹, Bozena Cukrowska², Sabina Gorska³, Tomas Hudcovic³

¹Laboratory of Gnotobiology, Institute of Microbiology of the CAS, Czech Republic  
²Department of Pathology, The Children’s Memorial Health Institute, Warsaw, Poland  
³Ludwik Hirszfeld Institute of Immunology and Experimental Therapy of the Polish Academy of Sciences, Department of Medical Microbiology, Wroclaw, Poland

**Background:** *Lactobacilli* are currently the most frequently used bacteria in probiotic products. We recently demonstrated that mixture of *Lactobacillus (L.) rhamnosus* LOCK0900, *L. rhamnosus* LOCK0908 and *L. casei* LOCK0919 (L919) have synergistic effect in induction of anti-allergic and regulatory cytokines in human blood cell culture.

**Method:** Germ-free mice were colonized with mentioned lactobacilli strains. The bacterial colonization was evaluated at weekly intervals to Day 48. Vortexed feces were plated into MRS agar and cultivated. Bacteria were distinguished on the colonies morphology basis. To distinguish between *L. rhamnosus* strains specific qPCRs were used. We investigated that L919 is mice gut dominant colonizer. The signaling pathways were evaluated using HEK 293 cells transfected with TLR2, TLR4 and NOD2 and immunomodulatory properties (cultivation with bone marrow-derived dendritic cells – BM-DC) were assessed with formalized bacteria and isolated and purified polysaccharides from L919.

**Results:** Whole bacterium L919 was recognized by TLR2 and NOD2 receptors and stimulated production of IL-10, TGF-beta, IL-12p70 and TNF-alpha in culture of BM-DC. Two antigens L919/D and L919/E were isolated from molecular mass of L919 according to their sugar composition. The polysaccharides structural analysis was determined by classical chemical analysis, nuclear magnetic resonance spectroscopy (NMR) and mass spectrometry. We showed that polysaccharides L919/D and L919/E did not signal through the TLR2, TLR4 nor NOD2 receptors and did not induce immune response by themselves in culture of BM-DC.

**Conclusion:** We found that *L. casei* LOCK0919 is a dominant colonizer of mice gut. We observed that probably polysaccharides contribute to the host colonization which may promote some benefits of the host. L919 produce two different polysaccharides which have distinct immunomodulatory properties compared to the whole bacteria. Supported by CSF grant No: 15-07268S.
Component resolved diagnosis (CRD) in patients with food allergy

Oznur Abadoglu\textsuperscript{1} and Sedef Nehir El\textsuperscript{2}

\textsuperscript{1}Cumhuriyet University, Faculty of Medicine, Sivas Turkey, 
\textsuperscript{2}Ege University, Food Engineering Department, Izmir, Turkey

BACKGROUND: Making the correct diagnosis of a food allergy is necessary to provide appropriate and potentially life-saving preventive measures. Allergy diagnosis needs to be improved in poly-sensitized patients. Component resolved diagnosis (CRD) is a new concept in the investigation of poly-sensitized patients to discriminate between genuine allergy and merely sensitization.

AIM: The aim of this study was to evaluate the utilization of “Component Resolved Diagnosis (CRD)” as a tool in the diagnosis of food allergy.

METHOD: In this study the literature was reviewed on the advantages of CRD of food allergy in clinical practice. References were identified by searches of PubMed published until June 2016 was included.

RESULTS: IgE reactivity to casein (Bos d 8), the major protein in cow’s milk (CM) may be markers for persistent milk allergy. In hen’s egg (HE) allergic patients, it was showed that sIgE to Gal d 1 was superior in the diagnosis of allergy to heated egg. It was reported that the Gly m 8 sIgE had the greatest accuracy in the diagnosis of soy allergy among the available soy components. In peanut allergic patients, Ara h 2 has been shown to be the most important predictor of clinical reactivity, though Ara h 1, 3, and 6 have also been associated with severe reactions. It was demonstrated an important role for Cor a 9 and Cor a 14 in predicting clinical reactivity to hazelnut. Some food allergens are responsible for the patient’s symptoms presenting as an oral allergy syndrome. CRD can distinguish between fruit allergy due to LTP sensitization and a pollen-related apple allergy. Patients with IgE antibodies to Mal d 2 and 3 (LTP stable proteins) are at higher risk of developing systemic reactions.

CONCLUSION: While CRD can be helpful in the evaluation of possible food allergy to peanut or hazelnut, further investigations will be needed to evaluate the benefit of CRD in the evaluation of HE, cow’s milk and soy allergy.
Greetings from the plant side – transcriptomic characteristics of pollen allergens in the region of Ukraine.

Jana Žiarovská¹ – Natalia Nikolaieva² – Tatiana Shevtsova² – Katerina Garkava² – Katarína Ražná¹ - Ján Brindza¹

¹Slovak University of Agriculture in Nitra; jana.ziarovska@uniag.sk
²Institute of Ecological Safety, National Aviation University, Kyiv

Background

Many immune-reactive and biochemical properties of plant allergens are actually well known, but the transcriptomic characteristics of the genes that encode for them in plants are still very limited. The application of real-time PCR in the analysis of the expression level of allergens in the pollen from different *in situ* conditions are only in the experimental phase and no normalized methods actually exist. Real-time PCR is efficient in pollen monitoring but the questions of the expression of allergens directly in the plants are to be solved.

Method

Expression level of Bet v1 homologous was analyzed by using of the qRT-PCR. Silver birch and hazelnut pollen was sampled from different environmental conditions: forest, city park, urbanized area of housing estate, city and industry area. Primers for qRT-PCR were designed on the basis of Betv1 homologous and the following references genes were used for the normalization of the expression level quantitation: alpha-tubulin, cyclophylin, transcription factor CBF1 and 18S rRNA. Allergen qRT-PCR results were compared to the expression of the same allergen gene in pollen sample from forest that was chosen as the calibrator.

Results

The expression levels were analysed for Bet v 1 allergen type genes in the in situ samples of birch and hazelnut pollen from different localities of Ukraine. The expression of BetV1 allergen for both of the analysed plant species was higher when comparing to the forest sample in samples from urbanized area (table). qRT-PCR showed only a little variation in the abundance of allergen transcripts among the samples from different places of growth for hazelnut, but a crucial differences for the samples of silver birch. specie/ increased expression level

<table>
<thead>
<tr>
<th>Specie</th>
<th>City Park</th>
<th>Housing Estate</th>
<th>City</th>
<th>Industry Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>silver birch</td>
<td>0,61</td>
<td>0,58</td>
<td>1,3</td>
<td>4,1</td>
</tr>
<tr>
<td>hazelnut</td>
<td>0,73</td>
<td>1,1</td>
<td>0,53</td>
<td>0,62</td>
</tr>
</tbody>
</table>
P16

Serum diamine oxidase activity as a diagnostic test for pseudoallergy in pediatric population- the pilot study

Joanna Kacik¹, Agata Będzichowska¹, Bolesław Kalicki¹, Barbara Wróblewska²

¹. Department of Pediatrics, Pediatric Nephrology and Allergology, Military Institute of Medicine, Warsaw, Poland
². Department of Immunology and Food Microbiology, Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland.
Email address: jkacik@wim.mil.pl

Background:
Histamine intolerance, also known as pseudo-allergy, is non-immunological mediated type of food hypersensitivity responsible for wide range of symptoms, mimicking an allergy reaction. It is triggered by imbalance between histamine accumulation due to ingestion histamine-rich foods along with drugs and other factors releasing histamine and the capacity for histamine degradation. The main enzyme involved in the degradation of ingested histamine is diamine oxidase (DAO).

Method:
We evaluated 36 patients with clinical symptoms of food intolerance for our pilot study. By means of laboratory tests and skin prick tests, 29 patients, of an average age of 8 years, were qualified to the further investigation. We divided them into two groups: (1) with negative laboratory tests for allergy- 7 patients (24%) and (0) with positive skin prick tests and/or with positive laboratory tests for allergy- 22 patients (76%). In both of the groups we determined total IgE concentration and DAO serum activity.

Result:
There was a significant reduction (p=0.0007) of the DAO serum activity in the group (0) in comparison to group (1). On the other hand, a significantly higher (p= 0.00012) total IgE concentration was observed in the patients of group (1) rather than in group (0).

Conclusion:
Our results confirm the evidence of a significant reduction in serum diamine oxidase activity in patients presenting symptoms suspected to be related to pseudo-allergy type of food hypersensitivity, unlike the group with test results correlating with atopy. Our findings reveal that determining the serum diamine oxidase activity should be considered a diagnostic test in pseudo-allergy and further studies on the topic are necessary.
**P17**

Is mealworm allergy indicative for allergy to other insects?

*Henrike CHP Broekman 1,4, Kitty CM Verhoeckx 1,2,4, Marco Gaspari 3, Constance F den Hartog Jager 1,4, Govardus de Jong 2,4, Geert F Houben 1,2,4, André C Knulst 1,4*

**Scope:** The growing world population motivates the exploration of new and more sustainable protein sources to ensure food security. Mealworm and other insects are promising candidates, with active ongoing marketing efforts within America and Europe. In previous studies we found that patients allergic to shrimp are at risk for mealworm allergy, and mealworm can also induce a primary allergy.

**Objective:** investigate the allergic potential of other edible insect candidates, suggested for human consumption by WHO/FAO and EFSA, in both the mealworm and shrimp allergic population. The following insects were investigated: *Tenebrio molitor*; mealworm (larvae), *Acheta domesticus*; house cricket (bug), *Zophobas morio*; superworm (larvae), *Alphitobius diaperinus*; lesser mealworm (larvae), *Locusta migratoria*; African grasshopper (bug), *Galleria mellonella*; large waxmoth (larvae) and *Hermetia illucens*; black soldier fly (larvae).

**Methods and results:** Fifteen shrimp allergic patients and four primary mealworm allergic subjects from our previous studies were included. In 13/15 of the shrimp allergic patients and in 2/4 of the mealworm allergic patients food allergy to mealworm was confirmed by double blind placebo controlled challenge. All shrimp allergic patients were sensitized to multiple insects, on immunoblot. The basophil activation test (BAT) showed reaction with similar responses within each individual patient to the different species. Primary mealworm allergic patients, reacted with serum binding to some insects on immunoblot, in contrary to the shrimp allergic patients. Positive basophil activation to all insects was seen.

**Conclusion:** All shrimp allergic patients showed sensitization, to all tested edible insects, with protein recognition similar to their mealworm sensitization. Food allergy to other insects due to cross-reactivity seems very likely, but a challenge should be performed to confirm this clinically.

The primary mealworm allergic subjects showed sensitization to known and unknown arthropod allergens, different from the shrimp allergic patients, but only show clinical reaction to mealworm.
P18

The nature of wheat gliadins modifies the immune response in a mice model of food allergy

Grégory Bouchaud¹, Laure Castan¹,²,³,⁴, Mathilde Claude¹,⁴, Philippe Aubert³,⁵,⁷, Michel Neunlist³,⁵,⁷, Antoine Magnan²,³,⁴,⁶,⁷ and Marie Bodinier¹.

¹INRA, UR1268 BIA, rue de la Géraudière, BP 71627, F-44316 Nantes, France
²INSERM, UMR1087, l’institut du thorax, Nantes, F-44000 France;
³CNRS, UMR6291, Nantes, F-44000 France;
⁴Université de Nantes, Nantes, F-44000 France
⁵INSERM UMR913, Institut des Maladies de l’Appareil Digestif (IMAD), Faculté de Médecine, Nantes, F-44000 France.
⁶CHU de Nantes, l’institut du thorax, Service de Pneumologie, Nantes, F-44000 France;
⁷DHU2020 médecine personnalisée des maladies chroniques, Nantes, F-44000 France;

Aim: Food allergies result from a complex immune response involving both innate and adaptive immune cells. Major proteins of wheat flour, gliadins, appear as important allergens and have a special role in wheat-dependent exercise-induced anaphylaxis. Allergen characteristics can influence the allergic response. In this context, chemically modified gliadins by industrial processes impact immune mechanisms orchestrating allergic reaction in an undefined manner. Our study investigates immune reaction development during food allergy with gliadins under native, deamidated or hydrolyzed forms.

Methods: Mice were sensitized with native (NG), deamidated (DG) or hydrolyzed gliadin (HG). Subsequently, mice were challenged by oral gavage with the corresponding allergens. Then, organs were collected at different time points and analyzed for immune and physiological parameters such as gastro-intestinal functions, cytokine secretion or allergenspecific IgE.

Results: Preliminary data clearly show an increase of specific IgE and IgG1 level in serum after challenge when mice were sensitized with DG compared with NG and a level comparable to unsensitized mice with HG. This was accompanied with an increase of intestinal permeability and histological score reflecting intestinal integrity. Moreover, a more pronounced Th2-polarization together with a decrease in regulatory immunosuppressive response was observed in lymph nodes from mice sensitized with DG compared with NG sensitized mice.

Conclusion: Altogether, our data tend to demonstrate that industrial processes such as deamidation or hydrolysis impact food allergenicity through immune modulation and help us to develop tools to define how they can influence this reaction and encourage or decrease allergic reactions.
Molecular characterization of recombinant Mus a 5 allergen from banana fruit

Andrijana Nesic¹, Uros Andjelkovic², Djuro Josic², Marija Gavrovic-Jankulovic¹

¹Faculty of Chemistry, University of Belgrade, Serbia
²Faculty of Biotechnology, University of Rijeka, Croatia

BACKGROUND
Banana (Musa acuminata) is an important fruit in human nutrition. In spite of positive influence on human health, banana has been recognized as a food allergen source. Among five allergens that have been identified, beta-1,3-glucanase, denoted as Mus a 5, was identified as a candidate allergen for the component-resolved allergy diagnosis of banana allergy. The plant-derived food extracts employed for allergy diagnosis represent mixtures of allergens and non-allergenic material. Replacement of allergen extracts with a panel of IgE reactive recombinant molecules from particular allergen source is a promising strategy for the improvement of allergy diagnosis.

METHODS
Gene for Mus a 5 was cloned in pET-23b vector and protein was expressed in BL21 (DE3) E. coli cells. Recombinant protein was purified by a combination of chromatographic steps under native conditions (Patent P-2015/0783). The primary structure was analyzed by mass fingerprint. The secondary structures of the purified recombinant Mus a 5 were assessed by CD spectroscopy. IgE reactivity of rMus a 5 was evaluated in ELISA and dot blot.

RESULTS
Recombinant Mus a 5 cDNA encodes a protein with molecular mass of 33 kDa and pI of about 6.0. The yield of purified rMus a 5 was about 15 mg per liter of the cell culture. By mass analysis of in-gel tryptic digest, the cDNA sequence of rMus a 5 was confirmed on the protein level. CD spectroscopy revealed that spectrum of recombinant and natural Mus a 5 are comparable. IgE reactivity of rMus a 5 was tested in ELISA and dot blot with six patient sera indicating that natural and recombinant protein have comparable IgE reactivity.

CONCLUSION
Recombinant Mus a 5 is a homogenous protein species and its expression yield in the prokaryotic expression system was about 15 mg per liter of the cell culture. Recombinant Mus a 5 showed IgE reactivity comparable to nMus a 5 and it is a novel reagent suitable for the component-resolved allergy diagnosis of banana allergy.
Assessment of the allergenicity of egg protein hydrolysates

Daniel Lozano Ojalvo

Background
Egg allergy is of major concern, notably in infancy, and the production of egg white hydrolysates as a potential source of hypoallergenic protein and/or specific immunotherapy, has been previously reported [1]. The residual in vitro allergenicity of hydrolysates needs to be assessed to ensure they will not induce anaphylactic reactions in allergic patients. In the reverse enzyme allegro-sorbent test (EAST) serum IgE antibodies are first captured by immobilized anti-human IgE, and then, the binding of specific IgE to labelled allergens is measured [2]. The major goal of this Short Term Scientific Mission (STSM) was to assess the allergenicity of egg white protein hydrolysates obtained by the group BIOPEP (home group, Instituto de Investigación en Ciencias de la Alimentación) by learning and implementing the reverse EAST inhibition techniques developed by the UIIA INRA-CEA (host group, Laboratoire d’Immuno-Allergie Alimentaire CEA-INRA).

Methods
Ovalbumin (OVA), lysozyme (LYS), ovomucoid (OM) and whole egg white (EW) tracers were prepared using a biotinylation procedure. The IgE-binding capacity of the different proteins and hydrolysates (with both, pepsin and alcalase), was assessed by evaluating their capacity to inhibit IgE binding to labelled proteins in a reverse EAST inhibition test, as previously described [2].

Results
The results showed that the native proteins, EW, OVA, LYS and OM, displayed a high capacity to inhibit the binding of labelled proteins to IgE Abs of sera from egg allergic patients. In contrast, the IgE reactivity was substantially decreased in the hydrolysates produced with pepsin and drastically reduced in the hydrolysates generated with alcalase.

Conclusions
In conclusion, the STSM spent in the UIIA INRA-CEA have allowed the optimization of a reverse EAST inhibition protocol to assess the IgE-binding of egg protein hydrolysates obtained by the group BIOPEP. The EAST assay showed that the IgE binding, tested in both a pool of sera and individually for each serum, was drastically reduced in the hydrolysates produced with alcalase.

Peach allergy is the most prevalent plant food allergy in the Mediterranean area, and its major allergen, the non-specific lipid transfer protein is Pru p 3. However, increasing evidence suggests that it is not the Pru p 3 protein itself, which is allergenic, but perhaps the lipid transported by Pru p 3. Many major allergens have been described as lipid carriers, such as Pru p 3, Bet v 1 and Ara h 2 and this feature could be linked to their allergenic activity. Thus, the allergic sensitization could be based on the recognition of lipid ligands by receptors on cells of immune system. Preliminary data suggests that the lipid antigen contained within Pru p 3 may be transported and presented to the immune system by CD1d.

The objective of this work is to study the role of Pru p 3 lipid in the activation of the innate immune system by CD1 receptors.
Degranulation ability of native and aggregated ovalbumin after in vitro simulated digestion

Claude M1, Picariello G2, Lupi R1, Rogniaux H1, Bodinier M1, Larré C1, Brossard C1, Denery-Papini S1.

1INRA, UR 1268 BIA (Biopolymers, Interactions, Assemblies), F-44316 Nantes, France
2Istituto di Scienze dell’Alimentazione (ISA) – CNR, Via Roma 64, I-83100 Avellino, Italy

Background
Aggregation is an irreversible modification of proteins involving the formation of a network of intermolecular bonds. The morphology of the aggregates may vary depending on physico-chemical conditions of protein association. In a previous study, we demonstrated that thermal aggregation of ovalbumin as large particles reduces its allergenicity in egg allergic children and in sensitized mice (Claude et al., 2016). The aim of the present work was to investigate whether the structure of ovalbumin aggregates can modulate their antigenicity. We compared basophil activation capacity of the native proteins to that of two types of ovalbumin aggregates, i.e. small and large aggregates obtained under opposite electrostatic repulsions, taking into account the impact of simulated gastrointestinal digestion.

Methods
An ovalbumin solution was extensively heated (80°C for 6h) under repulsive electrostatic conditions (pH9-0.03M) to form small linear and under minimal electrostatic repulsions (pH5-0.8M) to form large spherical-agglomerated aggregates. Native ovalbumin and aggregates were subjected to a static in vitro gastrointestinal digestion model, which included degradation with peptidases of the jejunal brush border membranes. The extent of digestion was monitored with HPLC and SDS-PAGE analysis. Using sera from egg allergic children, we measured the capacities of the different forms of ovalbumin submitted or not to digestion to induce degranulation of humanized RBL cells.

Results
Before digestion, aggregates displayed lower degranulation ability compared to the native form and no difference between large and small aggregates was observed. In line with Nyemb (2014), ovalbumin aggregates are more susceptible to gastric and duodenal enzymes than the native protein. However, the degranulation ability of native ovalbumin was more affected by digestion compared to the aggregates. The digestion step with brush border membrane peptidases did not induce any appreciable change in degranulation ability, suggesting that antigenic sequences could be already hydrolyzed upstream, at the level of the duodenal phase of digestion.

Conclusion
The native ovalbumin contains specific epitopes which are sensible to the digestion. Even if the aggregates are more susceptible to digestion, the basophil degranulation ability is less affected by digestion compared to native form. In the case of ovalbumin and aggregates, no direct link between digestibility and biological activity was evident.

References
Investigation on protein profiles of raw and roasted peanuts submitted to two different *in vitro* digestion models.

**Simona L. Bavaro** a*, Giuseppe Meca b, Jordi Manes b, Gianfranco Mamone c, Linda Monaci a.

aInstitute of Sciences of Food Production, National Research Council of Italy (ISPA-CNR), Via Amendola 122/O, Bari (IT)
bLaboratory of Food Chemistry and Toxicology, Faculty of Pharmacy, University of Valencia, Av. Vicent Andrés Estellés s/n, 46100 Burjassot, Spain.
cInstitute of Food Sciences (ISA–CNR), Via Roma 52 A/C, 83100 Avellino (IT)

*a*simona.bavaro@ispa.cnr.it

Peanut allergy is one of the most severe food allergies for its life-threatening nature and persistency. The prevalence seems to have increased in the North America and several European countries during the past decades. Differences in the types of processing applied to peanuts have shown to influence the degree of sensitization of allergic consumers to this legume (1). Modifications occurring during processing could alter food allergenicity, i.e. increasing or decreasing IgE reactivity (2, 3). In addition, food processing can influence the behaviour of proteins upon digestion, also affecting peptides release enhancing or quenching the allergenic potential. Due to the high complexity of the human gastrointestinal tract the employment of physiologically relevant in vitro digestion models for the evaluation of allergens digestibility, represents a critical aspect that should be taken into consideration to produce more realistic results. In addition more insights are needed to assess stability of raw and roasted peanut allergens throughout digestion and the allergenic potential of allergenic peptides present in the final gastro-duodenal digest. In this work, we have compared digestibility of raw and roasted peanuts by testing two different *in vitro* digestion models, static and dynamic. The generated protein patterns were first analysed by SDS-PAGE and the peptide mixture was further submitted to HPLC-MS/MS analyses.

References

Effects of Maillard reaction on immunogenicity of β-lactoglobulin

Marija B. Perusko1, Manon van Roest2, Dragana J. Stanic-Vucinic3, Raymond H. Pieters2, Tanja Cirkovic Velickovic3 and Joost J. Smit2

1University of Belgrade - Faculty of Chemistry, Innovation Center, 2Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands 3University of Belgrade - Faculty of Chemistry, Centre of Excellence in Molecular Food Sciences

BACKGROUND Maillard reaction occurs during food processing and results in formation of Advanced Glycation Endproducts (AGE) of proteins. Here, we aimed to investigate whether AGE of major milk allergen, β-lactoglobulin (BLG), influence transfer across epithelial barrier, interactions with dendritic cells (DCs), and the T-cell immunogenicity.

METHODS The degree of transcytosis of both native or glycated BLG was measured in a Caco-2 transwell system. Proteins were labeled with FITC for uptake experiments or with beads for degradation experiments. DCs were cultured from mouse bone marrow in the presence of GM-CSF. These DCs were exposed to 10µg/mL FITC-labeled protein or 1x108 protein coupled beads. CD4+ T cells were obtained from BLG-alum immunized mice and cocultured for 72h with BLG samples pulsed DCs. Cytokines production was measured using commercial ELISA.

RESULTS Glycation of BLG did not influence protein secondary structure but reduced its transport through Caco-2 monolayer. Uptake of glycated BLG by DCs was significantly increased compared to native BLG. Glycated BLG undergoes faster degradation inside endolysosomal compartments of DCs. Compared to native BLG, glycated BLG diminished the cytokine production (IL-5, IL-13, INFγ) by BLG-specific CD4+ T cells cocultured with DCs.

CONCLUSIONS Our data indicate that Maillard reaction plays a significant role in immunogenicity of BLG, influencing its gastrointestinal bioavailability, interactions with crucial cells of immune system, DCs, and its ability to stimulate CD4+ T cells.
## Participants

<table>
<thead>
<tr>
<th>First name</th>
<th>Name</th>
<th>Country</th>
<th>e-mail</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antunes</td>
<td>Celia</td>
<td>Portugal</td>
<td><a href="mailto:cmma@uevora.pt">cmma@uevora.pt</a></td>
</tr>
<tr>
<td>Bavaro</td>
<td>Simona Lucia</td>
<td>Italy</td>
<td><a href="mailto:simona.bavaro@ispa.cnr.it">simona.bavaro@ispa.cnr.it</a></td>
</tr>
<tr>
<td>Bouchaud</td>
<td>Gregory</td>
<td>France</td>
<td><a href="mailto:gregory.bouchaud@nantes.inra.fr">gregory.bouchaud@nantes.inra.fr</a></td>
</tr>
<tr>
<td>Broekman</td>
<td>Henrike</td>
<td>The Netherlands</td>
<td><a href="mailto:H.C.Broekman-3@umcutrecht.nl">H.C.Broekman-3@umcutrecht.nl</a></td>
</tr>
<tr>
<td>Claude</td>
<td>Mathilde</td>
<td>France</td>
<td><a href="mailto:mathilde.claude@nantes.inra.fr">mathilde.claude@nantes.inra.fr</a></td>
</tr>
<tr>
<td>Constable</td>
<td>Anne</td>
<td>Switzerland</td>
<td><a href="mailto:anne.constable@rdfs.nestle.com">anne.constable@rdfs.nestle.com</a></td>
</tr>
<tr>
<td>Costa</td>
<td>Joana</td>
<td>Portugal</td>
<td><a href="mailto:joanabcosta@gmail.com">joanabcosta@gmail.com</a></td>
</tr>
<tr>
<td>Crevel</td>
<td>René</td>
<td>United Kingdom</td>
<td><a href="mailto:Rene.Crevel@unilever.com">Rene.Crevel@unilever.com</a></td>
</tr>
<tr>
<td>Cubells Arandia</td>
<td>Nuría</td>
<td>Spain</td>
<td><a href="mailto:nuria.cubells@upm.es">nuria.cubells@upm.es</a></td>
</tr>
<tr>
<td>Cukrowska</td>
<td>Bożena</td>
<td>Poland</td>
<td><a href="mailto:B.Cukrowska@IPCZD.PL">B.Cukrowska@IPCZD.PL</a></td>
</tr>
<tr>
<td>Denery</td>
<td>Sandra</td>
<td>France</td>
<td><a href="mailto:sandra.denery@nantes.inra.fr">sandra.denery@nantes.inra.fr</a></td>
</tr>
<tr>
<td>Diaz Perales</td>
<td>Araceli</td>
<td>Spain</td>
<td><a href="mailto:araceli.diaz@upm.es">araceli.diaz@upm.es</a></td>
</tr>
<tr>
<td>el Nehir</td>
<td>Sedef</td>
<td>Turkey</td>
<td><a href="mailto:sedef.el@ege.edu.tr">sedef.el@ege.edu.tr</a></td>
</tr>
<tr>
<td>Epstein</td>
<td>Michelle</td>
<td>Austria</td>
<td><a href="mailto:michelle.epstein@meduniwien.ac.at">michelle.epstein@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>Fernandez Canton</td>
<td>Rocio</td>
<td>Belgium</td>
<td><a href="mailto:rocio.fernandez.canton@monsanto.com">rocio.fernandez.canton@monsanto.com</a></td>
</tr>
<tr>
<td>Ferranti</td>
<td>Pasquale</td>
<td>Italy</td>
<td><a href="mailto:ferranti@unina.it">ferranti@unina.it</a></td>
</tr>
<tr>
<td>Gelencsér</td>
<td>Éva Mária</td>
<td>Hungary</td>
<td><a href="mailto:e.gelencser@cfri.hu">e.gelencser@cfri.hu</a></td>
</tr>
<tr>
<td>Giavi</td>
<td>Stavroula</td>
<td>Greece</td>
<td><a href="mailto:stagia14@otenet.gr">stagia14@otenet.gr</a></td>
</tr>
<tr>
<td>Glogowski</td>
<td>Robert</td>
<td>Poland</td>
<td><a href="mailto:robert_glogowski@sggw.pl">robert_glogowski@sggw.pl</a></td>
</tr>
<tr>
<td>Gologan</td>
<td>Elena</td>
<td>Romania</td>
<td><a href="mailto:elena.gologan@umfiasiro.ro">elena.gologan@umfiasiro.ro</a></td>
</tr>
<tr>
<td>Graversen</td>
<td>Katrine</td>
<td>Denmark</td>
<td><a href="mailto:katgr@food.dtu.dk">katgr@food.dtu.dk</a></td>
</tr>
<tr>
<td>Hazebruck</td>
<td>Stephane</td>
<td>France</td>
<td><a href="mailto:stephane.hazebruck@cea.fr">stephane.hazebruck@cea.fr</a></td>
</tr>
<tr>
<td>Heijink</td>
<td>Irene H.</td>
<td>The Netherlands</td>
<td><a href="mailto:H.I.Heijink@umcg.nl">H.I.Heijink@umcg.nl</a></td>
</tr>
<tr>
<td>Hermanova</td>
<td>Petra</td>
<td>Czech Republic</td>
<td><a href="mailto:hermanova.peta@seznam.cz">hermanova.peta@seznam.cz</a></td>
</tr>
<tr>
<td>Herouet-Guicheney</td>
<td>Corinne</td>
<td>France</td>
<td><a href="mailto:corinne.herouet-guicheney@bayer.com">corinne.herouet-guicheney@bayer.com</a></td>
</tr>
<tr>
<td>Holzhauser</td>
<td>Thomas</td>
<td>Germany</td>
<td><a href="mailto:Thomas.Holzhauser@pei.de">Thomas.Holzhauser@pei.de</a></td>
</tr>
<tr>
<td>Janković</td>
<td>Vesna</td>
<td>Serbia</td>
<td><a href="mailto:vessna@inmesbgd.com">vessna@inmesbgd.com</a></td>
</tr>
<tr>
<td>Karakaya</td>
<td>Sibel</td>
<td>Turkey</td>
<td><a href="mailto:sibel.karakaya@ege.edu.tr">sibel.karakaya@ege.edu.tr</a></td>
</tr>
<tr>
<td>Kruizinga</td>
<td>Astrid</td>
<td>The Netherlands</td>
<td><a href="mailto:astrid.kruizinga@tno.nl">astrid.kruizinga@tno.nl</a></td>
</tr>
<tr>
<td>Larre</td>
<td>Colette</td>
<td>France</td>
<td><a href="mailto:colette.larre@nantes.inra.fr">colette.larre@nantes.inra.fr</a></td>
</tr>
<tr>
<td>Lasić</td>
<td>Dario</td>
<td>Croatia</td>
<td><a href="mailto:dario.lasic@stampar.hr">dario.lasic@stampar.hr</a></td>
</tr>
<tr>
<td>Lesmes</td>
<td>Uri</td>
<td>Israel</td>
<td><a href="mailto:lesmesu@tx.technion.ac.il">lesmesu@tx.technion.ac.il</a></td>
</tr>
<tr>
<td>Leszcynska</td>
<td>Joanna Anna</td>
<td>Poland</td>
<td><a href="mailto:joanna.leszcynska@p.lodz.pl">joanna.leszcynska@p.lodz.pl</a></td>
</tr>
<tr>
<td>Lindholm Bogh</td>
<td>Katrine</td>
<td>Denmark</td>
<td><a href="mailto:kalb@food.dtu.dk">kalb@food.dtu.dk</a></td>
</tr>
<tr>
<td>Lozano-Ojalvo</td>
<td>Daniel</td>
<td>Spain</td>
<td><a href="mailto:daniel.lozano@csic.es">daniel.lozano@csic.es</a></td>
</tr>
<tr>
<td>Madsen</td>
<td>Charlotte B.</td>
<td>Denmark</td>
<td><a href="mailto:charm@food.dtu.dk">charm@food.dtu.dk</a></td>
</tr>
<tr>
<td>Mafra</td>
<td>Isabel</td>
<td>Portugal</td>
<td><a href="mailto:isabel.mafra@ff.up.pt">isabel.mafra@ff.up.pt</a></td>
</tr>
<tr>
<td>Majak</td>
<td>Iwona Alicja</td>
<td>Poland</td>
<td><a href="mailto:iwona.majak@p.lodz.pl">iwona.majak@p.lodz.pl</a></td>
</tr>
<tr>
<td>Mazzucchelli</td>
<td>Gabriel</td>
<td>Belgium</td>
<td><a href="mailto:gabiela.mazzucchelli@ulg.ac.be">gabiela.mazzucchelli@ulg.ac.be</a></td>
</tr>
<tr>
<td>McClain</td>
<td>Scott</td>
<td>United States</td>
<td><a href="mailto:scott.mcclain@syngenta.com">scott.mcclain@syngenta.com</a></td>
</tr>
<tr>
<td>Mills</td>
<td>Clare</td>
<td>United Kingdom</td>
<td><a href="mailto:clare.mills@manchester.ac.uk">clare.mills@manchester.ac.uk</a></td>
</tr>
<tr>
<td>Molina</td>
<td>Elena</td>
<td>Spain</td>
<td><a href="mailto:e.molina@csic.es">e.molina@csic.es</a></td>
</tr>
<tr>
<td>Monaci</td>
<td>Linda</td>
<td>Italy</td>
<td><a href="mailto:linda.monaci@ispa.cnr.it">linda.monaci@ispa.cnr.it</a></td>
</tr>
<tr>
<td>First name</td>
<td>Name</td>
<td>Country</td>
<td>e-mail</td>
</tr>
<tr>
<td>----------------------------</td>
<td>-------------------</td>
<td>---------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Nesic</td>
<td>Andrijana</td>
<td>Serbia</td>
<td><a href="mailto:ananesic22@gmail.com">ananesic22@gmail.com</a></td>
</tr>
<tr>
<td>Nygaard</td>
<td>Unni C</td>
<td>Norway</td>
<td><a href="mailto:unni.cecilie.nygaard@fhi.no">unni.cecilie.nygaard@fhi.no</a></td>
</tr>
<tr>
<td>Perusko</td>
<td>Marija</td>
<td>Serbia</td>
<td><a href="mailto:mperusko@chem.bg.ac.rs">mperusko@chem.bg.ac.rs</a></td>
</tr>
<tr>
<td>Picariello</td>
<td>Gianluca</td>
<td>Italy</td>
<td><a href="mailto:picariello@isa.cnr.it">picariello@isa.cnr.it</a></td>
</tr>
<tr>
<td>Raposo</td>
<td>Cláudia</td>
<td>Portugal</td>
<td>cl <a href="mailto:sof.raposo@gmail.com">sof.raposo@gmail.com</a></td>
</tr>
<tr>
<td>Remposo</td>
<td>Benjamin</td>
<td>The Netherlands</td>
<td><a href="mailto:ben.remington@tno.nl">ben.remington@tno.nl</a></td>
</tr>
<tr>
<td>Rodrigues Costa</td>
<td>Ana</td>
<td>Portugal</td>
<td><a href="mailto:acrc@uevora.pt">acrc@uevora.pt</a></td>
</tr>
<tr>
<td>Roggen</td>
<td>Erwin L</td>
<td>Denmark</td>
<td><a href="mailto:3rsmc.eu@gmail.com">3rsmc.eu@gmail.com</a></td>
</tr>
<tr>
<td>Sánchez Mohya</td>
<td>Teresa</td>
<td>Spain</td>
<td><a href="mailto:tsm09382@um.es">tsm09382@um.es</a></td>
</tr>
<tr>
<td>Schall</td>
<td>Eszter</td>
<td>Hungary</td>
<td><a href="mailto:s.eszter@mail.bme.hu">s.eszter@mail.bme.hu</a></td>
</tr>
<tr>
<td>Savvaidis</td>
<td>Ioannis</td>
<td>Greece</td>
<td><a href="mailto:isavvaid@uo.i.gr">isavvaid@uo.i.gr</a></td>
</tr>
<tr>
<td>Schrama</td>
<td>Denise</td>
<td>Portugal</td>
<td><a href="mailto:dschrama@ualg.pt">dschrama@ualg.pt</a></td>
</tr>
<tr>
<td>Sienkiewicz-Szląpka</td>
<td>Edyta</td>
<td>Poland</td>
<td><a href="mailto:edyta.sienkiewicz@uw.edu.pl">edyta.sienkiewicz@uw.edu.pl</a></td>
</tr>
<tr>
<td>Smit</td>
<td>Joost</td>
<td>The Netherlands</td>
<td><a href="mailto:j.j.smit@uu.nl">j.j.smit@uu.nl</a></td>
</tr>
<tr>
<td>Tranquet</td>
<td>Olivier</td>
<td>France</td>
<td><a href="mailto:olivier.tranquet@nantes.inra.fr">olivier.tranquet@nantes.inra.fr</a></td>
</tr>
<tr>
<td>Untersmayr-Elsenhuber</td>
<td>Eva</td>
<td>Austria</td>
<td><a href="mailto:eva.untersmayr@meduniwien.ac.at">eva.untersmayr@meduniwien.ac.at</a></td>
</tr>
<tr>
<td>van Bilsen</td>
<td>Jolanda</td>
<td>The Netherlands</td>
<td><a href="mailto:j.vanbilsen@tno.nl">j.vanbilsen@tno.nl</a></td>
</tr>
<tr>
<td>van der Wal</td>
<td>Marloes</td>
<td>The Netherlands</td>
<td><a href="mailto:marloes.vanderwal@tno.nl">marloes.vanderwal@tno.nl</a></td>
</tr>
<tr>
<td>Vassilopoulou</td>
<td>Emilia</td>
<td>Cyprus</td>
<td><a href="mailto:vasilopoulou.e@unic.ac.cy">vasilopoulou.e@unic.ac.cy</a></td>
</tr>
<tr>
<td>Verhoeckx</td>
<td>Kitty</td>
<td>The Netherlands</td>
<td><a href="mailto:kitty.verhoeckx@tno.nl">kitty.verhoeckx@tno.nl</a></td>
</tr>
<tr>
<td>Wróblewska</td>
<td>Barbara</td>
<td>Poland</td>
<td><a href="mailto:b.wroblewska@pan.olsztyn.pl">b.wroblewska@pan.olsztyn.pl</a></td>
</tr>
<tr>
<td>Zrimszek</td>
<td>Petra</td>
<td>Slovenia</td>
<td><a href="mailto:petra.zrimszek@vf.uni-lj.si">petra.zrimszek@vf.uni-lj.si</a></td>
</tr>
<tr>
<td>Martínez-Blanco</td>
<td>Mónica</td>
<td>Spain</td>
<td><a href="mailto:monilay91@gmail.com">monilay91@gmail.com</a></td>
</tr>
<tr>
<td>Kordos</td>
<td>Karolina</td>
<td>Poland</td>
<td><a href="mailto:aa.jozwik@ighz.pl">aa.jozwik@ighz.pl</a></td>
</tr>
<tr>
<td>Katarzyna</td>
<td>Janyzt</td>
<td>Poland</td>
<td><a href="mailto:aa.jozwik@ighz.pl">aa.jozwik@ighz.pl</a></td>
</tr>
<tr>
<td>Maiga</td>
<td>Aicha</td>
<td>France</td>
<td><a href="mailto:aicha.maiga@cea.fr">aicha.maiga@cea.fr</a></td>
</tr>
<tr>
<td>Mamone</td>
<td>Gianfranco</td>
<td>Italy</td>
<td><a href="mailto:mamone@isa.cnr.it">mamone@isa.cnr.it</a></td>
</tr>
<tr>
<td>Wagenaar</td>
<td>Laura</td>
<td>Netherlands</td>
<td><a href="mailto:l.wagenaar@uu.nl">l.wagenaar@uu.nl</a></td>
</tr>
<tr>
<td>Dabkowska-Fotschki</td>
<td>Joanna</td>
<td>Poland</td>
<td><a href="mailto:j.dabkowska@pan.olsztyn.pl">j.dabkowska@pan.olsztyn.pl</a></td>
</tr>
<tr>
<td>Markiewicz</td>
<td>Lidia</td>
<td>Poland</td>
<td><a href="mailto:l.markiewicz@pan.olsztyn.pl">l.markiewicz@pan.olsztyn.pl</a></td>
</tr>
<tr>
<td>Szyrc</td>
<td>Anna</td>
<td>Poland</td>
<td><a href="mailto:a.szyrc@pan.olsztyn.pl">a.szyrc@pan.olsztyn.pl</a></td>
</tr>
<tr>
<td>Szymkiewicz</td>
<td>Agata</td>
<td>Poland</td>
<td><a href="mailto:a.szymkiewicz@pan.olsztyn.pl">a.szymkiewicz@pan.olsztyn.pl</a></td>
</tr>
</tbody>
</table>
Local Information
Conference will be held in:

Golden Tulip
Towarowa 2
00-811 / Warsaw
Poland
Telephone +48 22 582 75 00
Fax +48 22 582 75 01
info@goldentulipwarsawcentre.com
www.goldentulipwarsawcentre.com

How to reach Warsaw:
Warsaw can be reached by plane. There are two airports in Warsaw,

Chopin Airport that is located 8 km from the city center and the meeting venue, and the second is Warsaw Modlin Airport that is situated 43 km from the city.

Transportation from the airport:
The options for getting to the city from the airport.

(Please be aware that for tickets you have to pay in PLN. You can change it at exchange office, located at the airport; 1EUR = 4,33 PLN (zloty)
From Chopin Airport to Golden Tulip Hotel

By 175 bus
By S2 train

From Warsaw Modlin Airport to Golden Tulip Hotel
Sponsors & organizers

European Commission

COST

European Cooperation in Science and Technology

ImpARAS

Innovaction for life

TNO

Instytut Rozrodu Zwierząt i Badań Zdrowotno-Nakładowych Profesjonalnego Akademickiego

Anima

SANLAB

Vivari

OLYMPUS

BD

2nd ImpARAS conference September 20-22, 2016
Warsaw Poland