

COST 927 Action

Thermally processed foods: possible health implications



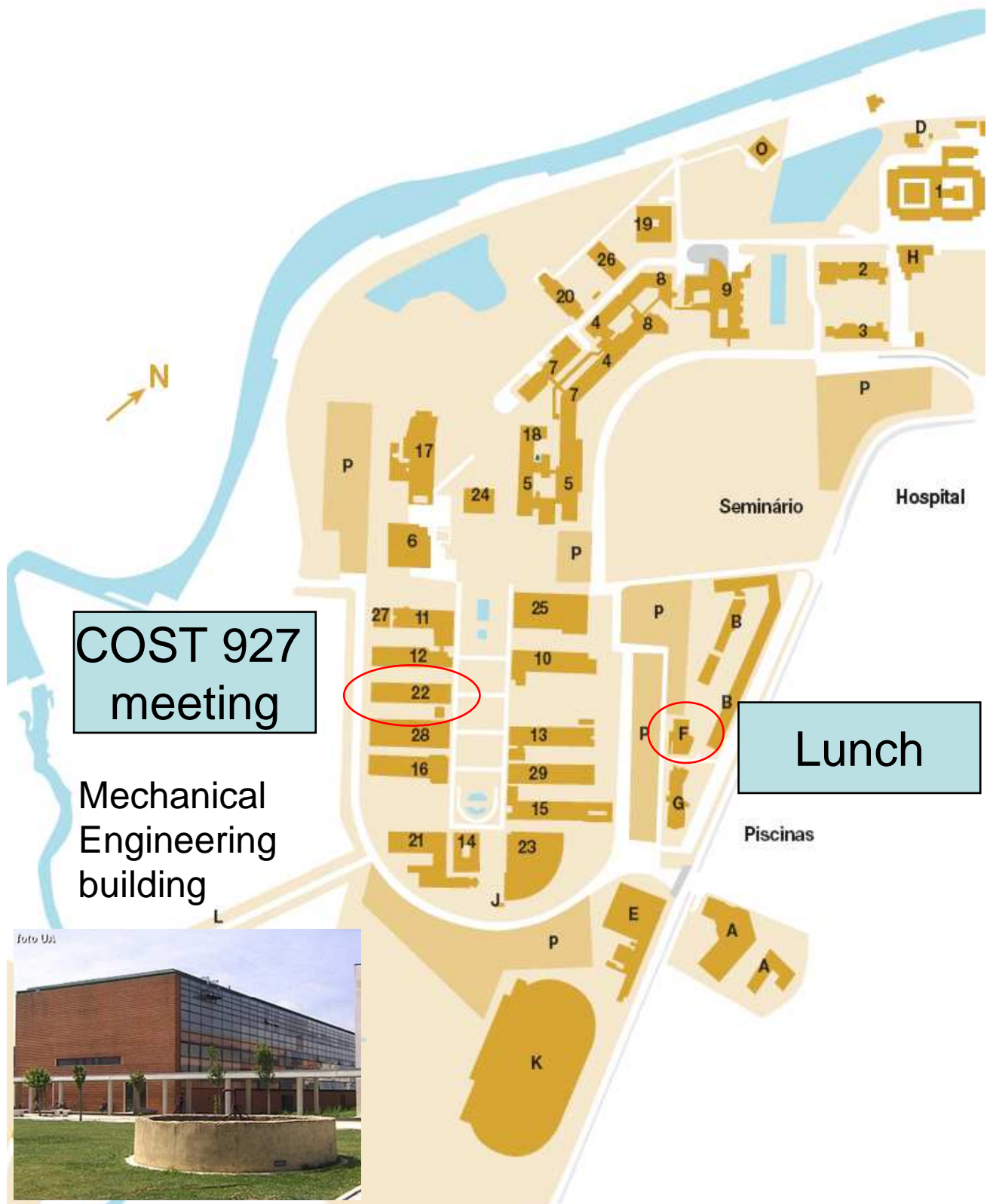
Analytical and chemical aspects related to thermally processed foods

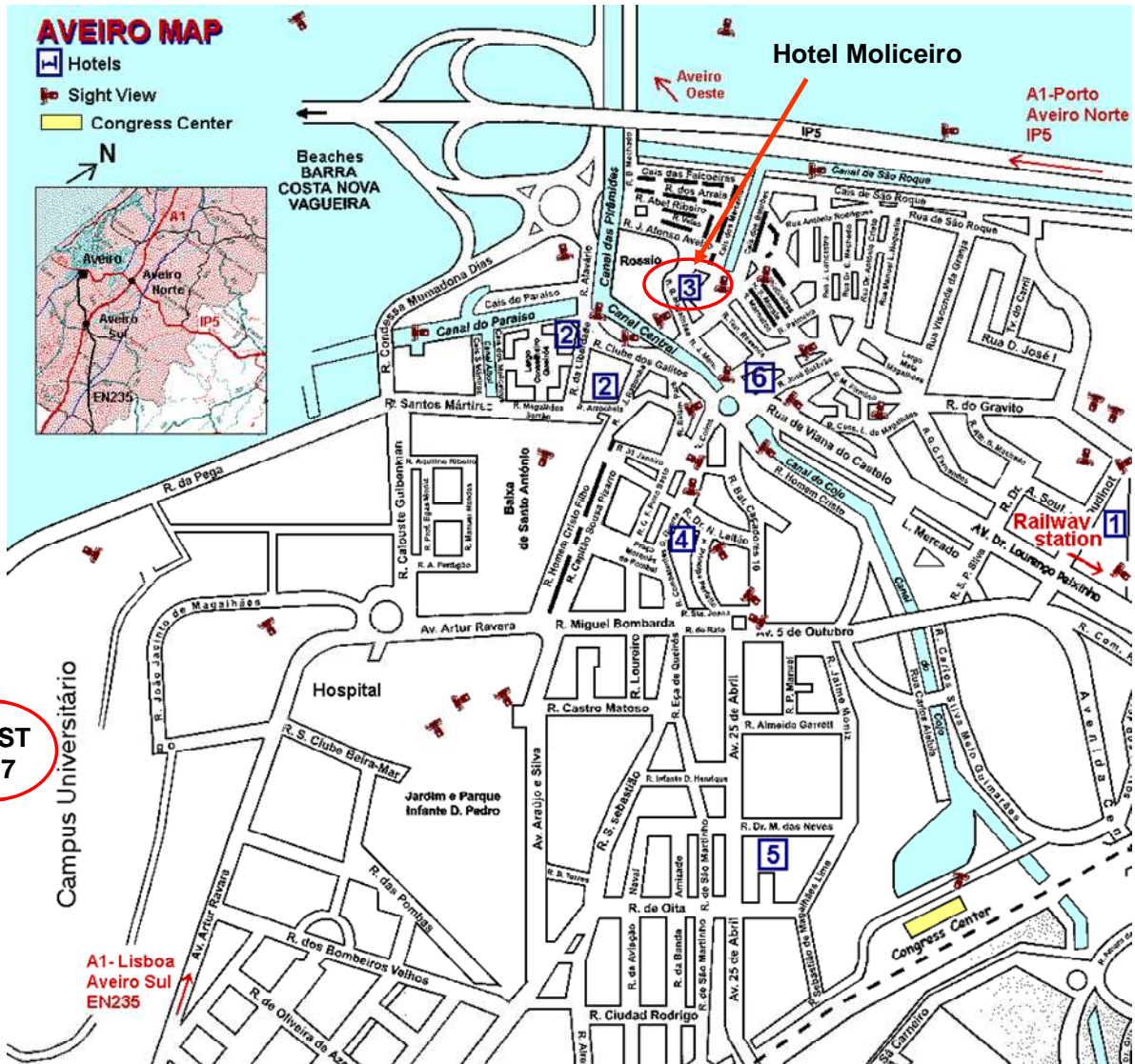


Department of Chemistry

University of Aveiro, Portugal

16-17 April 2009





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SUMMARY PROGRAMME

| Thursday, 16 | | | Friday, 17 | | |
|---|------|---------------------|-------------------------------------|------|------------------------|
| 09:00 | | Opening | <i>Session 5 – MAJS van Boekel</i> | | |
| <i>Session 1 – Vincenzo Fogliano</i> | | | 09:00 | IL11 | Francisco J Hidalgo |
| 09:15 | IL01 | MAJS van Boekel | 09:30 | IL12 | José O. Fernandes |
| 09:45 | IL02 | Susana Casal | 10:00 | IL13 | Zuzana Ciesarova |
| 10:15 | IL03 | Gabrilla Gazzani | 10:30 | | Coffee break |
| 10:45 | | Coffee break | <i>Session 6 – Mirko Bunzel</i> | | |
| <i>Session 2 – Henk A Schols</i> | | | 11:00 | IL14 | P Rimantas Venskutonis |
| 11:15 | IL04 | Vincenzo Fogliano | 11:30 | IL15 | Isabel MPLVO Ferreira |
| 11:45 | IL05 | Mirko Bunzel | 12:00 | IL16 | Rosa Buskets |
| 12:15 | OC01 | Joana Simões | 12:30 | OC05 | Jorge Saraiva |
| 12:30 | OC02 | Cláudia Nunes | 12:45 | | Lunch |
| 12:45 | | Photograph | <i>Session 7 – Beatriz Oliveira</i> | | |
| 13:00 | | Lunch | 14:30 | IL17 | Michael Murkovic |
| <i>Session 3 – Francisco J. Hidalgo</i> | | | 15:00 | IL18 | Anna Arnoldi |
| 14:30 | IL06 | Luciano Navarini | 15:30 | IL19 | Dietmar R. Kammerer |
| 15:00 | IL07 | Fernando Nunes | 16:00 | | Coffee break |
| 15:30 | IL08 | Henk A Schols | <i>Session 8 – Michael Murkovic</i> | | |
| 16:00 | | Coffee break | 16:30 | IL20 | Jorge Ruiz |
| <i>Session 4 – Jana Hajslova</i> | | | 17:00 | IL21 | Beatriz Oliveira |
| 16:30 | IL09 | Jesus Simal-Gándara | 17:30 | IL22 | Jana Hajslova |
| 17:00 | OC03 | Giuseppe Meca | 18:00 | | Closing |
| 17:15 | OC04 | Henrik Frandsen | 20:00 | | Symposium dinner |
| 17:30 | IL10 | Bettina Cämmerer | | | |
| 18:00 | | Jana Hajslova | | | |
| 18:30 | | Closing of day 1 | | | |
| 20:00 | | Welcome dinner | | | |

SCHEDULE OF SCIENTIFIC PROGRAMME

Thursday – 16th April 2009

9:00 Welcome

Session 1 – Chair Vincenzo Fogliano

9:15 IL01. **MAJS van Boekel** - Univ. Wageningen, The Netherlands
Quality matters of heated foods: a question of balance

9:45 IL02. **Susana Casal** - Univ. Porto, Portugal
Coffee roasting: accurate control for bioactive beverages

10:15 IL03. **Gabriella Gazzani** - Univ. Pavia, Italy
Melanoidin antioxidant and anticaries properties

10:45 Coffee break

Session 2 – Chair Henk A. Schols

11:15 IL04. **Vincenzo Fogliano** – Univ Naples, Italy
The physiological relevance of antioxidant dietary fibre

11:45 IL05. **Mirko Bunzel** – Univ. Minnesota, USA
Enrichment, characterization and microbial fermentation of melanoidin fractions from coffee brews

12:15 OC01. **Joana Simões** – Univ. Aveiro, Portugal
Increase on the extractability of immunostimulatory mannans from coffee residue by a roasting procedure

12:30 OC02. **Cláudia Nunes** – Univ. Aveiro, Portugal
Effect of candying on volatiles and cell wall polysaccharides of “Ameixa d’Elvas” plums

12:45 Photograph

13:00 Lunch at the University Restaurant

Session 3 – Francisco J. Hidalgo

14:30 IL06. **Luciano Navarini** – Illycaffè SpA Trieste, Italy
Reinvestigation of an arabinogalactan isolated from pure *Coffea arabica* freeze dried instant coffee

15:00 IL07. **Fernando Nunes** – UTAD, Vila Real, Portugal
Coffee melanoidins: structural changes during the roasting process

15:30 IL08. **Henk A Schols** - Univ. Wageningen, The Netherlands
Coffee melanoidins: characteristics and possible health aspects

16:00 Coffee break

Session 4 – Chair Chair Jana Hajslova

- 16:30 IL09. **Jesus Simal-Gándara** – Univ. Vigo, Ourense, Spain
Influence of toasting on the occurrence of polycyclic aromatic hydrocarbons in toasted bread
- 17:00 OC03. **Giuseppe Meca** – Univ. Valencia, Spain
Study of the formation of *N*-(carboxymethyl)fumonisin B1 in a model system of crispy corn bread
- 17:15 OC04. **Henrik Frandsen** – Tech. Univ. Denmark
Biomonitoring of urinary metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) following human consumption of cooked chicken.
- 17:30 IL10. **Bettina Cämmerer** - Berlin Univ Technol, Germany
 α -Dicarbonyls: common knowledge and recent findings
- 18:00 **Jana Hajslova** - Presentation/discussion on the establishment of a possible consortium in the aim of 7th framework call KBBE-2010-2-4-02: Identification of the effect of processing on food contaminants
- 18:30 Closing of day 1
- 20:00 Welcome dinner

Friday – 17th April 2009

Session 5 – Chair M.A.J.S. van Boekel

- 9:00 IL11. **Francisco J Hidalgo** – CSIC Seville, Spain
The role of amino compounds in the fate of acrylamide
- 9:30 IL12. **José O Fernandes** – Univ. Porto, Portugal
Towards simpler and faster GC-MS procedures for acrylamide analysis in food matrices
- 10:00 IL13. **Zuzana Ciesarova** – VUP FRI, Bratislava, Slovakia
Asparaginase treatment: the impact on acrylamide, amino acids, and other MRPs in processed products
- 10:30 Coffee break

Session 6 – Chair Mirko Bunzel

- 11.00 IL14. **Petras Rimantas Venskutonis** – Kaunas Univ. Technol., Lithuania
Analysis of heterocyclic amines and effects of antioxidants on their formation in meat
- 11.30 IL15. **Isabel MPLVO Ferreira** – Univ. Porto, Portugal
Heterocyclic Aromatic Amines Formation in Barbecued Sardines (*Sardina pilchardus*) and Atlantic Salmon (*Salmo salar*)
- 12.00 IL16. **Rosa Busquets** - Univ. Barcelona, Spain
Advances in the detection of heterocyclic amines in food and biological fluids
- 12:30 OC05. **Jorge Saraiva** – Univ. Aveiro, Portugal
Chemical changes in foods processed by pressure-assisted thermal processing (PATP)
- 12:45 Lunch at the University Restaurant

Session 7 – Chair Beatriz Oliveira

- 14:30 IL17. **Michael Murkovic** – Graz Univ. Technol., Austria
Formation of hazardous compounds during heating of foods
- 15:00 IL18. **Anna Arnoldi** – Univ. Milan, Italy
The effects of various processing conditions on a protein isolate from *Lupinus angustifolius* investigated by different approaches
- 15:30 IL19. **Dietmar R Kammerer** – Hohenheim Univ., Stuttgart, Germany
Heat stability of anthocyanins from black carrot, elderberry and strawberry
- 16:00 Coffee break

Session 8 – Chair Michael Murkovic

- 16:30 IL20. **Jorge Ruiz** – Univ. Extremadura, Cáceres, Spain
Vacuum cooking of meat at moderate to low temperatures: physico-chemical changes and formation of volatile flavour compounds
- 17:00 IL21. **Beatriz Oliveira** – Univ. Porto, Portugal
Influence of frying procedure on quality and safety of fried foods
- 17:30 IL22. **Jana Hajslova** – Inst. Chem. Technol., Prague, Czech Republic
Novel strategies to monitor transfer of processing contaminants across the beer making chain
- 18:00 Closing
- 20:00 Symposium dinner

**Invited Lectures
(IL01 – IL 22)**

Quality matters of heated foods: a question of balanceM.A.J.S. van Boekel*Product Design & Quality Management, Wageningen University*

Foods are heated for many reasons and the overall objective is, of course, to come to a better quality as compared to unheated foods. Quality is, unfortunately, a vague concept, and the challenge is to get a grip on this in scientific terms. It is proposed to do this via the QACCP approach: Quality Analysis Critical Control Points. The basic idea behind this is to decompose quality from the consumer perspective into manageable quality indicators that can be measured via performance indicators. In addition, it is suggested to consider the fate of relevant quality attributes in the perspective of the food chain, i.e., from production up until consumption, and to find the critical control points that affect the most relevant quality attributes. Since usually more than one quality attribute needs to be considered, this can become quite complex, and for that reason changes in the most relevant quality attributes need to be modelled mathematically. In doing so, the most optimal strategy can then be predicted from models to find the right balance between desired and undesired changes that will determine quality in the end. This lecture will discuss what is needed to come to such an approach.

Coffee roasting: accurate control for bioactive beverages

Susana Casal, Rita Alves, Beatriz Oliveira

Requimte, Serviço de Bromatologia, Faculdade Farmácia, Universidade do Porto, Portugal

A “special” coffee is controlled by several standards, from the field to the cup: the growing region, variety and climate, the care given during processing and transportation, and the expertise in the roasting and brewing methods, will determine the consumer’s acceptability, based ultimately on the pleasure brought by the beverage ingestion. These standards, however, are related mostly with the consumers delight and not with the consumer’s health.

Coffee is increasingly the headline of medical journals as important factor in the reduction of the risk for several diseases, almost as a new global coffee health paradox. In most cases caffeine, the single most studied compound in medical science, is unable to justify the findings, at least alone. Indeed, modern science knows almost everything about caffeine but really very little about coffee and human health.

Green coffee is far richer in chlorogenic acids (6-9%) compared with caffeine (1-2.5%). The chlorogenic acids present in the green coffee, with potent antioxidant activities, give birth during roast to several compounds, including lactones with powerful opioid antagonism activity and capacity to enhance insulin action. Trigonelline degradation with roast (80-90%) gives rise to hundreds of important volatiles, including some with high antioxidant capacity and a vitamin – niacin. From the array of compounds formed from Maillard reactions, melanoidins are receiving increased attention for their antioxidant capacities. The aminoacids degradation products include beta-carbolines, with potent competitive and reversible MAO inhibition capacities.

These might be only a few examples from an array of bioactive compounds formed during coffee roasting with possible health implications. An underdeveloped roast will keep many of the original green coffee constituents, chlorogenic acids included, but will be poor in important compounds formed during the roast process. A dark roast will be depleted of both, except for caffeine, that is thermostable. Also, the amounts of each arabica and robusta in the blend, with different chemical compositions, will provide the raw compounds for the reactions taking place during roast.

The strength of the roast and the roasting time will define the balance of bioactive compounds, and thus the final roasted coffee health potential.

Melanoidin antioxidant and anticaries properties

M. Daglia¹, M. Stauder², A. Papetti¹, G. Giusto³, C. Pruzzo³, G. Gazzani¹

¹ Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Pavia University, Pavia, Italy; ² Istituto di Microbiologia e Scienze Biomediche, Marche Polytechnic University, Ancona, Italy; ³ DIBIO, Genova University, Genova, Italy

Maillard reaction takes place at high rate in thermal treated foods, such as bakery products, coffee, barley coffee, beer, giving rise to a large number of compounds with different chemical features, molecular weight (MW) and health-promoting or toxicological properties. Among Maillard reaction products (MRPs), melanoidins, generally defined as brown and acidic macromolecular compounds containing nitrogen, attracted researchers' interest for their heterogeneous and complex chemical structure, still poorly defined, and their biological properties, such as antioxidant [1-3], antiallergenic [4], antiadhesive [5-6], antimicrobial [7], antimutagenic and cytotoxic capacities [4].

In our previous investigations high molecular weight nitrogen containing components with different chemical features, were isolated from coffee and barley coffee and studied for their antioxidant and anticaries properties. As regards antioxidant capacity, five HMW coffee components and a very HMW barley coffee melanoidin showed high activity in an ex vivo system consisting of rat hepatocyte microsomes. Such activity could be attributed to radical scavenger properties, metal chelating activity and reducing power showed in different extent by the isolated components when tested in different chemical systems.

Considering coffee and barley coffee melanoidin anticaries properties, they are supported by the capacity, showed also in this case in different extent by each isolated component, to prevent adhesion to hydroxyapatite (HA), to induce detachment from HA, or to prevent biofilm formation by *Streptococcus mutans*, considered the main aetiological agent of caries.

- [1] M. Daglia, A. Papetti, C. Gregotti, F. Bertè, G. Gazzani *J. Agric. Food Chem.* 48, 1449-1454 (2000)
- [2] R.C. Borrelli, A. Visconti, C. Mennella, M. Anese, V. Fogliano *J. Agric. Food Chem.* 50, 6527-6533 (2002)
- [3] L. Goya, C. Delgado-Andrade, J.A. Rufian-Henares, L. Bravo, F.J. Morales *Mol. Nutr. Food Res.* 51, 536-545 (2007)
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- [7] J.A. Rufian-Henares, F.J. Morales *J. Agric. Food Chem.* 56, 2357-2362 (2008)

The physiological relevance of antioxidant dietary fibre

V. Fogliano, P. Vitaglione

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Cereals such as wheat, barley, oat, rye, and maize, are staple foods for the population of Western countries contributing about 50% of dietary fibre (DF) intake. The relation between DF intake and chronic heart and gastrointestinal diseases, namely “fibre hypothesis”, has been observed over thirty-five years ago. From that observation many epidemiological studies associate whole grain consumption to a reduced risk of many diseases. This paper focuses on the antioxidant component of cereal dietary fibre starting from its chemical structure, bioavailability and biological meaning.

By the critical assessment of the intervention studies performed using cereal bran and whole grains, the hypothesis that the slow and continuous release in the gut of the dietary fibre bound antioxidants determines the health benefits of cereals, is illustrated. According to this picture the cereal antioxidant dietary fibre is a suitable vehicle to bring phenolic compounds into the lower gut. These compounds cannot be absorbed when are bound to the polysaccharide moiety, becoming available for gut microflora. Moreover, those linked to the soluble dietary fibre can be hydrolyzed by bacterial esterases and absorbed into the bloodstream where they might prevent LDL oxidation, thus exerting several beneficial effects.

For these reasons it is advisable to convert IDF into SDF to maximise the possible health benefits of cereal DF phenolic compounds. This goal can be achieved by different approaches and in the last part of the work, new perspectives and technological possibilities to enhance the health potential of this cereal component will be also highlighted.

Keywords: Cereal antioxidant activity, ferulic acid, gut microflora

References:

- Vitaglione P, Napolitano A., Fogliano V Cereal dietary fibre as natural functional ingredient to deliver phenolic compounds into the gut. *Trends in Food Science and Technology*, 2008, 19, 451-463.
- Serpen A., Gökmen V., Pellegrini N., Fogliano V. Direct measurement of total antioxidant capacity of cereal products. *Journal of Cereal Science* 2008, 48 (2008) 816–820.
- Napolitano A, Lanzuise S, Rocco M, Arlotti G, Ranieri R, Knutsen SH, Lorito M, Fogliano V Treatment of cereal products with a tailored preparation of *Trichoderma* enzymes increases the amount of soluble dietary fibre. *J. Agric Food Chem*, 2006, 54, 7863-7869.

Enrichment, characterization and microbial fermentation of melanoidin fractions from coffee brews

Mirko Bunzel¹, Diana Gniechwitz²

¹ *University of Minnesota, Department of Food Science and Nutrition, St. Paul, USA;* ² *University of Hamburg, Institute of Biochemistry and Food Chemistry Hamburg, Germany*

Coffee brews contain polysaccharides which make up most of the soluble dietary fiber complex of the coffee beverage. These polysaccharides are predominantly type II arabinogalactans and galactomannans with a low degree of substitution. However, melanoidins can also contribute to the fiber content of coffee brews. Melanoidins are reported to be physiologically active components, not exclusively but also due to their antioxidant activities. They might, for example, also be a substrate for the gut microflora. Structural information about coffee melanoidins is rare and current models are supported by few data. Coffee melanoidins were proposed to be of high molecular weight themselves, but it was also suggested that merely low-molecular weight chromophoric structural units are bound to intact polysaccharides and proteins. Therefore, questions to be answered include whether or not proteins and polysaccharides are indeed integral components of coffee melanoidins. Another often discussed issue is how phenolic compounds are integrated into this macromolecular assembly.

In our studies, we isolated ethanol soluble fractions with molecular masses greater than 2kDa from differently roasted Arabica coffee brews. Chemical characterization of these fractions showed that they contained about 13% polysaccharides, mostly arabinogalactans, and about 20% proteins/peptides. However, the main part was composed of structurally unknown Maillard reaction products. From NMR spectroscopy we assume that intact caffeic and ferulic acid derivatives were not present in these fractions.

In a different enrichment approach, we used hydrophobic interaction chromatography (HIC) with Sephadex LH-20 and Octylsepharose as stationary phases to get melanoidin fractions from high-molecular weight fraction of the brews. These melanoidins contained less than 6% releasable carbohydrates (mostly arabinose and galactose) and amino acids. NMR spectroscopy revealed an enrichment of phenolic/aromatic/olefinic structural units, but, again, did not hint on intact hydroxycinnamates in these fractions. The antioxidant activities of the HIC-fractions were three- to four fold higher as compared to the original molecular weight fractions and they were intensively dark coloured. Although these fractions made up only a small part of the original high-molecular weight material they largely contributed to their antioxidant activities.

From these studies we support the concept of melanoidins being high-molecular-weight chromophores themselves as opposed to other models.

The ethanol soluble fractions were fermented by using human fecal bacteria. It was demonstrated that these fractions were less fermentable than soluble fibers from coffee. Destructive chemical analysis and NMR spectroscopy indicated that the carbohydrate constituents of ethanol soluble, high-molecular weight fractions were the preferred substrates for colonic microbiota. As the carbohydrate proportion in these fractions was comparably low less short-chain fatty acids were formed as compared to soluble coffee fibers. However, NMR spectra, absorbances at 405 nm, and non-protein nitrogen contents showed that non-carbohydrate and non-protein compounds were also utilized to some extent but the bacterial species involved in this degradation remain to be identified.

IL05

Reinvestigation of an arabinogalactan isolated from pure *Coffea arabica* freeze dried instant coffee

L. Navarini¹, P. Capek², M. Matulova², F. Suggi-Liverani¹

¹ *illycaffè s.p.a., Research & Innovation, Trieste, Italy;* ² *Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Bratislava, Slovakia*

It is well known that raw *Coffea arabica* beans are composed, approximately, by 15% of arabinogalactan-proteins, AGP, about half of which, loosely bound to cell walls. These proteoglycans have been recognized very important as roasted coffee aroma precursors and play an important role in determining some functional properties to coffee brews. In spite of the relevant steps ahead performed up to now in the knowledge of both green and roasted coffee beans arabinogalactans chemistry, little effort has been dedicated to investigate arabinogalactans from instant coffee powder (soluble coffee). Industrial soluble coffee processing, in addition to roasting, includes further thermal treatments: the extraction, which is normally performed at high temperature and it is accompanied by hydrolysis and the drying step which can be performed by using high temperature as in the case of spray drying process. These drastic conditions may remarkably alter the chemical structure of the polysaccharides originally present in the green coffee beans and for this reason more attention has been given to investigate mono and oligosaccharides. As far as we know, a part the pioneering work by Wolfrom and Anderson in 1967 and some observations regarding the presence of arabinogalactan-derived polymer material in soluble coffee, no detailed structural characterization of arabinogalactan-protein from soluble coffee has been reported in the literature and the experimental data by Wolfrom and Anderson have not yet been rediscussed.

In the present work, AGP has been isolated, according to Wolfrom and Anderson, from freeze-dried instant coffee powder industrially produced from dark roasted *C. arabica*. The isolation procedure reproducibility, in terms of yield and chemical identity, has been assessed. AGP has been characterized by chemical (fractionation, partial hydrolysis and methylation analysis) and spectroscopic methods (¹H and ¹³C NMR and FTIR). The polymer, Mw 5400 Da, was composed of Gal (85% w/w), Ara (8.2%), Man (2.7%), Glc (1.4%), Hep (2.7%) and traces of Xyl residues with 1.6% protein. No rhamnose and glucuronic acid as well as incorporated chlorogenic acid have been found. The preliminary results of chemolytic and spectroscopic investigation revealed the expected 3-linked β -galactosyl backbone branched at C-6 by side oligomeric chains. Whereas galactose/arabinose (12.0) and t-Araf/5-Araf (4.92) molar ratios, in the purified unfractionated polymer are consistent with those expected for both roasting and processing conditions, the degree of branching is higher than that expected. NMR analysis 1D and 2D ¹H-¹³C NMR spectra of the AGP indicate the presence of internal 1,3-linked β Gal, 3-linked reducing end α , β Gal, terminal 1,3-linked β Gal, 1,3,6-linked β Gal, 1,6-linked nonsubstituted and terminal β Gal. No Rha and GlcA signals were detected. The most of arabinose was found as terminal Araf and internal 1,5- α Araf. However, in COSY spectra, other low intensity signals indicate the presence of Araf in unknown linkage types. The role played by soluble coffee processing in determining yield and chemical features of AGP is emphasized.

IL06

Coffee melanoidins: structural changes during the roasting process

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Melanoidins are the high molecular weight brown final products of Maillard reaction. They are formed during the heat processing of foods like coffee, bread, malt, and beef. A chemical definition of these food polymers is still today impossible, despite several efforts to determine their structure. In the last years, the interest on research of melanoidins as increased, due to their biological activities. Coffee brew is one of the main sources of melanoidins in human diet.

A method involving fractionation in ethanol aqueous solutions, anion exchange chromatography, and immobilized copper chelating chromatography was developed to obtain high molecular weight anionic melanoidin populations from coffee infusions. Fractions with different physicochemical properties and chemical composition regarding carbohydrate as well as protein nature and content were isolated. Fractions with similar chemical composition were obtained for light-, medium-, and dark-roasted coffee infusions. These melanoidin fractions accounted for 30-33% of the cold-water soluble high molecular weight material, independently of the degree of roast of the coffee. Nevertheless, the amount and structural features of the carbohydrate and protein components of these melanoidin fractions varies with the roasting degree, showing a structural change in the melanoidins with the roasting process.

Coffee melanoidins: characteristics and possible health aspects

H.A. Schols

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The aim of the work presented was the identification of structural and functional properties of coffee brew melanoidins, and their formation mechanisms, that are formed upon roasting of coffee beans. To this end, coffee brew was fractionated on the basis of e.g. molecular weight, charge, and hydrophobicity and the composition of the isolated coffee brew melanoidin populations was determined.

A new parameter, K_{mix} 405nm, was introduced that allowed the quantification of the melanoidin level in coffee brew fractions. The determined melanoidins levels correlated with both the protein content and the nonprotein-nitrogen content, from which it was concluded that proteins become part of melanoidin structures upon roasting. Additionally, it was found that intact chlorogenic acids are incorporated into melanoidin structures via the phenolic acid moiety through nonester-bonds. The extent of chlorogenic acid incorporation correlated with the melanoidin level, indicating that phenolic oxidation contributes to the brown color as well as Maillard reactions. Another finding was that coffee brew melanoidins were shown to expose negative charges at the pH of coffee. Furthermore, arabinogalactan proteins (AGPs) were found to participate in melanoidin formation upon roasting. A 'pure' AGP-melanoidin population could be isolated from coffee brew due to the high specificity of the reagent used for precipitation. Characterization of low molecular weight melanoidins provide strong indications that sucrose is involved in the formation of melanoidins too. Electron spin resonance studies revealed that roasting leads to formation of antioxidative structures in coffee brew melanoidins. This was expected to be due to the formation of novel roasting-induced antioxidative structures or due to the incorporation of chlorogenic acids in melanoidins. Investigation of the effect of the degree of roast on coffee brew melanoidins properties confirmed that proteins and chlorogenic acids are primarily involved in melanoidin formation. Furthermore, arabinogalactans seem to be more involved in melanoidin formation than galactomannans. Additionally, it was found that prolonged roasting especially led to accumulation of HMw coffee brew melanoidins.

Finally, a scheme that describes melanoidin-related formation pathways for coffee beans compounds was introduced. The reaction pathways involved are explained in detail per coffee bean compound.

References:

Coffee brew melanoidins, Structural and Functional Properties of Brown-Colored Coffee Compounds, E.K.Bekedam, PhD thesis Wageningen University, 2008

Influence of toasting on the occurrence of polycyclic aromatic hydrocarbons in toasted bread

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Some polycyclic aromatic hydrocarbons (PAHs), particularly those with a high molecular weight, have been classified as probably carcinogens to humans by the International Agency for Research on Cancer (IARC). The significance of the determination of PAHs is reflected by the special attention of the European Union, which is paying to regulate the maximum allowed levels of PAHs in foodstuffs such as smoked foods. Like other thermally processed foodstuffs, toasted bread can contain these carcinogenic chemicals, not only due to a contamination at source but also during toasting. In order to check PAHs generated from toasting in sandwich bread, several treatment conditions were evaluated: direct toasting (flame-toasting, coal-grilling or gas oven-toasting) or indirect toasting (electric oven-toasting) [1].

PAHs were extracted by solid-liquid extraction (SLE) and determined by liquid chromatography with fluorescence detection (LC-FD). Based on the results, the used toasted technique would strongly affect in PAH levels in the final product. No samples obtained by electric oven and toaster were polluted; otherwise the samples toasted by charcoal and flame grilling presented very important levels. Up to 350 µg/kg of total PAHs were detected in toasted samples by wood flame. Differences between different ways of toasting could be ascribed to deposition of PAHs from smoke. Finally, several commercial toasted samples of bread were tested to determine PAHs. Overall, the PAH levels were very low. Benzo[a]pyrene ranged from no detectable to 0.23 µg/kg.

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α -Dicarbonyls – common knowledge and recent findings

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α -Dicarbonyl compounds are known as highly reactive compounds which play an important role as intermediates of nonenzymatic browning reactions in heat treated food. They are proved to be involved in colour and flavour formation as well as in undesirable reactions like the formation of toxic acrylamide or heterocyclic compounds. It is assumed that they are also responsible for changes in antioxidant properties of food after cooking or roasting.

In this presentation some Maillard analogous reactions resulting in α -dicarbonyls will be introduced, but the focus lies on the Maillard reaction. At the example of the three main and best known α -dicarbonyl compound formed during Maillard reaction – 1-deoxyhexosuloses, 3-deoxyhexosulose and methylglyoxal – the similarities and differences of these reactive intermediates concerning their reactivity and possible reaction products should be shown. For this purpose conditions influencing their formation reactions as well as their degradation pathways will be discussed. It should attempt to distinguish α -dicarbonyls regarding to their responsibility for colour formation and antioxidant action respectively. Some recent research shows that C6- or C5- α -dicarbonyl compounds can also be formed from smaller sugar degradation products.

The role of amino compounds in the fate of acrylamideRosario Zamora, Rosa M. Delgado, Francisco J. Hidalgo*Instituto de la Grasa, CSIC, Avenida Padre García Tejero 4, 41012-Seville, Spain*

Acrylamide is formed during heating of foods in the course of the Maillard reaction, including potato and bakery products, cereals, and roasted coffee, among others. However, in some food products, acrylamide content reaches a maximum within the initial roasting period, followed by a decline toward the end of the roasting process. Furthermore, in some other foods acrylamide levels decrease during storage depending on time and temperature by pathways which are still unknown. In an attempt to clarify the mechanisms responsible for the acrylamide fate in foods, this study analyzes in depth the reaction of acrylamide with amino compounds. Reactions were carried out between acrylamide and amines, amino acids, and proteins in model systems, which were heated at several temperatures for different periods of time. These reactions produced the Michael addition of the amino compound to the carbon-carbon double bond of acrylamide. The formed products have been characterized. In addition, kinetic parameters as well as the corresponding activation energies for these reactions were also determined. The obtained results contribute to understand the changes produced in the acrylamide content in foods.

Towards simpler and faster GC-MS procedures for acrylamide analysis in food matrices

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Reliable and accurate data of acrylamide concentration in foods are needed for different kinds of reasons: i) for intake estimates and exposure assessment studies, ii) to support mitigation attempts made by Food Industry and food scientists, iii) to support mechanistic and modeling studies in the pathways formation of the compound, and iv) to distinguish seasonal/regional/varietal differences in crops and products therefrom.

Analysis of acrylamide by bromination and GC determination on a benchtop GC-MS instrument is a well suited method for routine analysis, requiring only common laboratory equipment. Briefly, acrylamide is extracted with water, then derivatized to 2,3-dibromopropionamide at low temperature during 1 hour, the resulting derivative is back-extracted with ethyl acetate and finally analyzed by GC-MS in SIM mode. The method presents a number of advantages over alternative methods which analyze acrylamide without derivatization, such as GC-MS(CI), GC-MS(TOF) and LC-MS/MS. Unlike what happens with these techniques, particularly the last ones, it makes use of costly-effective and largely available equipments, and it is less demanding concerning the thoroughness of the clean-up procedure, namely in what respects the absence of acrylamide precursors that can cause in-site formation of the compound when sample extracts are subjected to high temperatures, such as in the GC injectors and LC-MS/MS desolvation chambers.

The major shortcoming that can be pointed to the "bromination method" is the excessive time spent to execute the whole procedure. In order to overcome this problem our group has focused attention at two different levels: development of faster extraction/clean-up procedures, and more recently, application of new concepts regarding "fast GC methods" and multidimensional GC (MDGC) to the chromatographic determination of the compound.

Concerning the extraction/clean-up procedures, MSPD (matrix solid-phase dispersion) was selected as the method of choice, and optimizations were made aiming to apply the technique to the different type of matrices that usually contain acrylamide, including the most difficult, such as coffee and coffee surrogates. In what respect the GC-MS analysis itself, a heart-cutting MDGC procedure was developed making use of a combination of 2 columns, a 5-m x 0.32 mm x 1.0 μ m HT-5, followed by a 15-m x 0.25 mm x 0.25 μ m DB-5, allowing a full chromatographic time cycle of 8 minutes, cooling time included. Furthermore the method avoids excessive contamination of the detector because the flow of carrier gas containing the sample is directed to the mass spectrometer only in a small "time window" of a few seconds, being diverted to the outside just before and just after the time corresponding to the retention time of the acrylamide derivative (1.7 minutes).

The coupling of the MSPD extraction/clean-up procedure with the MDGC determination allows a significant increase in the rate analysis, making possible the duplicate determination of 18 samples plus 6 standards in about 9 hours (1 working day).

IL12

Asparaginase treatment – the impact on acrylamide, amino acids, and other MRPs in processed products

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A perspective way of acrylamide elimination in processed products seems to be L-asparaginase treatment which efficiently reduces the final acrylamide content in many foodstuffs. The application of asparaginase is supposed not to have any impact on sensorial properties, but the enzyme action is associated with alterations in amino acid composition and consequently in substrates entering Maillard reaction and formation of MRPs. Moreover, keeping conditions for optimal enzyme activity can require sufficient surroundings, and vice versa a formulation of particular products can affect asparaginase activity through pH, water content, salt addition, etc. These relations were illustrated on examples of asparaginase treatment of gingerbreads, cookies, and fried bakery ware. The action of asparaginase resulted not only in a decrease of asparagine content, but also in changes in other amino acid proportion (aspartic acid, glutamine, glutamic acid) jointed in transamination and transamidation reactions in the systems. Other parameters (colour, browning, fluorescence, furosine, HMF, DPPH radical scavenging activity), meeting the expectations, were not significantly affected by the enzyme treatment; substantial differences were observed as a consequence of heat treatment conditions and a salt presence.

Acknowledgement

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Analysis of heterocyclic amines and effects of antioxidants on their formation in meat

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The studies of heterocyclic amines (HAs) in foods were inspired by the idea that smoke produced during cooking might be carcinogenic. The first studies of HAs in foods were started more than 30 years ago and since that time various analytical methods and particularly chromatographic techniques have been used for the identification and quantitative assessment of HAs. Nevertheless, various problems in the quantification of very low concentrations of HAs as well as some controversial results, for instance, in the evaluation of the effects of antioxidants on the formation of this class of compounds in foods, require further studies for the improvement of the existing methods and development of new techniques.

The use of antioxidants is one of the proposed ways to decrease the mutagenic effects of HAs or to inhibit their formation. In our study, beef meat samples heated in diethylene glycol in open crucibles (1st experiment) and in olive oil in tightly closed test tubes (2nd experiment) were used to assess the influence of basil, oregano, marjoram, rosemary, lovage, sweet grass, savory, thyme and coriander extracts on the formation of HAs using HPLC-fluorescence detection. The results obtained in 1st experiment indicate that addition of 0.2 and 0.5 % of thyme, savory and oregano extracts may slightly decrease the formation of PhIP in meat. Other extracts did not have any influence to the concentration of PhIP, while in some cases they even promoted its formation. The highest increase of PhIP was observed in case of addition of basil extract. The extracts of oregano and basil, which showed most significant positive and negative effects on the formation of PhIP in the 1st experiment were tested against 6 HAs in a 2nd experiment. In this case both of them increased the amount of PhIP, and decreased the concentration of Trp-P-2. The content of Trp-P-1 decreased in the samples with basil and increased in the samples with oregano. Other known in meat HAs, AaC, MeAaC and Glu-P-1 were not detected in the tested beef samples. The correlation between antioxidant activity of extracts and formation of PhIP was not found.

Heterocyclic aromatic amines formation in barbecued sardines (*Sardina pilchardus*) and Atlantic salmon (*Salmo salar*)

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The consumption of fish provides utilization of proteins of high biological value, certain minerals, and vitamins. Additionally, fish and fish oil are rich sources of omega-3 fatty acids. However, grilling and barbecuing, common methods for preparation of fatty fishes, usually, requires high temperatures of cooking and heterocyclic aromatic amines (HAs) are sometimes formed. Several studies show that charcoal-cooked meat presents higher amounts of these compounds. Concerning fish samples studies are scarce but indicate similar trend [1].

As HAs are candidates in the aetiology of human cancer, the search for ways to minimize their intake by limiting their occurrence in cooked foods is very important. In the present study, we focused on conditions favouring the formation of thermic and pyrolytic HAs during barbecuing of sardines (*Sardina pilchardus*) and Atlantic salmon (*Salmo salar*) to varying degrees of doneness and grilling conditions. Additionally, the influence of charcoal and electric heat source on formation of HAs in grilled salmon was compared. Thus, the main objective of this study was to examine in which way the formation of HAs can be reduced in barbecued fatty fish and which HAs can be better indicators of drastic conditions used. These informations are needed to make health hazard assessments. It is hoped that with subsequent consumer education about precautions that are needed during fish barbecuing, exposure to these carcinogens by humans can be reduced, thus making such cooked foods safer for human consumption.

Concerning sardine samples barbecued at 280 to 300°C, "rare" samples produced not detected amounts of HAs, "medium done sardines" presented IQ, MeIQx, PhIP, and AαC, with levels of 1.9, 4.4, 3.3 and 2.0 ng/ g, respectively and "well done sardines" presented IQ, MeIQx, Trp-P-1, Trp-P-2, PhIP, AαC and MeAαC, with levels of 0.9, 2.2, 1.8, 8.2, 6.5, 17.7 and 10.6 ng/ g, respectively. Different qualitative and quantitative profiles of HAs were observed in sardine and salmon samples cooked under similar conditions of temperature and doneness. Levels of 13.3, 3.5, 1.13 and 3.18 ng/g were obtained, respectively, for PhIP, AαC, MeAαC and Glu-P-1 in salmon samples barbecued at 280-300°C. The contents of HAs were significantly higher in these samples than in salmon samples barbecued at 180-200°C or in the electric device. However, MeIQx content (0.5 ng/g) was lower in salmon samples barbecued at 280-300°C than in the other samples.

The results obtained in barbecued sardines and salmon indicated that the pyrolytic HA AαC can be a good indicator of excessive temperature or time during cooking of these two fishes.

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Advances in the detection of heterocyclic amines in food and biological fluids

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Heterocyclic amines (HCAs) are genotoxic compounds that are formed at ng/g levels during heating of protein-rich food. The impact of the dietary exposure to HCAs on human health is not clear to date, but analytical strategies are progressing towards obtaining data that will shed light on this issue. The complexity of food and the low concentration of HCAs in these matrices has so far limited the information available. We have taken advantage of multistep mass spectrometry (MSⁿ) to identify uncommonly detected HCAs in meat, such as a putative DMIP regioisomer and 4'-OH-PhIP. Advances in sample preparation to quantify HCAs and their metabolites in biological fluids from subjects with normal exposure to HCAs are necessary to study the exposure. For example, protein adducts of PhIP would integrate the exposure over several weeks. An automated method using restricted access materials (RAM) coupled to liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) has been developed and applied to the analysis of globin adducts in blood samples from subjects eating a normal diet, but the adduct levels were below the detection limit of the method (0.1 fmol PhIP/mg globin). Another example is HCAs in urine that are indicative of the exposure for the last 24 h. A high throughput method based on liquid-phase microextraction (LPME) and LC-MS/MS has been used to detect HCAs and some metabolites in urine samples. Successful detection of urinary HCAs after a moderate exposure to cooked foods points out this method as a valuable strategy for future research.

Formation of hazardous compounds during heating of foods

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During heating of foods many chemical reactions take place in which hazardous chemicals can be formed. In the Maillard reaction many different compounds are formed that contribute to colour, aroma and texture. Some of the products of this reaction of amino acids with carbohydrates can pose a risk to humans like the heterocyclic amines with a carcinogenic potential (mainly in meat) as well as acrylamide (foods rich in carbohydrates and asparagine) and even HMF (5-hydroxymethyl-2-furfural) (carbohydrate rich foods, long stored). For these reactions the temperature has to be in the range of about 180 °C to 250 °C for the formation of heterocyclic amines, ca. 180 °C for acrylamide and room temperature to 300 °C for HMF. The concentration of the highly active heterocyclic amines is in the low ng/g range whereas HMF – which has a low DNA binding activity – can occur up to 1 mg/g.

The formation of heterocyclic amines is linked to the presence of creatinine, amino acids, and carbohydrates in the foods. Depending on the distribution of amino acids and carbohydrates the formation of different heterocyclic amines (quinolines, quinoxalines, pyridines) is induced. Using high temperatures and long heating times the concentration of the HAs increases up to a certain point. Especially strongly fried meats (dark brown) can contain extremely high concentrations of these mutagenic/carcinogenic compounds. Many efforts were undertaken to decrease the HA formation while maintaining the well accepted sensory properties of the heated products. These include the use of different (antioxidant) spices and increasing the concentration of residual sugar in the meat. However, the reduction of heat/temperature during cooking is the most efficient way of reducing the HA content. During boiling of meat these HAs are formed at practically not detectable levels.

Recent results of the group of HR Glatt have shown that HMF might be activated with sulfotransferases forming SMF which is a very potential mutagen. Although renal elimination of HMF (mainly oxidation to HMFA and urinary excretion) is very efficient in the healthy population the exposure to HMF can be rather high leading to a cancer risk. The main food groups resulting in a high exposure were identified by T Husøy being beer, coffee, dried fruits, and caramels.

Significant amounts of HMF are formed at practically any temperature during heating or storage from carbohydrates in presence of absence of amino acids. Several possible pathways of formation were published recently. There is currently no legal limit for HMF for health reasons. A detailed investigation of the exposure and possible health implications (e.g. cancer, kidney diseases) in the healthy population and in patients with kidney failure would be necessary to judge in detail the first disconcerting results of the biological activity of HMF.

This presentation will give an overview on the biological relevance, the content in foods, and the ways of formation of these compounds during preparation. Although the Maillard reaction is a common starting point these compounds are formed in different pathways in a later stage of the Maillard reaction.

The effects of various processing conditions on a protein isolate from *Lupinus angustifolius* investigated by different approaches.

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Food industry has an increasing interest for food ingredients with improved technological and nutritional properties: plant proteins, for example, may replace milk and egg proteins in numerous applications. Lupin kernel may provide innovative ingredients, since it has valuable nutritional characteristics, such as useful contents of vitamins and macro/microelements and a protein endowed with satisfactory essential amino acid contents. For its techno-functional properties, lupin can be used as emulsifying and coloring agent, for taste enhancing, water retention, texture improvement, and shelf-life extension.

In addition, recent literature has reported some useful health benefits related to lupin consumption, such as hypocholesterolemic ¹⁻⁵, anti-atherogenic ⁶, and hypotensive ^{3,7} activities.

With the objecting of investigating the consequences of processing on protein integrity, a lupin protein isolate from *Lupinus angustifolius* was treated thermally and mechanically and the effects on the protein profile were determined.

As a preliminary step, a proteome analysis was performed in order to identify the main protein components of *L. angustifolius*. A combination of two experimental approaches was used: a) the canonical proteomic approach including 2D-separation and mass spectrometry analysis of tryptic peptides; b) the "de novo peptide sequencing". Fifty-five out of 57 main spots were found to belong to the major seed protein families: α -conglutin (legumin), β -conglutin (vicilin), γ -conglutin, and δ -conglutin.

The protein degradation was studied via differential scanning calorimetry, 2D-electrophoresis, and mass spectrometry, which enabled the fingerprint of the available peptides after processing. The results indicate that, even after prolonged industrial processing conditions, α -, β - and δ -conglutin are still able to release intact peptides, although they are completely or partially dissociated as shown by the 2D protein profiles and the DSC graphs.

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IL18

Heat stability of anthocyanins from black carrot, elderberry and strawberry

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Color is one of the most important attributes of fruit and vegetable products significantly determining consumers' choice [1]. Thus, synthetic colorants have commonly been applied to improve visual appearance of processed food and to restore initial color shades. In recent years, these are increasingly substituted by their natural counterparts, such as anthocyanins, due to health concerns associated with the use of synthetic pigments. However, the poor stability of anthocyanins as compared to synthetic compounds is their major drawback. For this reason, numerous studies on intrinsic and extrinsic factors affecting pigment stability and color evolution, such as compound structure, temperature, illumination, SO₂, and the pH value have been performed [2]. Since the degradation mechanisms of anthocyanins in the presence of sugars and ascorbic acid are still unknown and contradictory results have been reported in the literature, further investigations on heat induced degradation of anthocyanins and the effect on color and antioxidant capacity were performed.

Juices were prepared from black carrot, elderberry and strawberry concentrate as well as from fresh plant material. Heat stability of anthocyanins was assessed in stress tests by adjusting the pH value (3.5) and heating the solutions at 95 °C for 2 and 4 hours, respectively. The impact of supplemented saccharides (glucose, fructose, sucrose; 50 g/L each) and ascorbic acid (200 mg/L) on pigment stability and color properties were evaluated using HPLC-DAD and UV-Vis spectrophotometry.

Pigment stability and color evolution significantly depended on anthocyanin structure, which can be seen from major differences in the behavior of black carrot, elderberry and strawberry juices upon heating. The changes of the color coordinates L*, C* and h°, which may be expressed as total color difference ΔE^* , of the juices prepared from fresh material were smaller as compared to the juices from concentrates, demonstrating that color stability depends on the genuine food matrix. These differences, which were also observed for anthocyanin half-life values, might be due to the retention of polymeric matrix compounds in the former products. The matrix effect was also seen from the half-life values of black carrot anthocyanins in juice prepared from concentrate, which significantly increased when glucose, fructose, sucrose and ascorbic acid, respectively, were added to the juices. For strawberry and elderberry juices these effects were less pronounced or even opposite, thus, demonstrating the complex interdependencies between pigment stability, structure and matrix composition. The present study may contribute to the development of suitable technological processes for the production of coloring foodstuff and food with improved color stability.

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Vacuum cooking of meat at moderate to low temperatures: physico-chemical changes and formation of volatile flavour compounds

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Masseter muscles from 20 Iberian pigs were vacuum-packaged and subsequently cooked for either 5 or 12 hours, and at two different temperatures (60°C and 80°C). Physico-chemical characteristics (moisture, weight losses, instrumental colour, texture profile analysis, TBARs and histological structure) and volatiles (by means of SPME coupled to GC/MS) from cooked meat samples were studied.

Cooking temperature significantly affected weight losses, moisture content, instrumental colour parameters (L, a* and b*) and all instrumental texture variables analyzed, but did not influence TBARs. Cooking time significantly affected moisture losses and moisture content, but not any of the instrumental colour traits, neither the TBARs. However, this latter factor showed a great influence in most TPA variables considered. In this sense, the interaction cooking time x cooking temperature significantly affected all TPA variables under study, pointing out to the importance of the structural changes in the protein network formed (either that of the myofibrillar and sarcoplasmic proteins or that formed by the denatured collagen) on the physical characteristics of the product. Vacuum packaging of samples did not exert any effect on any of the studied variables.

The profile of volatile compounds was strongly influenced by both cooking time and temperature. As a general rule, compounds from lipid oxidation showed a marked increase when cooking at 80°C for 5h, although a longer cooking time led to a decrease in the observed amounts. On the other hand, the longer the time and the higher the temperature, the higher the amount of compounds coming from degradation of amino acids (either by Strecker or thermal degradation). Some compounds, such as 1-hydroxy-2-propanone, dimethyl disulfide or 2-octanone, were presented only in samples cooked at 80°C, while others, such as 2-acetyl-2-thiazoline, were not in samples cooked at 80°C.

Influence of frying procedure on quality and safety of fried foods

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In Mediterranean countries, including Portugal, frying with oil is a traditional cooking procedure. Fried foods are consumed at home as frequently as away from home, and can be processed either by pan-frying or deep-frying. Deep-frying, which consists in a total immersion of foods in oil, and pan-frying, in which foods are only partially immersed, have different effects on fat degradation, depending on the food, the oil used and the frying conditions.

Fried foods are crunchy, highly palatable, and with unique organoleptic and sensorial characteristics, including flavour, texture and appearance.

Although frying is considered an inexpensive, fast and efficient method for cooking, and a food surface sterilization, fried foods in the Western diet are perceived negatively. However, to date, no overall agreement exists concerning the direct relationship between frying and health risks in an adequate diet and according to normal frying conditions.

Several studies on frying stability of vegetable oils have been performed under real frying conditions (at 180°C, with consecutive cycles of French fries and traditional frozen products). Samples were collected until 25 % total polar compounds was achieved, the single limitation for compulsory discharge within EU. Other evaluated parameters were free fatty acids, fatty acid composition, oxidative stability, vitamin E contents and fried foods acceptance. Deep-frying (domestic and semi-industrial fryers) and pan-frying behaviours were assessed using different vegetable oils (soybean, sunflower, colza, peanut, corn, olive oil and oil blends).

Taking into account the actual knowledge, there are no health concerns associated with consumption of fried foods processed in fats and oils that have not exceeded normal frying conditions.

Food industry, professional societies and academic communities should make an effort to educate consumers, retailers and caterers on proper cooking methods and food handling.

Novel strategies to monitor transfer of processing contaminants across the beer making chain

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**Oral Communications
(OC01 – OC05)**

Increase on extractability of immunostimulatory mannans from coffee residue by a roasting procedure

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A large number of plant derived polysaccharides with immunostimulatory activity have already been reported. Among these are the mannans of coffee residue [1]. Mannans are polysaccharides containing single Galp and Araf residues as side chains of a β -(1 \rightarrow 4)-Manp backbone. Mannans with higher degree of polymerisation and lower content of side chain residues have a tendency to aggregate and become insoluble [2]. Coffee residue mannans are insoluble in water but their partial acetylation rendered them soluble, allowing their handling for possible applications, namely, their use as compounds able to *in vitro* stimulation of murine B and T lymphocytes, as evaluated by the expression of the surface lymphocyte activation marker CD69 [1].

Considering the huge amount of coffee residue produced all over the world, the reutilization of this by-product by its utilization as a source of polysaccharide with immunostimulatory activity is very promising. By extraction of coffee residue with 4 M NaOH solutions, a total amount of 16.5% of mannans can be extracted. In order to improve the extraction, different roasting and alkali procedures were assayed, which allowed to improve the extraction of mannans up to 73%.

To evaluate the structural features of these mannans, they were submitted to a selective degradation by an *endo*- β -mannanase and the oligosaccharides (OS) obtained were fractionated by size exclusion chromatography, using a Biogel-P2. The fractions were analyzed by Electrospray Mass Spectrometry (ESI-MS), allowing to observe dimmers and trimmers and, although in very small amount, some acetylated structures and OS containing arabinose residues. These results allowed to conclude that these samples, even under high thermal and strong alkali conditions, keep acetylated and branched residues.

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Effect of candying on volatiles and cell wall polysaccharides of "Ameixa d'Elvas" plums

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Plums (*Prunus domestica* L.) of a special type of 'Green Gage' variety, "Rainha Cláudia Verde", from Alto Alentejo (South-East of Portugal) can be utilised to obtain a traditional candied plum, "Ameixa d'Elvas" (Fig. 1). This product has a Protected Designation of Origin (PDO) recognized by the European Union. The candying process consists in boiling the intact plums in water for 15 min and then put them in sucrose syrup, which is successively concentrated until 75 °Brix (75 g of sucrose per 100 g of solution). The plums can be consumed with this syrup or, alternatively, can be stored in the syrup until being washed and packed in a solid state.



Fig. 1 – Ameixa d'Elvas plums

The volatile composition of "Ameixa d'Elvas" candied plums includes compounds from 10 chemical groups: acids, esters, furans, aldehydes, alcohols, phenols, ketones, terpenoids, lactones, and alkanes. Nineteen compounds were identified as having a potential individual contribution to the aroma, since the concentration found in the plum pulp is higher than the sensory perception limit for the compound. From these 19 compounds, 11 compounds were detected in "Ameixa d'Elvas" plums headspace: ethyl octanoate, nonanal, 2-methoxy-4-(2-propenyl)phenol, 2-phenylethylacetate, linalool, ethyl benzoate, benzaldehyde, 2-heptenal, hexadecanoic acid, 3-methyl butanoic acid, and β -citronellol. Therefore, these are the would-be impact odourants of "Ameixa d'Elvas", which are associated with sweet, cooked, and fruity odours. This volatile composition reflects the complexity of the reactions and rearrangements that happen during the fruit ripening plus those occurring due to the thermal processing in presence of sucrose. All would-be impact odourants of "Ameixa d'Elvas" fruits, except 2-heptenal, were also detected in the sucrose syrup headspace where the fruits have been submersed. These indicate the occurrence of transference of volatile components from the processed fruits to the syrup during storage, revealing that syrup contribute to the aroma of candied fruits [1].

Plum cell wall polysaccharides are composed mainly by pectic polysaccharides and cellulose. During the boiling step of the processing to "Ameixa d'Elvas" these polymers are degraded and solubilised, which is related with the decrease of cell wall adhesion and loss of firmness of the fruit tissues. However, surprisingly, candied plums showed recovery of the microstructure and texture properties. This recovery might be related to pectic polysaccharides, since they are highly esterified and their gelification inside the fruits in the presence of sucrose seems to be the reason for the recovery of the fruits consistency upon candying. Analysis of the syrup showed also the presence of highly methylesterified pectic polysaccharides, which confirms the diffusion of the cell wall polysaccharides from the fruits to the sucrose syrup during candying [2]. The presence of these pectic polysaccharides increases the syrup viscosity, which explains the retention of the fruit volatile compounds that contribute to its aroma.

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[2] Nunes, C.; Saraiva, J.A.; Coimbra, M.A. *Food Chemistry*, 111 (2008), 538-548.

OC02

Study of the formation of N-(carboxymethyl)fumonisin B1 in a model system of crispy corn bread

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Fumonisin is a family of mycotoxins produced mainly by the maize pathogens *Fusarium proliferatum* and *Fusarium moniliforme* [1] in certain weather conditions. The presence of fumonisins has been demonstrated worldwide in corn cereals and processed corn based food products. FB₁, the most abundant and toxic metabolite, is known to cause a range species-specific toxic responses. It has been associated with a high incidence of esophageal cancer in the Transkei region of South Africa [1] and some provinces of China. Recently, fumonisin B1 was declared a class 2B carcinogen or "possibly carcinogenic to humans" [2].

Some authors have reported that FB₁ present in corn or in cereal products decreases during heat treatment and long cooking times with variable values depending on temperature, exposition time, contamination level and reducing sugar level [3]. Murphy et al. [4] suggested that FB₁ could be detoxified by the formation of a FB₁-reducing sugar adduct (N-carboxymethylfumonisin B1 (NC-FB₁)) using the nonenzymatic browning reaction. Preliminary cell tissue culture test suggested that these adducts were less toxic than FB₁ [4]. Moreover, NC-FB₁ was found to be nontoxic in female B6C3F mice fed with 140 µmol/kg of FB₁ [4]. The aim of this study was to investigate the kinetic of FB₁ degradation and the formation of Maillard's adduct in a model system constituted by crispy corn bread contaminated with 1 mg kg⁻¹ of FB₁ and heated at 160, 180 and 200°C for 3, 6, 10, 15, 20 minutes by and liquid chromatography/electrospray ionization (ESI) tandem mass spectrometry with a triple quadrupole (QqQ) analyzer. The extraction of FB₁ and its adduct was produced by 3g of sample extracted with 20 mL of CH₃OH:H₂O (70:30), and the toxin and its adduct was detected with LC-MS/MS operating in MRM, selecting for FB₁ *m/z* 722 [M+H]⁺ as the precursor ion and *m/z* 352 and 334 as product ions, and for NC-FB₁, the precursor ion was *m/z* 780, and the product ions *m/z* 428 and 410. The reduction of FB₁ is proportional to the increment of temperature and time, with a decrease from 0.96 to 0.30 mg kg⁻¹, and an increase of NC-FB₁ from 0.025 mg kg⁻¹ to 0.100 mg kg⁻¹. The effect on temperature and time was also studied in naturally contaminated crispy corn bread. These findings indicate that heat treatment used for the production crispy corn bread might be a possible way for the detoxification of fumonisin.

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Biomonitoring of urinary metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) following human consumption of cooked chicken.Henrik Frandsen*Technical University of Denmark*

Human risk assessment of exposure to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) through the diet may be improved by conducting biomonitoring studies comparing metabolism in humans and rodents. Eleven volunteers ingested a meal of cooked chicken containing 4'-OH-PhIP and PhIP in amounts of 0.6 and 0.8 µg/kg, respectively and urine was collected for the next 16 hours. The large number of PhIP metabolites was by treatment of the urine samples with hydrazine hydrate and hydrolytic enzymes reduced to three substances, 4'-OH-PhIP, PhIP and 5-OH-PhIP of which the first is a biomarker for detoxification and the last a biomarker for activation. The eleven volunteers eliminated large amounts of 4'-OH-PhIP in the urine. The majority of which could be accounted for by the presence of 4'-OH-PhIP in the fried chicken, showing that PhIP only to a small extent (11%) was metabolised to 4'-OH-PhIP. A larger fraction of the PhIP exposure, 38%, was recovered as PhIP and the largest fraction (51%) was recovered as 5-OH-PhIP suggesting that PhIP in humans to a large extent is metabolised to reactive substances. In rats, less than 1% of the dose of PhIP was eliminated as 5-OH-PhIP, suggesting that human cancer risk from exposure to PhIP is considerable higher than risk estimations based on extrapolation from rodent bioassays.

Chemical changes in foods processed by pressure-assisted thermal processing (PATP)

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Pressure-assisted thermal processing (PATP) is now being investigated as a novel sterilization method for low-acid foods that are heat-sensitive. In PATP, the adiabatic compression/decompression heat increases/decreases temperature almost instantaneously, and the simultaneous application of high pressure (~600-700 MPa) and temperature (~100-120°C) accelerates spore inactivation. Some data are already available in the literature concerning PATP effects on chemical components of foods and are coming mostly from EU researchers as a direct consequence of novel food laws. Changes are analyzed using a reaction kinetics approach including activation volume (V_a) and activation energy (E_a) values. The complex effects of food matrix, pH, dissolved oxygen and presence of antioxidants show that optimization of vitamin, pigment and flavor retention while ensuring PATP microbial and enzyme inactivation will require substantially more chemical reaction kinetics research. This presentation will summarize a review of worldwide efforts of pressure and temperature effects on reaction kinetics in different food matrices.

OC05

