8th Young AGErs Symposium

16th and 17th October 2025 in Berlin

Book of Abstracts

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From field to oven: asparagine as a precursor of acrylamide formation in cereals

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Free asparagine naturally present in cereal grains is a major precursor acrylamide formation in bread and other baked goods. Within the framework of the COST Action CA21149 (ACRYRED), our research focuses on identifying both agronomic and technological factors influencing its occurrence in cereal-based foods — from field management to thermal processing.

An extensive two-year screening (harvests 2023–2024) comprising 945 cereal samples revealed substantial variability in free asparagine concentrations across cereal species, localities, and cultivation systems. Building on these findings, controlled field experiments were established for the 2024–2025 growing seasons to investigate the combined effects of nitrogen and sulphur fertilisation on free asparagine accumulation in rye, oats, and wheat, each represented by two cultivars.

For mor in-depth understanding how these factors influence precursor distribution, selected grain samples were milled into defined flour and bran fractions and analysed for free asparagine by HPLC-MS. Consistent with previous observations, the highest concentrations were found in bran-rich fractions, reflecting the metabolic location of asparagine in the outer kernel layers. Preliminary data indicate that fertilisation regime and cereal could affect both the total asparagine concentration and its distribution between bran and flour fractions.

Baking trials performed with wheat flours and model cereal products supported the link between precursor concentrations and acrylamide formation during thermal processing. Doughs enriched with bran generally showed higher acrylamide levels, reflecting the elevated asparagine content in the outer kernel layers.

The presented results illustrate the importance of joint research involving not only assessment of agronomic conditions and analysis of basic grain parameters, but also investigation of subsequent processing steps enabling better understand of asparagine distribution in various milling fraction thus estimation of risk of acrylamide formation on thermally processed cereal-based foods.

Mechanistical Insights into Browning reactions of Hydroxycinnamic acids in the Maillard Reaction

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Food browning occurs throughout every step of food production, including harvesting, fermentation, thermal processing, and storage. Important contributors are the oxidative conversion of phenolic compounds to colored melanins and the heat-induced conversion of sugars and amino acids to brown melanoidins. Melanin formation can be induced enzymatically, e.g., in freshly cut or damaged fruits and vegetables, and chemically [1]. The latter is associated with an undesired discoloration during the storage of beverages. However, the incorporation of phenolic compounds into melanoidins, which occurs during roasting of cocoa or coffee, is poorly described so far. This might be owed to the fact that heat-induced browning reactions are primarily associated with the MAILLARD reaction despite the evidence that phenolic compounds are vital constituents of coffee melanoidins [2].

To investigate this, hydroxycinnamic acids – a ubiquitous group of phenolic compounds – were heated in binary and ternary reaction systems. Caffeic acid and ferulic acid were heated in equimolar reaction mixtures with key reactants of the MAILLARD reaction, including α-dicarbonyl compounds (glyoxal, methylglyoxal, diacetyl), heterocyclic carbonyl compounds (furfural, 5-hydroxymethylfurfural, pyrrole-2-carbaldehyde), sugars (galactose, arabinose), and alanine. The reactivity was characterized by analysis of the browning (420 nm) and the conversion of the reactants (HPLC-DAD, GC-MS). Changes in the antioxidant properties were monitored using a TEAC assay. Browning intermediates were isolated (preparative HPLC) and structurally elucidated (NMR). Furthermore, the composition of oligomer colorants was elucidated using high-resolution mass spectrometry.

The findings revealed that hydroxycinnamic acids form heat-induced colorants, individually and in combination with the aforementioned melanoidin precursors. In this regard, decarboxylation reactions were identified as the driving force of this novel type of color formation. These reactions are catalyzed in the presence of amines, which subsequently partook in color formation. Furthermore, hydroxycinnamic acid-derived vinylphenols were found to react as donors and acceptors in electrophilic aromatic substitution reactions, enabling crosslinking reactions and thus the formation of heterogenous and conjugated chromophores.

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Plasma metabolomics identifies systemic biomarkers of skin advanced glycation end products (AGEs) not explained by dietary intake: a population-based study

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Background. Advanced glycation end products (AGEs) are generated from intermediate products of glucose and lipid metabolism, such as dicarbonyls. AGEs accumulate over time and contribute to age-related diseases, with risk factors including aging, smoking, diabetes, and impaired renal function. Advances in metabolomics enable the identification of novel biomarkers and pathways associated with AGE formation and accumulation.

Objectives. This study aimed to: (1) identify circulating metabolites and metabolic pathways associated with skin AGEs through metabolomics analysis in a population-based cohort, and (2) explore dietary data linked to these metabolites and their correlation with skin AGEs.

Methods. In the Rotterdam Study, skin AGEs were assessed non-invasively as skin autofluorescence (SAF) using the AGE Reader®. Metabolomic profiling was performed using Nightingale and Metabolon platforms, together measuring nearly 1,300 metabolites. After adjusting for multiple testing correction by FDR, metabolites significantly associated with SAF were identified. Dietary intake of omega-3 and omega-6 fatty acids, including alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), total omega-3, total omega-6 and the omega-6/omega-3 ratio, was assessed using food frequency questionnaires (FFQs). Associations of plasma metabolites and dietary omega fatty acid intake with SAF were examined separately using linear regression models adjusted for demographic, clinical, and lifestyle factors.

Results. Associations between metabolites and SAF were analyzed using the Nightingale (n = 1,910) and Metabolon (n = 845) platforms. In total, five metabolites from Nightingale and 36 from Metabolon were significantly associated with SAF after multiple testing correction (FDR < 0.05). On the Nightingale platform, DHA and omega-3 were inversely associated with SAF, while the omega-6/omega-3 ratio showed a positive association. Among the significant metabolites from the Metabolon platform, 4-vinylcatechol sulfate and 4-vinylguaiacol exhibited the strongest positive associations with SAF. Among 1,581 participants with available FFQs, dietary EPA and DHA showed a suggestive, but non-significant inverse association with SAF (EPA: $\beta = -0.14$, $p = 0.14$; DHA: $\beta = -0.12$, $p = 0.09$). Correlations between dietary intake and corresponding plasma metabolite levels were generally weak, being strongest for DHA ($r = 0.24$, $p < 0.01$) and the omega-6/omega-3 ratio ($r = 0.14$, $p < 0.01$).

Conclusion. In this study, investigating nearly 1,300 metabolites, we found inverse associations of omega-3 and DHA with SAF, whereas the omega-6/omega-3 ratio showed a positive association. Dietary analyses showed a suggestive but non-significant inverse association for dietary EPA and DHA with SAF, and weak correlations between dietary intake and corresponding plasma metabolites. These findings suggest that circulating omega-3 fatty acids may influence SAF accumulation, for example by reducing oxidative stress and inflammation. The weak diet-metabolite correlations may reflect limitations of FFQs, inter-individual differences in fatty acid absorption and metabolism, or other unmeasured factors such as supplement use. Further studies, including replication of our findings, are needed to clarify a potential causal role of fatty acids in AGE accumulation.

Monitoring of Thermal Process Contaminants in Soy-Based Milk Alternatives During Storage

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Cow's milk is nutritious due to its unique properties, but sustainability efforts and ethical concerns are driving demand for plant-based alternatives. Soy is a key ingredient in plant-based milk alternatives (PBMAs) due to its protein content, similar to cow's milk. During the thermal processing of milk, high-temperature leads to Maillard reaction and glycation forming α-dicarbonyl compounds leading to the formation of the thermal process contaminants like fructosyl-lysine, N-ε-carboxymethyllysine (CML), N-ε-carboxyethyllysine (CEL). Similar to cow's milk, the production of soy-based milk alternatives (SBMAs) undergoes ultra-high temperature (UHT) treatment process that could cause thermal damage. In this study, different SBMAs and one UHT cow's milk samples bought from a local market in Türkiye were investigated for the formation of glycation markers during six months of storage at room temperature. Furosine, an early-stage glycation marker, was found at substantially higher levels in UHT cow's milk (3.07±0.14 – 4.37±0.90 g/kg protein) compared to SBMAs (0.34±0.01 – 1.78±0.16 g/kg protein). Over six months of storage, furosine content significantly decreased in all samples. As an advanced glycation marker, CML concentration increased significantly after six months of storage, showing a rise from 48.9±10.53 – 96.8±12,9% for SBMAs and 152.7±24.3% for UHT milk. Meanwhile, CEL concentrations in SBMAs approximately doubled within two months. The CEL content in cow's milk (11.43±1.3 – 15.7±1.14 mg/kg protein) was found to be lower than in SBMAs (27.09±7.18 – 94.97±6.05 mg/kg protein). The decreasing content of 3-deoxyosone (3-DG) in all samples, glyoxal (GO) in SBMAs and methyglyoxal (MGO) in SBMA-1 and -2 are related with the increasing formation of AGEs in all samples. On the other hand, there was no statistically significant change in the total lysine content after six month of storage. These results indicate that thermal processing of SBMA causes the Maillard reaction, leading to the formation of both early- and advanced-stage glycation products. During the six months of storage, glycation reactions continue, resulting in a decrease in early-stage markers, furosine and α-dicarbonyl compounds, and an increase in advanced glycation end products, i.e. CML and CEL.

Optimization of extraction for the determination of acrylamide in coffee products

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Thermally processed foods for acrylamide analysis are mostly solid foods, however acrylamide is not distributed homogeneously. For this reason, it is necessary to use a larger quantity for appropriate analysis. Larger sample quantities significantly slow down the extraction of the acrylamide from the sample, and the preparation time itself has a considerable impact on the overall duration of the analysis. In this study, the ISO 16618:2015 method was modified by optimizing the extraction procedure to improve acrylamide extraction and reduce the sample preparation time for analysis. The introduced modification refers to a triple-stage extraction, which is necessary for the complete extraction of acrylamide from the sample. By using Carrez clarification, the need for preliminary sample purification using cleanup columns can be eliminated, allowing the prepared sample to be directly extracted using solid-phase columns. In order to assess the efficiency of this modification, various types of coffee products were tested to evaluate whether there is a significant difference in acrylamide concentrations across different preparation methods. A total of 35 samples were divided into five categories: roasted coffee and coffee beans, instant coffee, a mixture of ground roasted coffee in capsules and filter coffee. The highest acrylamide concentrations were observed in roasted coffee, with levels ranging from 254 to 739 μg/kg, as well as in instant coffee, where regulatory limits are higher, resulting in concentrations of around 700 μg/kg. In contrast, other forms showed significantly lower concentrations, ranging from 50 to 323 μg/kg. Analysis of the same samples with the optimized extraction procedure showed increased acrylamide concentrations — in some cases by up to 20%, while in most samples the results were about 7% higher compared to those obtained by the ISO method. In this way, satisfactory reproducibility of results was achieved, along with a significant reduction in analysis time, through the shortening of the sample preparation process and the elimination of purification columns, which also considerably reduces the overall cost of the analysis. Improving overall extraction from the matrix is particularly important for borderline samples, where a slight concentration difference can determine whether the sample is suitable for human consumption. Given that these types of products are consumed daily, determining acrylamide and evaluating the potential risk associated with consuming coffee products are significant for human health.

Acknowledgements: This research was supported by the COST Action (European Cooperation in Science and Technology) CA21149 Young Researcher and Innovator Conference Grant, Grant
period 15/10/2025 - 17/10/2025, E-COST-GRANT-CA21149-c9ea9a8cnce

The Effects of Maillard and Caramelisation Reactions on the Sweetness Perception of Biscuits

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Sugar is not only one of the major components that affects taste and technological properties, such as texture, but also primary substrate for reactions occurring during thermal processing of bakery products, directly influencing the formation of volatile compounds, colour, and taste substances. Therefore, the sugar content has a direct impact on both technological properties of the product and consumer acceptance, reducing sugar in baking goods is a highly challenging issue. Flavouring substances are one of the promising approaches to reduce sugar in bakery products, and Maillard and caramelisation reactions are the primary ways to form flavour compounds in heat-treated foods. This study investigated the differences in sweetness perception by adding caramel and enhancing the Maillard reaction in biscuits, as well as their relationship with aroma compounds.

The caramel-added and Maillard reaction-boosted biscuits were baked with minor modifications to the AACC 10.54 biscuit recipe. For caramel-added biscuits, 5 g of the icing sugar was replaced with caramel and Maillard reaction-boosted biscuits were prepared by replacing 5 g of refined wheat flour with calcium caseinate. Each type of biscuit was baked for 7, 11, and 13 min. Sensory analysis was conducted using a line scale and untrained panellists to observe the changes in sweetness perception of different recipes at varying baking times. Volatile compounds were analysed to explain differences in perceived sweetness resulting from the formulation changes by SPME-GC-MS.

As a result, the Maillard reaction and caramel were found to increase the perceived sweetness of biscuits for all baking times; however, this effect is more distinct in Maillard reaction-boosted biscuits. The addition of caramel to the formulation increased the levels of sweetness-related volatile compounds such as Strecker aldehydes, diketones, and furan derivatives. It was considered that the lower sweetness perception of caramel-added biscuits compared to MR-boosted biscuits might be associated with taste molecules. Moreover, an increase in sweetness scores for all types of biscuits was observed when the baking time was extended from 7 minutes to 11 minutes due to the increase in pyrazines, Strecker aldehydes, furan derivatives, lactones, and pyrroles. Despite this, extending the baking time from 11 min to 13 min decreased the sweetness perception, which may be caused by the formation of bitter taste molecules in the advanced stages of MR and caramelisation. In conclusion, the Maillard and caramelization reaction products, as they enhance the aromas linked to sweetness, could be added to the biscuit recipe to reduce sugar.

Vertical farming meets novel processing techniques for reducing thermal process induced contaminants in native Chilean potatoes

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The *Chilotanum* potato group from southern Chile, notable for its diverse colored tubers offer potential for developing innovative processed food products. Colored vegetable chips, especially those made from native colored potatoes, are gaining popularity and availability as alternatives to conventional snacks. However, the processing methods employed for these products, such as frying, baking, and extrusion, often involve high temperatures, similar to conventional potato-derived snack production. Consequently, these processes can lead to high fat content and the formation of thermal process contaminants like acrylamide, which can compromise the nutritional quality and chemical safety of the final product. Developing safer thermal processed foods involves managing raw material composition. High levels of reducing sugars and free amino acids, particularly asparagine, in raw potatoes increase the formation of NFCs (Acrylamide, furanoids) and dietary AGEs (CML, CEL). Strategies to lower these precursors include cultivar selection, breeding, gene editing, optimizing agronomic practices and adequate postharvest handling, which significantly influence tuber composition. However, potato production faces challenges from climate change (extreme temperatures, resource scarcity) and urbanization (reduced land and labor), threatening conventional and native varieties. In that context, vertical farming emerges as a potential solution, offering controlled, resource-efficient production that can preserve biodiversity, enhance crop quality consistency, and meet the demand for healthy food. Furthermore, novel processing technologies, like microwave heating, have potential for improving final product quality. Microwave heating can help reducing oil uptake and processing time in fried snacks while maintaining sensory quality, enabling the development of lower-calorie products with lower levels of undesirable compounds.The effects of growing factors under a vertical farming production system (e.g.: light spectrum and nutrient solution salinity) in native potato processing suitability have not been evaluated yet. And so do the effects of novel processing technologies on thermal process derived contaminants, sensory and nutritional quality of colored snacks. In this proposal we postulate that the modification of crucial growing factors for improving potato quality along with novel processing technologies like microwave heating can produce lower-calorie, chemically safer snacks from colored native potatoes. Therefore, the main objective is to evaluate the effect of the growing conditions (light spectrum and nutrient solution salinity) on the productive traits, sweetening potential and processing quality of two native potato genotypes cultivated in a vertical farming system followed by the application of novel and conventional processing technologies to further improve the chemical innocuity, without affecting the physicochemical and sensorial characteristics of their derived processed products. We expect that by applying this “from farm to fork” approach, we will be able to find suitable conditions for the production of potatoes in vertical farming systems and an innovative process to produce healthier snacks with good sensorial characteristics and reduced amounts of NFCs and dietary AGEs. This in turn will be very valuable for the main stakeholders in the potato production chain, from producers to food processors and to the final consumers.

Formation of color and glucose degradation products in peritoneal dialysis fluids depending on sterilization time

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Peritoneal dialysis is an important type of renal replacement therapy in which the peritoneum serves as a natural dialysis membrane. [1] Peritoneal dialysis fluids (PDFs) commonly contain glucose as an osmotic agent, electrolytes, and a buffer such as lactate. To reduce the risk of bacterial infections, PDFs are usually heat sterilized. [2] This leads not only to the formation of glucose degradation products (GDPs), but also to the browning of the solutions [3], which is considered a lack of quality and can lead to rejection of the PDF by the patient.

In order to better understand the molecular processes behind the color formation during sterilization, PDFs containing 4.25 % glucose were subjected to different sterilization times. Afterwards, the concentrations of glucose, fructose, lactate and formic acid (FA) were determined by enzymatic assays. Concentrations of the α-dicarbonyls glucosone, 3-deoxyglucosone (3-DG), 3-deoxygalactosone (3-DGal), glyoxal, methylglyoxal (MGO) and 3,4-dideoxyglucosone-3-ene (3,4-DGE) were analyzed by ultrahigh-performance liquid chromatography coupled with diode array detection (UHPLC-DAD) after derivatization with o-phenylenediamine. Concentrations of the monocarbonyls formaldehyde, acetaldehyde, 5-hydroxymethylfurfural (5-HMF) and furfural were determined by UHPLC-DAD after derivatization with 2,4-dinitrophenylhydrazine. Furthermore, the pH value, the absorbance at 278 nm and the color parameters in the CIELAB color space (Commission Internationale de l'Eclairage, model with coordinates L*, a*, b*) were determined for all samples. Subsequently, changes of these parameters with sterilization periods were analyzed.
Longer sterilization times decreased the concentrations of glucose and glucosone and increased the concentrations of fructose, 3-DG, 3-DGal, glyoxal, MGO, 3,4-DGE, formaldehyde, acetaldehyde, 5-HMF, furfural, and FA. Of these, furfural, 5-HMF, and formaldehyde show the largest relative increase. Lactate concentrations did not change. Additionally, longer sterilization leads to solutions with lower pH values, higher absorbances at 278 nm and darker, slightly greener and considerably yellower coloration.

To observe not only how PDFs change with longer sterilization times, but also the direct influence of analytes on the color formation during sterilization, an additional experiment was conducted. Here, PDFs were individually spiked with fructose, FA, glucosone, 3-DG, 3-DGal, glyoxal, MGO, 3,4-DGE, formaldehyde, acetaldehyde, 5-HMF, and furfural, followed by color measurements before and after sterilization. 3,4-DGE had the strongest influence on the color formation leading to the highest intensity for darkness, red and yellow color. 3-DG and 3-DGal had the same effect, but to a lesser extent. Fructose made the PDFs yellower and darker but had no effect on the green-red axis. FA reduced the yellowness of the PDFs but had no effect on the darkness and the green-red axis. GO, MGO, acetaldehyde, furfural, glucosone, formaldehyde, and 5-HMF had little to no effect on the coloration of the PDFs. Therefore, it

was concluded that, of all the GDPs investigated, particularly 3,4-DGE and to a lower extent also 3-DG and 3-DGal play the most important role in the formation of brown structures in PDFs during sterilization.

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Maillard reaction compounds in fermented pea

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In response to the transition toward more sustainable dietary practices, fermentation offers an alternative to the process intensive plant-based foods derived from protein extracts. Furthermore, fermentation can increase the quality and bioavailability of protein, due to the degradation of complex proteins into smaller polypeptides, peptides and free amino acids (Feng et al., 2024). Some microbes have shown the ability to produce the highly reactive α-dicarbonyls but also possess enzymes that can reduce α-dicarbonyls (Akinrimisi et al., 2025). Thus, fermentation might increase precursors in the Maillard reaction, due to protein degradation and α-dicarbonyl production.

Given the recent emergence of fermented plant-based foods, research into the formation of Maillard reaction compounds within these products remains limited. This study aims to quantify advanced glycation end-products (AGEs) in solid-state fermented peas inoculated with *Aspergillus*, *Rhizopus*, or *Bacillus* species. In addition, a commercial product was included for comparison. The four products were oven-heated for 20 minutes at 190 °C before being freeze-dried and homogenised. Maillard reaction compounds were analysed using LC-MS equipped with a HILIC column enabling the simultaneous quantification of eight Maillard reaction compounds (Akillioglu & Lund, 2022).

Preliminary results showed that the most abundant compounds were methylglyoxal-derived hydroimidazolone isomers (MG-Hs), ε-carboxymethyl-lysine (CML) and furosine. Although microbial species influenced AGE formation, the heating parameters prior to fermentation had a greater impact, with the highest amounts of AGEs found in autoclaved peas.

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Non-invasive measurement of hair AGEs levels as markers for metabolic abnormalities in diabetic rats

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Introduction: Continuous metabolic monitoring is important for assessing the risk of lifestyle-related diseases. However, visiting the hospital periodically for collecting blood samples and assessment is challenging. In Japan, approximately 35% of people are suspected to have diabetes, but they do not receive therapy because they do not have time to visit the hospital. Non-invasive methods using skin or hair are useful tools for diagnosis, and some AGEs levels have been detected in non-invasive samples such as skin. However, research on measuring AGEs levels in hair is scarce, and the AGE precursor fructoselysine has been detected in hair samples. Therefore, we hypothesized that hair AGEs levels could be used to detect diabetes in rats.

Method: We established a non-invasive method for measuring the levels of AGEs [*N*$^{\varepsilon}$-(carboxymethyl)lysine (CML), *N*$^{\varepsilon}$-(carboxyethyllysine) (CEL), and methylglyoxal-derived hydroimidazolone-1 (MG-H1)] in hair with ESI- Quadrupole Time-of-Flight-Mass spectrometry (QTOF) using internal standards. Individual hair was collected 1 cm from the root of the rat, and 5 hairs were hydrolyzed, cation exchange, and analyzed. Hair and serum AGEs levels were measured in non-diabetic(control) and diabetic (DM) rats induced by streptozotocin.

Result: All AGEs and internal standards from hair were detected using QTOF. Hair CEL and MG-H1 increased in DM rats, however, hair CML, and all AGEs from serum did not change in DM rats. To evaluate whether easily obtainable hair samples could effectively distinguish diabetes status with receiver operating characteristic (ROC) analysis. Both hair CEL and MG-H1 in ROC showed that the area under the curve (AUC) values were 1.

Discussion: Those results showed that AGEs levels were detected from hair samples, and hair AGEs levels were higher than serum AGEs levels. From ROC analysis, hair CEL and MG-H1 levels completely distinguished between DM and control rats. This suggests that hair AGEs levels can be used to detect the risk of diseases such as diabetes, and are a useful tool for continuous metabolic monitoring.

Unveiling the complexity of Advanced Glycation End products (AGEs): A new HILIC MS/MS method for simultaneous analysis of 19 AGEs and their coeluting isomers in food model systems

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Advanced Glycation End products (AGEs), a group of process-induced contaminants, have garnered significant attention in food safety research [1]. They are formed through the Maillard reaction, a non-enzymatic process occurring in both food systems and the human body. This reaction involves the glycation of reducing sugars with amino groups and is induced by heat. AGEs play a dual role in food and health: while contributing to the flavor and quality of processed foods, excessive accumulation in the human body is potentially linked to adverse health effects [2]. The increasing shift toward a Western-style diet, characterized by high consumption of ultra-processed foods, sugar-sweetened beverages, and fructose-rich sweeteners, further elevates dietary AGE (dAGE) exposure [3] . Earlier work from our laboratory has shown that dietary AGEs can induce an inflammatory response in human macrophage-like cells [4]. Therefore, the development of improved methods for the accurate quantification of AGEs in food and biological samples is crucial.

From an analytical perspective, the detection of AGEs presents significant challenges due to their high polarity, which complicates retention in chromatographic analysis. Moreover, some AGEs exist in various isomeric forms, and separating these isomers chromatographically has been an unmet challenge until now. While previous methods, such as reversed-phase liquid chromatography (RPLC), have provided high detection accuracy, they have not succeeded in separating AGE isomers or resolving issues related to retention without using mobile phase additives like ion-pairing agents and/or serious co-elution effects [5].

This study addresses these analytical difficulties by developing a novel hydrophilic interaction liquid chromatography (HILIC)-based method that successfully achieves both retention and isomer separation of AGEs. Specifically, the method enables the simultaneous detection of 19 AGEs in a single run. The method includes AGEs derived from arginine, lysine, and cysteine, as well as complex cross-links between lysine-lysine and lysine-arginine residues. To enhance chromatography, four different HILIC columns, the composition of the mobile phase, the gradient profile, run-time and temperature were assessed and optimized to achieve high resolution and symmetric peak shapes. Subsequently, the developed method was effectively applied to standard solutions and food model systems (i.e. a protein and reducing sugar mixed and heated to mimic food processing conditions).

Looking ahead, these data suggest that, after method validation, this HILIC chromatography approach may be applied not only to food model systems, but also to real food and biological samples. This can substantially advance the database on AGEs occurrence in foods under processing conditions, allowing more accurate exposure assessments. To further advance the scientific quality of Maillard research, it would be beneficial to measure various AGEs in target organs or providing conclusive evidence that the compounds reach reaction sites, such as the kidneys [6].

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Functional Food Development through Maillard Reaction: Electrolyzed Watermelon Rind in Wheat-Based Flatbread

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Fruit and vegetable processing produces massive amounts of bio-waste, such as peels, seeds, stones, and unused flesh. These by-products have the potential for utilization in food products as they are rich sources of protein, fiber, vitamins, minerals, and bioactive compounds. Watermelon (*Citrullus lanatus*) is rich in bioactive substances, healthy tropical fruit that belongs to the Cucurbitaceae family. It is primarily grown in Asia (79.5%), Africa (7.5%), and America (6.9%). It is low in salt and calories, possesses sweetness, thirst-quenching, nutritive, refreshing taste, high water content, and appealing colors ranging from red to pink to yellow. Moreover, its byproducts' majorly rind is rich in bioactive compounds, phytochemicals, nutrients (arginine, citrulline, aspartic acid, ascorbic acid, beta-carotene, citrulline, leucine, and glutamic acid). Rinds are still discarded as bio-waste, even though they possess good nutritional potential and bioactives with therapeutic effects. Electrolysis technology was performed to improve the consumer preference and quality of watermelon rind (WR) incorporated into food products as a functional ingredient. In this study, wheat-based unleavened flatbread (UFB) was prepared by the incorporation of electrolyzed watermelon rind flour (WMRF) in ratios of 0%, 5%, 10%, 15%, and 20%, which increased ash, fiber, and essential minerals, as well as the functional, microstructure, thermal and antioxidant properties of UFB. Electrolysis technology was performed to achieve the sensory score of WR consumption and to improve its incorporation in food products as a functional ingredient. In-vitro protein digestibility showed an increase in the electrolyzed WMRF ratio, which resulted in a decrease in the protein digestibility. Moreover, the browning, color and flavor changes with the increase in WMRF ratio showed a significant difference in UFBs due to the Maillard reactions (MRs). These MRs not only contribute to browning but influence the thermal and functional properties. These findings demonstrate the role of Maillard reaction in potential use of electrolyzed WMRF as a functional ingredient in food products, offering improved nutritional and functional characteristics. Utilization of rind part will help to reduce agricultural by-products waste, providing nutrition to mitigate malnutrition and promote food security to meet sustainable development goals.

Promoting Youth Food Safety: An Educational Approach Based on Knowledge, Attitudes, and Practices (KAP)

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Introduction: Food security encompasses not only the availability of food but also its safety, hygiene, and compliance with health standards across all stages of the food chain, from production to consumption. Recent global studies increasingly identify food safety as a critical public health concern. Adolescence, a pivotal stage for shaping lifelong dietary habits, has drawn particular attention in this regard. According to the Food and Agriculture Organization (FAO, 2019), food security includes both the adequacy and safety of food. Education and awareness among young people are essential for preventing foodborne illnesses and promoting long-term health. Prior research has consistently shown that adolescents' knowledge, attitudes, and behaviors toward food directly influence their risk of obesity and other chronic conditions (Karimi et al., 2018; Lee et al., 2021).

Aim: This study aims to evaluate the knowledge, attitudes, and practices (KAP) of high school students regarding food safety. Specifically, it explores adolescents' understanding of food safety principles and identifies potential gaps between knowledge and practice. The findings are intended to inform the development of targeted educational interventions that promote healthier and safer food-related behaviors among youth (Almeida et al., 2017; Muhammet & Ozturk, 2019).

Materials and Methods: A quantitative research design was employed, using structured questionnaires distributed to a selected sample of secondary school students. The survey instrument was designed based on standardized methodologies recommended by international agencies such as the FAO, WHO, and UNICEF. It included items assessing theoretical knowledge of food safety, attitudes toward hygienic practices, and daily food-related behaviors. This model has been widely used and validated in prior studies on nutrition education among school-aged populations (Santos et al., 2018; Chen & Zhang, 2020).

Results and Discussion: Findings from the literature and initial analysis suggest a mismatch between students' knowledge of food safety and their real-life practices. While many demonstrate theoretical awareness, actual behaviors are often shaped by external influences such as family traditions, school environments, and cultural norms (Brown et al., 2019). These disparities underscore the importance of implementing context-specific and behaviorally focused education programs that extend beyond knowledge acquisition to promote consistent, healthy practices.

Conclusion: This study presents a nuanced view of adolescent food behavior, where biological, social, cultural, and economic factors collectively shape eating habits. While the majority of students were found to be within a healthy weight range and demonstrated relatively balanced habits, a subset of vulnerable individuals exhibited signs of nutritional risk, either underweight or overweight. These dual challenges demand targeted attention. The findings highlight the urgent need to incorporate structured, evidence-based nutrition and food safety education within school curricula. Such programs must aim not only to inform but also to empower students to make healthier and safer food choices. These recommendations align with the International Association for Nutrition Education (IAFNE, 2023) and support broader calls for proactive strategies in adolescent food literacy and health promotion (Peterson et al., 2020).

Investigating Maillard Reaction Pathways in Plant-Based Foods

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The Maillard reaction is one of many chemical processes that take place in food. It contributes significantly to the development of colour, aroma and texture and at the same time leads to the formation of a variety of complex Maillard reaction products (MRPs), which may have positive properties on the one hand, but can also influence nutritional value on the other.

While the Maillard reaction in animal-based foods such as dairy and meat products has been extensively studied, there have been few systematic studies on plant-based foods to date. With the growing importance of plant-based alternatives, this field of research is becoming increasingly relevant.

The aim is to investigate the formation of MRPs in plant-based foods in more detail and to find out how protein source, sugar composition and processing influence the reaction. The focus is on commercial plant-based products, whose changes during processing are observed. Model approaches can be used to supplement this in order to examine individual factors in a targeted manner.

A wide range of analytical methods is used. Selected MRPs are quantified using liquid chromatography coupled with mass spectrometry (LC-MS). An established HPLC-UV method is used to determine the amino acid composition. Reducing sugars can be examined after derivatisation using capillary electrophoresis (CE) or HPLC-RI. In addition, both raw products and processed foods will be examined in order to characterise the changes in MRPs during processing. If necessary, real manufacturing processes can also be included in collaboration with manufacturers.

It is expected that MRP formation depends on the amino acid composition of plant proteins, the type of sugars and the respective processing conditions. The planned investigations should show how these factors influence the formation of certain MRPs and whether there are differences between different protein sources. The results should contribute to a better understanding of the Maillard reaction in plant-based foods.

LC-MS/MS peptide profiling of differently extracted pea protein isolates to reveal non-enzymatical post-translational protein modification

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The market for plant-based food is estimated to grow [1]. The reason for this is increasing interest of consumers to eat more sustainably and to follow vegan/vegetarian diets. For the production of a wide range of plant-based alternatives, pea protein is used. The pea proteins undergo several processing steps during their production, such as alkalization, isoelectric precipitation or spray drying. During these processes, multiple protein modifications, such as early and advanced Maillard products, deamidation of asparagine or lysine oxidation can occur which can significantly affect the biological value of the proteins. Furthermore, protein modifications contribute to the techno-functional properties of these isolates. For example, higher acetylation rates lead to increasing oil binding capacity.

The aim of the study is a comprehensive profiling of non-enzymatical post-translational protein modifications in pea protein. Different extraction procedures based on alkaline extraction followed by different steps of purification such as ultrafiltration, ultrafiltration followed by diafiltration, ultrafiltration followed by thermal precipitation and isoelectric precipitation as well as salt-induced extraction followed by dilutive precipitation were carried out. Each protein isolate was neutralized prior to spray-drying and the resulting protein isolates were analyzed for their protein modifications. For this purpose, we established a LC-MS/MS method for a bottom-up proteome analysis [2] of pea protein modifications. Therefore, proteins were enzymatically hydrolyzed and the resulting peptides were quantified via LC-MS/MS. Prior to injection, the peptide contents of the digested samples are measured colorimetrically at 480 nm. The results from MS measurement are analyzed with PEAKS Online.

For each pea protein sample roughly 650 peptides were identified, of which one third were modified with at least one modification. The most common modification is deamidation of asparagine and glutamine, followed by carboxymethylation of lysine, hexose adducts on amino acids with nucleophilic side chains and acetylation of lysine. Maillard reaction products, for instance fructosyl-lysine, formyl-lysine, carboxymethyllysine, carboxyethyllysine, methylglyoxal-hydroimidazolone and 3-desoxyglucosone-hydroimidazolone were detected but represent only a part of all modifications of pea protein. Longer storage time and contact with air resulted in oxidation of arginine residues and methionine. Also, different extraction procedures lead to different levels of oxidation due to the influence of chemicals or heat.

The presented LC-MS/MS method and subsequent data evaluation allow a better understanding of how the different extraction procedures affect protein modifications like oxidation and glycation in qualitative ways. With the presented method, Maillard reaction products can be monitored comprehensively also in other food products.

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Formation of Intermediates and Colorants during Non-Enzymatic Browning in Model Reactions: Influence of Amino Acid and Sugar Composition

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During the formation of melanoidins, aldol reactions are considered the key formation mechanism of such high-molecular-weight, nitrogen-containing colorants. The sugar degradation product methylglyoxal (MGO) is an essential precursor for color formation due to its two electrophilic carbonyl groups and its C-H-acidic methyl group, which make it highly reactive and prone to undergo aldol reactions. [1] It is postulated that the kind of amino acid influences the the color formation and conversion of educts in binary reation systems. [1] To better understand the impact of the amino acid in the Maillard reaction of MGO, binary reaction systems of MGO with one of four different amino acids were investigated. At the same time, the type of sugar compound influences the profile of (intermediate) reaction products. To better understand this impact, honey and sugar syrups with varying fructose and glucose contents were analyzed. Although aldol reactions of sugar degradation products and dicarbonyl compounds like MGO are considered the key formation mechanism of melanoidins, the detailed structures of specific precursors involved in color fomration remain unclear. Improving this understanding requires studying how both amino acids and the type of sugar compound influence the range of intermediate compounds and final colorants.

Model Maillard systems were created by incubating MGO with alanine, proline, glutamic acid, or phenylalanine at 100 °C and pH 5 for up to 300 minutes. Intermediates from these reactions were isolated by preparative HPLC and their structures characterized using HRMS and NMR. Melanoidins were purified by dialysis (>12 kDa cutoff) and assessed for browning (absorbance at 420 nm), repetitive structural motifs (HRMS), and antioxidant properties (TEAC assay). In adddition, honey and sugar syrup samples were heated at 180 °C for up to 30 minutes to investigate the influence of the sugar compound. During heat treatment the samples were monitored for changes in fructose, glucose, and maltose content (HPLC-DAD/RID), browning (absorbance at 420 nm), changed in molecular weight (size exclusion chromatography), as well as formation of α-dicarbonyl compounds and heterocyclic intermediates (HPLC-DAD).

The contribution of high-molecular-weight products to the browning in aqueous solution strongly depends on the amino compound, ranging from 5% (MGO/proline) to 44% (MGO/alanine). These differences occur despite a comparable melanoidin yield of 2–4 wt% of dry mass. TEAC-assay showed that the melanoidin fraction of MGO/alanine contributes approximately 5% to the antioxidant activity of the entire mixture, corresponding with its weight proportion. Additionally, the most quantitatively relevant intermediates were isolated to reveal the structures and formation mechanisms of novel pyrrole and pyridine derivatives. During the heat treatment of honey and syrups, the increase in antioxidant activity correlates with the increasing browning. The α-dicarbonyl profile was closely linked to the starting fructose and glucose content; 3-deoxyglucosone was generally the most abundant, but 2-deoxyglucosone was newly detected as a relevant intermediate in heat-treated honey.

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Integrating Field and Molecular Approaches to Low-Asparagine Wheat: Outcomes of an ACRYRED Short-Term Scientific Mission

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Acrylamide is a process contaminant classified as a probable human carcinogen. It forms in wheat and other carbohydrate-rich foods during high-temperature processing, where free asparagine is a major precursor. One promising strategy to reduce acrylamide formation in food is to lower the concentration of free asparagine in the raw material, particularly wheat grain. My Short-Term Scientific Mission (STSM), funded by the COST Action ACRYRED (CA21149), was hosted at Rothamsted Research in the United Kingdom and aimed to explore a multidisciplinary approach to this problem by integrating agronomic, biochemical, and molecular biology techniques.

Over the three-week period, I participated in the full pipeline of low asparagine wheat research. Field activities included threshing and post-harvest processing of wheat samples, as well as straw collection from experimental plots. Straw was treated as a valuable biomass component, providing information on harvest index, nutrient allocation, and cell wall composition. This supported the biological validation of remote sensing indices such as NDVI and canopy structure obtained from UAV and ground-based platforms.

In the laboratory, I was trained in high-performance liquid chromatography (HPLC) for amino acid profiling, focusing on lysine and other essential amino acids in wheat grain. I also determined dry matter content in low asparagine wheat lines by weighing, drying, and reweighing grain samples, confirming consistency in sample moisture levels prior to further biochemical analysis.

The final phase of the STSM involved molecular biology work in Rothamsted's Crop Transformation and Genome Editing Unit. I extracted DNA from genetically modified low asparagine wheat lines and performed polymerase chain reaction (PCR) using primers specific to the asparagine synthetase genes located in the A, B, and D genomes of bread wheat. PCR results confirmed the presence of mutations linked to reduced asparagine levels in lines L59 and L23. Additionally, I screened genetically modified Brassica napus material for the FAE construct, with positive PCR amplification confirming successful transformation. I also participated in a wheat particle bombardment experiment, gaining first-hand experience in transformation techniques.

Failure of a Gold Standard: Uncovering the Critical Blind Spots of the Low method for Fruit Juice Adulteration

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The integrity of our food supply relies on robust and reliable analytical methods to detect economic adulteration. In the fruit juice industry, the "LOW method", a GC-FID based approach, has served for decades as the benchmark for identifying the illegal addition of invert sugar syrups. Despite its long-standing acceptance, its suitability has rarely been challenged in the context of today's diverse food matrices and the wide array of modern, low-cost sugar syrups used for adulteration.

This study presents a systematic re-evaluation of this cornerstone analytical method. We assessed its performance by analyzing authentic fruit juices with varying matrix complexity (apple, orange, etc.). These samples were spiked with both traditional invert sugar and, more importantly, modern alternative syrups such as rice and corn syrup. The investigation critically examined the method's vulnerability to chromatographic challenges, detectability of marker substances, and process variability for identifying adulteration.

Our results demonstrate three fundamental weaknesses that invalidate the method for modern quality control: First, the reliable quantification of target markers is impossible in real juice matrices due to severe co-elution with natural matrix components or occurrence of marker substances. Second, the method exhibits critical blind spots, proving systematically unable to detect common adulterants like rice and corn syrup, even at high concentrations. Third, the recovery of the analytical markers are highly dependent on the derivatization procedure, confirming a profound lack of method robustness.

In conclusion, this widely used method is unfit for its purpose in the modern food landscape and creates a false sense of security for regulators and consumers. Our findings underscore the urgent need to replace outdated analytical standards with more selective and powerful technologies, such as high-performance liquid chromatography coupled to mass spectrometry, to ensure the effective detection of food fraud and guarantee product authenticity.

Maillard Reaction Products and Inflammatory Bowel Disease: Effects of Dietary AGEs on Intestinal Permeability

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Inflammatory Bowel Disease (IBD) is a chronic relapsing-remitting inflammatory condition which is also characterised by an impaired intestinal barrier. Its prevalence is rising, which is mainly seen in developing countries, coinciding with a shift to a Western lifestyle. As diet is associated with the onset and development of IBD, this relationship warrants a closer look. The Western diet consists of foods high in sugar and protein that are processed under high temperatures before intake. This results in foods containing high amounts of Maillard reaction products (MRPs) and specifically Advanced Glycation Endproducts (AGEs). AGEs can also be formed endogenously and are linked to systemic inflammation by activation of the Receptor for AGEs (RAGE). RAGE activation can also impair the intestinal barrier function, leading to inflammation. The current research investigates whether dietary MRPs can increase intestinal permeability, using a well-established *in vitro* cell culture model.
Caco-2 monolayers (passage 30-40) were differentiated for 15 days. They were exposed for two hours to two concentrations (33% and 100%) of a model system for dietary MRPs (dMRP-model). This model system consisted of a combination of one protein (casein, whey, lentil or soy protein) and one sugar (fructose, glucose, lactose or sucrose), heated at 100°C for two hours. Each dMRP-model had a matched, unheated, control. Percent change in Trans Epithelial Electrical Resistance (TEER) after exposure was recorded and FITC-dextran permeation across the monolayers was measured, as indicators for intestinal permeability.

After 33% exposure, while some MRPs showed a lower TEER value, only lactose-whey significantly reduced TEER compared to its unheated control (mean difference=68.5%, $p=0.0004$). When comparing 33% exposure of dMRP-models to the medium control, no significant differences in change in TEER could be seen. At 100% exposure, almost all dMRP-models (except for fructose-whey, glucose-soy, lactose-soy and the lentil combinations) significantly decreased TEER values, both compared to their unheated controls and the medium control (mean differences ranging from 60 to 111%, $p<0.001$). For the other dMRP-models, lower TEER values could be observed, but this change was not significant. These same effects could be seen for FITC-dextran permeation. At a 33% exposure, no dMRP-models significantly increased intestinal permeability compared to their unheated controls or the medium control. At 100% exposure, most dMRP-models showed higher permeability than their unheated controls, with significant differences for the lactose-whey (mean difference=17.7%, $p=0.01$), lactose-casein (mean difference=26.7%, $p<0.0001$) and glucose-casein (mean difference=16.1%, $p=0.028$). Lactose-whey and lactose-casein, as well as glucose-casein, also showed a significantly higher FITC-dextran permeation than the medium control (mean differences are 13.1%, 22.7% and 17.6%, $p<0.01$ respectively).

Dietary MRPs can increase intestinal permeability, as shown by their effects on TEER and FITC-dextran permeation. Notably, the impact on intestinal barrier function appears to vary depending on the specific sugar and protein sources involved in MRP formation. These findings suggest a modulatory role for dietary MRPs in intestinal barrier integrity. Future research is needed to further elucidate the mechanisms by which dietary MRPs influence intestinal permeability and inflammation and their potential role in the management of IBD.

Targeting Methylglyoxal: a Novel Approach to Improve Vascular Function in Diabetes

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Background:
Chronic hyperglycemia in diabetes promotes cardiovascular complications, partly through accumulation of methylglyoxal (MGO), a reactive glycolytic byproduct that contributes to the formation of advanced glycation endproducts and induces endothelial dysfunction. This study investigates whether lowering endogenous MGO can improve vascular function in a mouse model of type 1 diabetes.

Methods:
Eight-week-old Tie2Cre mice (n=16/group) were injected intraperitoneally with streptozotocin (50 mg/kg) or citrate buffer (vehicle) for five consecutive days to induce diabetes. From 10 weeks of age, mice received either an MGO-lowering cocktail (2 g/L pyridoxamine, 0.1 g/L hesperidin, 0.1 g/L resveratrol) or vehicle in drinking water. Echocardiography was performed one week prior to sacrifice at 20 weeks of age. Vascular function of the thoracic aorta was assessed through wire myography, and plasma and urine samples were analysed for multiple biomarkers.

Results:
Diabetes impaired acetylcholine-induced vasorelaxation compared to controls ($p<0.001$), whereas cocktail treatment significantly improved endothelial function in the diabetic mice ($p<0.05$). Plasma MGO levels were significantly higher in diabetic mice than in non-diabetic controls ($p<0.01$), and the cocktail treatment showed a trend toward mitigating this increase ($p=0.06$). Plasma levels of E-selectin ($p<0.001$) and intercellular adhesion molecule 1 (ICAM-1, $p<0.001$) were elevated in diabetic mice compared to controls, and cocktail treatment significantly reduced ICAM-1 levels ($p<0.01$).

Conclusion:
Treatment with the MGO-lowering cocktail reduced plasma MGO levels, significantly restored endothelial function, and attenuated vascular inflammation in diabetic mice. These findings support targeting MGO as a promising therapeutic strategy to improve vascular function and to mitigate diabetes-related cardiovascular complications.

Untargeted profiling of Protein Pharmaceuticals: Simultaneous determination of Identity and Post Translational Modifications

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Protein pharmaceuticals are increasingly important in modern medicine but require rigorous quality control to ensure their safety and efficacy. A particular challenge is the detection of post-translational modifications (PTMs), especially non-enzymatic ones, which include for example early and advanced Maillard reaction products, oxidation, deamidation, or dehydration. These modifications frequently occur during production or storage and affect protein stability, biological activity, immunogenicity, or functionality. Conventional analytical methods are often targeted to known impurities or contaminants and therefore insufficient to capture the full spectrum of PTMs.

The aim of this project was to develop an untargeted mass-spectrometry-based protein profiling workflow that not only confirms the protein identity but also provides complete sequence coverage. This allows the reliable detection of sequence variants, truncation, mutations in addition to the analysis of PTMs in recombinant protein pharmaceuticals. Filgrastim, a recombinant form of human granulocyte colony-stimulating factor produced in E. coli, which is used to stimulate neutrophil regeneration in patients undergoing chemotherapy or immunosuppressive treatments, was chosen as a model protein for method development.

The analytical workflow included protein extraction, chymotryptic protein digestion, peptide extraction and microLC-ESI-IM-QTOF-MS/MS analysis. Data processing and PTM annotation were performed using PEAKS Online 12. To identify process parameters, which may lead to protein modifications, Filgrastim samples were also subjected to oxidative, thermal, and basic + thermal stress prior to sample preparation.

Protein identity was confirmed with 100% sequence coverage relative to the database sequence. In total, 22 potential PTM species were detected, of which 16 are considered plausible based on their reaction mechanism or the intrinsic properties of Filgrastim. Under stressed conditions, oxidation of methionine, histidine, and tryptophan correlated positively with oxidative stress, while dehydration of aspartic acid, threonine, and serine was strongly associated with thermal stress. Glutamine deamidation was observed at slightly increased levels under combined basic and thermal stress. In addition, sugar-related modifications such as a hexose adduct, were consistently detected, suggesting possible Maillard-type reactions, although the exact source and mechanism of sugar attachment remain to be clarified.

In summary, this study successfully established a workflow for untargeted protein profiling of recombinant protein pharmaceuticals. The approach not only confirms protein identity but also identifies potential PTMs within the sample, offering valuable information for the quality assessment of recombinant protein drugs

This work was supported by the Frankfurt Foundation Quality of Medicines and the Pharmacy of Uniklinikum Erlangen.

Unveiling AMPK pathways by AGEs and ALEs reactions: A frame of carbohydrate and lipid metabolism in human digestive system

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Advanced glycation end products (AGEs) and advanced lipoxidation end products (ALEs) are heterogeneous groups of toxic compounds formed endogenously (hyperglycemic conditions) and exogenously (dietary) during non-enzymatic reactions of carbohydrates and lipids, respectively. Their accumulation has been linked to oxidative stress, inflammation, and the pathogenesis of metabolic disorders such as type 2 diabetes, cardiovascular disease, and liver disease. Recent studies have shown that dietary AGEs/ALEs affect the function of AMP-activated protein kinase (AMPK), a central regulator of cellular energy metabolism. AMPK suppresses ATP-consuming anabolic processes (including lipid and protein synthesis) while shifting metabolism toward ATP-producing catabolic pathways (such as glucose uptake and fatty acid oxidation). This dual role places AMPK at the crossroads of carbohydrate and lipid metabolism, integrating nutritional, hormonal, and stress signals to maintain metabolic homeostasis. When ingested with food, AGEs and ALEs undergo digestion and absorption in the gastrointestinal tract. Small peptide-bound forms and free carbonyl adducts can cross the intestinal barrier and enter the systemic circulation. Within the gastrointestinal tract, these compounds can exert direct effects on epithelial cells, hepatocytes, and the gut microbiota, affecting nutrient absorption and metabolic responses. Importantly, their interactions with AMPK pathways may determine whether an organism maintains homeostasis or develops metabolic dysfunction.

AMPK is involved in carbohydrate metabolism. Chronic exposure to AGEs has been shown to impair insulin signaling and reduce AMPK phosphorylation, thereby limiting glucose utilization and promoting hyperglycemia. For example, Nε-(carboxymethyl)lysine (Nε-Lysine) (Nε-Lysine) (Nε-Lysine) has been reported to inhibit AMPK activation in hepatocytes, leading to impaired carbohydrate metabolism. Such findings suggest that AGEs may counteract the beneficial effects of AMPK by contributing to insulin resistance and the progression of type 2 diabetes. In lipid metabolism, AMPK phosphorylates and inactivates acetyl-CoA carboxylase (ACC), reduces malonyl-CoA levels, and thereby increases mitochondrial fatty acid β-oxidation. It also suppresses sterol regulatory element-binding protein 1c (SREBP-1c), a transcription factor important for lipogenesis. However, ALEs, such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)-derived adducts, can interfere with these mechanisms. ALEs can increase oxidative stress and mitochondrial dysfunction, leading to decreased AMPK activity. This suppression not only reduces fatty acid oxidation but also increases triglyceride accumulation, a hallmark of dyslipidemia and hepatic steatosis. Therefore, ALEs may impair lipid homeostasis by weakening AMPK-mediated regulation.

The gastrointestinal tract is the first site of exposure to dietary AGEs and ALEs, where they can interact with epithelial cells, gut microbiota, and immune components. Emerging evidence suggests that AGEs and ALEs alter the composition of the gut microbiota and promote dysbiosis, which further impacts host metabolism. Because AMPK also plays a role in intestinal barrier integrity, nutrient sensing, and microbiota interactions, the digestive tract serves as a central hub connecting food chemistry with systemic metabolic health. In summary, AGEs and ALEs represent a crucial interface between food chemistry, digestion, and metabolic regulation.