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Contents

Summaries

1. Structure and Function of Low Molecular Weight Food Components

1.1 Aroma and Taste (Hedonic Value) as Quality Parameters

1.1.1 Selection and characterization of "aroma yeasts" designed to improve wheat bread aroma

Sensory investigations demonstrated that a large number of pure yeast strains are able to generate characteristic wheat dough flavors. Regarding two doughs fermented with baker's yeast (*Saccharomyces cerevisiae*) and *Dekkera bruxellensis* as an example, the property of both yeasts to form significant different aroma was proved. Baker´s yeast produced a typical yeast-like dough aroma but an intense fruity note dominated the *Dekkera* dough. By application of Aroma Extract Dilution Analysis (AEDA), Stable Isotope Dilution Assays (SIDA) and calculation of Odor Activity Values (OAV), the differences in dough aroma were attributed in particular to 2-methylbutanoic acid ethyl ester and methylpropanoic acid ethyl ester, which were liberated by *Dekkera bruxellensis* in high amounts. Baking experiments provided the evidence that the characteristic odor of the *Dekkera* dough can be transferred into wheat bread crumb. The present systematic approach offers the possibility to influence bread aroma using doughs fermented with special "aroma yeasts" and to improve the quality of wheat products in the future.

1.1.2. Investigations on the formation of the characteristic fecal off-flavor in white pepper (*Piper nigrum* L.)

Screening for potent odorants in samples of white pepper showing an intense fecal, cowshed-like off-flavor by aroma extract dilution analyses revealed the fecal, swine-manure-like smelling compound 3-methylindole and the fecal, horse-like smelling compound 4-methylphenol, according to their odor qualities and their high FD-factors, as potential major contributors to this malodor. Further compounds possibly also contributing to this characteristic off-flavor were identified as 3-methylphenol (phenolic), butanoic acid, 2- and 3-methylbutanoic acid, pentanoic acid and hexanoic acid (cheesy). Quantification of these compounds in 50 different samples of white pepper by stable isotope dilution analyses showed that they are ubiquitously present in

commercial white pepper samples. The determination of breakthrough thresholds of these substances in a white pepper aroma model finally corroborated the relevance of particularly 3-methylindole, 4-methylphenol, 3-methylphenol and butanoic acid for the observed off-flavor. Monitoring the concentrations of these off-odorants showed that they are not increased during storage as sometimes assumed. In black pepper as well as in fresh, undried pepper of different maturity their concentrations were perspicuously lower, indicating that their formation most likely occurs during the retting process within white pepper manufacturing.

1.1.3. Changes in key odorants of sheep meat induced by cooking

Application of the Aroma Extract Dilution Analysis on two extracts prepared from cooked and raw lean sheep meat revealed 4-ethyloctanoic acid (mutton-like), trans 4,5-epoxy-(*E*)-2-decenal (metallic), (*Z*)-1,5-octadien-3-one (geranium-like) and (*E,E*)-2,4-decadienal (deep fried) as important odorants in the cooked as well as in the raw meat, thereby indicating the important role of the raw meat as source of sheep meat odorants. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone and 2-acetyl-1-pyrroline as well as 2-aminoacetophenone were among the few aroma compoundds, which were clearly increased in their Flavor Dilution (FD) factors by the cooking procedure. A stable isotope dilution assay showed that 4-ethyloctanoic acid was by a factor of 4 higher in uncooked intramuscular fat as compared to uncooked adipose tissue, in which 4,5-epoxy-(*E*)-2-decenal and further lipid degradation products were shown to be the most odor-active compounds. The cooking procedure did not much alter the concentration of 4-ethyloctanoic acid.

1.1.4. Influence of high hydrostatic pressure and enhanced temperatures on aroma compound formation in *Maillard*-type reactions

Application of an Aroma Extract Dilution Analysis on an extract isolated from a glucose/proline mixture thermally processed (90 min, 100 °C) at normal pressure (NP) in MPOS buffer confirmed the popcorn-like smelling 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine as the key odorants formed. However, reacting the same mixture under high hydrostatic pressure (HHP), completely changed the profile of key odorants by minimizing the formation of ACPY, but significantly enhancing the odor intensity of the caramel-like smelling odorants 2-hydroxy-3,4-dimethylcyclopent-2-en-1-one and also 2-hydroxy-3-methylcyclopent-2-en-1-one (HMC). Quantitative studies revealed that at HHP much higher amounts of carbohydrate degradation products, such as 2-oxopropanol, are formed. Based on labeling experiments using the CAMOLA technique, a new reaction sequence could be verified for HMC formation at HHP by an Aldol condensation of 2 molecules of 2-oxopropanol followed by elimination of 2 molecules of water.

1.1.5. Quantitation of the intense aroma compound 3-mercapto-2-methylpentan-1-ol in raw and processed onions (*Allium cepa*) of different origins and in other Allium varieties using a stable isotope dilution assay

A stable isotope dilution assay was developed for the quantitation of the potent onion odorant 3-mercapto-2-methylpentan-1-ol using mass chromatography and synthesized [$^2H_2$]-3-mercapto-2-methylpentan-1-ol as the internal standard. Application of the newly developed method on onions from different origins revealed amounts between 8 µg and 32 µg per kg in raw onions, whereas 34 µg up to 246 µg were found in sliced, stored (50 min) and then cooked onions. In extracts prepared by simultaneous steam distillation/extraction the highest concentrations of 1 were formed amounting to more than 1200 µg/kg. The much higher content of 3-mercapto-2-methylpentan-1-ol in cooked onions suggested its formation from specific, yet unkown precursors enzymatically formed during cutting of raw onions. 1 was for the first time identified and also quantified in other *Allium* species such as chives, scallions, and leek, whereas surprisingly garlic and bear's garlic did not contain the aroma compound.

1.1.6. Characterization of the astringent key taste compounds in black tea infusions

Application of taste dilution analyses on freshly prepared black tea infusions revealed neither the high-molecular thearubigen-like polyphenols, nor the catechins and theaflavins, but a series of 14 flavon-3-ol glycosides as the main contributors to the astringent taste perceived upon black tea consumption. Among these glycosides, the apigenin-8-C-[(-L-rhamnopyranosyl-(1→2)-O-(-D-glucopyranoside] was identified for the first time in tea infusions. Depending on the structure, the flavon-3-ol glycosides were found to induce a velvety and mouth-coating sensation at very low threshold concentrations which were far below those of catechins or theaflavins; for example, the threshold of 0.001 (mol/L found for quercetin-3-O-[α-L-rhamnopyranosyl-(1→6)-O-ß-D-glucopyranoside] is 190,000 or 16,000 times below the threshold determined for epigallocatechin gallate or theaflavin, respectively. Moreover, structure/activity considerations revealed that, besides the type of the flavon-3-ol aglycone, the type as well as the sequence of the individual monosaccharides in the glycosidic chain are key drivers for astringency perception of flavon-3-ol glycosides.

1.1.7. Studies on the molecular requirements of alapyridaine for taste enhancement

By application of the taste dilution analysis (+)-(S)-1-(1-carboxyethyl)-5-hydroxy-2-(hydroxymethyl)-pyridinium inner salt was recently successfully identified as a multimodal taste enhancer in beef bouillon. While being taste-less on its own, this so-called alapyridaine was found to intensify the human perception of sweet, salty and umami taste. To gain information on the molecular requirements of this novel class of taste enhancer, a range of structurally related pyridinium betaines were synthesized, purified, and their physiological activities sensorially evaluated. Removal or modification of the hydroxyl and the hydroxymethyl group, respectively, induced a loss in bioactivity, thus indicating the 2-(hydroxymethyl)-5-hydroxypyridinium moiety as an essential structural element for taste enhancement. Regarding the amino substituent, neither the prolongation or removal of the alkyl chain or the carboxy function in the 1-(1-carboxy-2-ethyl)-moiety, nor the incorporation of an additional carboxy function led to any active derivative, thus demonstrating that also the structure of the nitrogen substituent is rather conserved for taste enhancement. But substitution of the methyl group by a benzyl group yielded a compound showing similar taste enhancing activities as found for alapyridaine. Interestingly, additional insertion of glycine between the 1-(1-carboxy-2-phenylethyl)-moiety and the pyridinium ring resulted in a compound eliciting comparable taste enhancing effects as shown for the compound lacking the glycine spacer. In contrast to these multimodal taste enhancers, substitution of the alanine moiety in alapyridaine by an arginine moiety revealed an one-dimensional taste enhancer exclusively increasing the human sensitivity for salty taste.

1.2. Techno-Functional Properties of Food Constituents

1.2.1. Influence of the phosphoric acid derivative on the functional properties of phospholipids

Pure phospholipid classes were isolated by thin layer chromatography in a semi-preparative scale (1 - 2 g) from crude lecithins of different origins (soybean, rapeseed, sunflower, egg) and their functional properties have been studied by micro-scale baking tests and rheological methods. Baking tests with these phospholipid classes showed for the first time, that especially phosphatidyl inositol, which was supposed to play a secondary role in breadmaking, is the only phospholipid class which is active in very low concentrations of 0.02 to 0.1 %. None of the other phospholipid classes were active in this concentration range. The effects of individual phospholipid classes were compared to those of mixtures of them. In this way those phospholipid mixtures with a high baking activity were identified. A mixture of phosphatidyl inositol/phosphatidyl ethanolamine/ phosphatidic acid in a 2/1/1 (m/m/m) proportion was found to be most

effective in breadmaking (increase of the loaf volume up to 55 %). On this basis, crude lecithins were fractionated by phase partitioning to obtain samples with improved baking performance.

1.2.2. Influence of flour improvers on the concentration of dityrosine in wheat flour and wheat dough

A method for quantitative determination of dityrosine in wheat flour and wheat dough was developed. The reference compounds dityrosine and [3,3'-$^{13}C_2$]-dityrosine were synthesized by means of an enzymatic reaction and purified by sequential ultrafiltration, gel permeation chromatography and preparative HPLC. For quantitative release of dityrosine from gluten proteins acidic hydrolysis was optimized by using a mixture of propionic acid and hydrochloric acid. After hydrolysis the digests were subjected to solid phase extraction on C18 silica gel. Analysis was carried out by RP-HPLC with electrospray ionization and tandem mass spectrometry. Validation of the method gave a limit of detection of 80 ng/g, the limit of quantitation was 270 ng/g and the mean coefficient of variation was 7.5 %. By means of the new method the concentrations of dityrosine in wheat flour (cv. Flair) and wheat dough were determined. The influence of the mixing time (1, 7, 20 min) and of flour improvers (glucose oxidase, hexose oxidase, potassium bromate, ascorbic acid, hydrogen peroxide) was studied. During mixing of the dough the concentration of dityrosine increased by a factor of 2 within the first 10 min, for longer mixing times the concentration remained constant. The enzymes glucose oxidase and hexose oxidase as well as hydrogen peroxide caused a strong increase of the dityrosine concentration depending on the amount of additive. Especially a combination of the enzymes with glucose led to a drastic increase of dityrosine formation. On addition of ascorbic acid and potassium bromate no significant change of the dityrosine concentration was observed.

1.2.3. Production of foils from wheat gluten modified by variation of temperature and high pressure

Foils produced from wheat gluten are of increasing interest because they can be manufactured from a sustainable material, and are biodegradable and eatable. Previous studies have shown that physical and chemical properties of gluten can be strongly influenced by the application of high pressure. Effects on the characteristics of gluten foils, however, were not investigated until now. Therefore, a method was developed to produce gluten foils in a laboratory scale and, subsequently, the influence of temperature and high pressure on foil properties was studied. In preliminary experiments, foil production was optimized. The best procedure was found to be suspending gluten in concentrated formic acid with glycerol as plasticizer, casting into Teflon moulds and drying at 40 °C; the result was an almost transparent foil. In the following experiments, the effects of temperature, high pressure and additives on foil properties were measured by extension tests. The firmness of foils increased at high temperature (e.g. 70 °C), their cohesivity decreased and they tore after short extension. After treatment of gluten with relatively low pressure (200 MPa), foils got softer and more extensible; at 400 MPa they were adhesive and had properties like parafilm and at 800 MPa, they lost their viscoelastic properties. Pure gliadin did not form any foil, whereas glutenin formed an elastic and firm foil. Differences in foil properties could also be affected by the choice of raw material (e.g. gluten from different wheat varieties). Addition of reducing agents (e.g. cysteine, sulfite) to gluten in combination with high pressure (300 - 400 MPa) produced relatively soft foils with unique elastic properties. Altogether, the results demonstrated that the variation of temperature, pressure and additives leads to numerous variable foil properties.

2. Development of Special Analytical Techniques

2.1. Retronasal persistence of Espresso aroma

Recently, the influence of physiological parameters as well as matrix composition on the immediate retronasal perception of Espresso aroma has been elucidated. It was found that

addition of milk led to significant decreases not only in immediate aroma intensity during consumption but also changed the overall aroma profiles and shortened the aroma persistence considerably. Generally, highly different aroma profiles were observed during the timecourse of "afterodor" development.

For this reason, the intra-oral persistence of trace key odorants of Espresso was followed after consumption by means of analytical and sensory terms. The BOSS analysis proved to be a highly sensitive and selective way for the characterization of Espresso compounds even 32 min after swallowing, as well as for the investigation of the influence of milk addition on single odorant's persistence. Obviously, milk addition prevented interactions of aroma compounds (especially the heteroaromatic compounds) with oral mucosa during consumption so that no, or only minor aroma adsorption to the mucosal tissue occurred. The analytical finding of a significantly reduced persistence of e.g. the heteroaromatic compounds may be regarded as the explanation for the reduced intensity of characteristic Espresso aroma impressions, but needs further confirmation by sensory model experiments.

2.2. Simultanous analysis of folic acid and pantothenic acid in foods enriched with vitamins by stable isotope dilution assays

Folic and pantothenic acid were quantified in multivitamin products by stable isotope dilution assays using [$^{2}H_{4}$]-folic acid and [$^{13}C_{3}$,^{15}N]-pantothenic acid as the internal standards. Detection was achieved by liquid chromatography-mass spectrometry which enabled unequivocal determination of the vitamins. Due to the very simple extraction procedure, analysis of the vitamins was completed within two hours. When analyzing multivitamin sweets, the intra-assay and inter-assay coefficient of variation was 3.2 % (n = 5) and 3.1 % (n = 5) for folic acid and 4.5 % (n = 5) as well as 6.5 % (n = 7) for pantothenic acid, respectively. Along with the precision data, recovery values of 99.4 % for folic acid and 103 % for pantothenic acid at addition levels of 6 mg/kg and 600 µg/kg, respectively, to starch products proved the accuracy of the new method. Application of the stable isotope dilution assay to fruit juices, whey products, cereals, sweets, pharmaceuticals, wheat flour and salt fortified with one or both vitamins revealed that for the majority of products the labeled pantothenic acid contents were exceeded by about 30 %, whereas for folic acid also significantly lower contents than the label claim were found.

2.3. Stable isotope dilution assay proves rapid degradation of the mycotoxin patulin in the human organism

The absorption and degradation of the mycotoxin patulin in humans was quantified by using a recently developed stable isotope dilution assay. Application of this currently most sensitive method revealed a patulin content less than 200 ng/L in the blood serum of five consumers of apple juice. Likewise, no patulin could be found in the serum of a volunteer, whose blood was drawn shortly after consumption of a juice containing a maximum tolerable amount of patulin. In further in vitro experiments the degradation of patulin by reacting with whole blood was investigated. After addition of 100 µg patulin to 9 mL of blood, only 6.1 % of the mycotoxin was detected after two minutes. It was concluded, therefore, that even high natural occuring concentrations of patulin in foods are quickly degraded before reaching other tissues than the gastrointestinal tract.

2.4. Quantification of ochratoxin A in foods by a stable isotope dilution assay using high-performance liquid chromatography-tandem mass spectrometry

A stable isotope dilution assay (SIDA) was developed for quantification of the mycotoxin ochratoxin A (OTA) by using [$^{2}H_{5}$]-OTA as internal standard. The synthesis of labeled OTA was accomplished by acid hydrolysis of unlabeled OTA and subsequent coupling one of the products, ochratoxin (, to [$^{2}H_{5}$]-L-phenylalanine. The mycotoxin was quantified in foods by LC-tandem MS

after extraction with buffers containing [$^2H_5$]-OTA and clean-up by immuno affinity chromatography or by solid phase extraction on silica. The method showed a sufficient sensitivity with a low detection and quantification limit of 0.5 and 1.4 µg/kg, respectively, and good precision in inter-assay studies showing a CV (n = 3) of 3.6 %.

2.5. Development of a general quantitative gliadin extraction procedure for unheated and heat-processed foods

Coeliac patients have to maintain a strict lifelong gluten-free diet. According to the recent draft revised standard of Codex Alimentarius, an immunochemical method (ELISA) is recommended for the quantitative determination of contaminations with coeliac-toxic proteins (prolamins from wheat, rye and barley) in gluten-free foods. In the past, one of the major problems has been the low extraction rate of prolamins in heat-processed foods when conventional 60 % ethanol was used. This study was, therefore, designed to develop a universal extraction procedure capable to extract the totality of coeliac-toxic prolamins from unprocessed and heat-processed foods. The extract should be completely compatible with subsequent ELISA containing R5 antibodies developed previously. The conditions for extraction were optimized by means of a gliadin standard and wheat doughs differently heated from 22 - 230 °C. A combination of 2-mercaptoethanol (250 mmol/L) and guanidine (2 mol/L) (extraction solvent E2) was shown to extract gliadins completely, even from breads heated at 230 °C, whereas the extraction rate of gliadins with 60 % ethanol (extraction solvent E1) was strongly reduced. 50 °C and 40 min are recommended as extraction temperature and time. Further comparative experiments were performed with fourteen maize breads spiked with different amounts of gliadin standard (0 - 156 mg/kg). ELISA revealed a recovery of 91 - 105 % with E2 and only 31 - 64 % with E1. Similar differences were found in numerous commercial heat-processed gluten-free foods. Even from unheated products E2 extracted 10 % more gliadins than E1, probably because E2 could additionally extract glutenin-bound gliadins. In summary a quantitative gliadin extraction procedure based on reducing and disaggregating agents were developed for both unheated and heat-processed food. The solvents are compatible with R5-ELISA and can be used for corresponding proteins from rye and barley. Extraction procedure plus R5-ELISA was successfully tested by an international ring test and was endorsed by the Codex Committee on Methods of Analysis and Sampling (CCMAS).

3. Relationship Between the Structure of Biopolymers and Their Technological Properties

3.1. Fractionation of wheat flour by non-aqueous systems: optimization of the method and determination of the protein composition of the fractions

Since the functional properties of wheat flour are not maintained during fractionation in aqueous solvents, an approach using non-aqueous solvents was made. The method is based on a physical separation of starch and protein in a mixture of toluol and tetrachoroethene due to the difference of the density. Therefore, wheat flour was firstly crushed in a ball mill, suspended in the afore mentioned solvent mixture and centrifuged. Crude protein was collected from the upper layer and the sediment represented the starch fraction. The crude protein fraction was purified by a second sedimentation step yielding a pure protein fraction and a fraction with higher starch content which was termed "residual flour". The fourth fraction were lipids, which were isolated by evaporation of the solvent. The proteins present in the fractions were quantified by means of a combined extraction/HPLC method. It was shown that the protein composition of the starch and the protein fraction differed only quantitatively but not qualitatively. Compared to the native flour, the protein fraction contained more glutenins and less gliadins as well as albumins and globulins. The fraction "residual flour" had almost the same protein composition as the native flour, whereas in the starch fraction albumins / globulins were enriched and glutenins were decreased. Previous studies postulated fundamental differences of the protein composition between the starch ("Haftprotein") and the protein ("Zwickelprotein")

fractions. The results of the present study impressively show that there are only quantitative differences between the proteins present in these fractions.

3.2. Identification of a ferulic acid-tyrosine crosslink between arabinoxylans and proteins of rye and wheat

Since the degree of dimerization of ferulic acid is not changed significantly during the breadmaking process, the formation of high-molecular-weight arabinoxylan fractions, as they were identified in the bread samples, is caused by other effects than ferulic acid dimerization. Therefore, 8-^{14}C-(*E*)-ferulic acid-(D-galactopyranos-6'-yl)ester was synthesized as a radiotracer and added to wheat and rye flour prior to breadmaking. Breads were lyophilized, extracted by means of a modified Osborne fractionation and the radioactivity of the fractions was determined by scintillation analysis. The major portion of the radioactivity remained in the water soluble fraction. However, a significant enrichment of the tracer was also detected in the prolamin and glutelin fractions in comparison to the control experiment. Separation of the prolamin fraction by RP-HPLC and scintillation measurement of the fractions gave evidence for a chemical modification of the tracer. To determine the structure of the reaction product, the prolamin fractions were hydrolyzed completely by means of an enzyme cocktail and the digests were studied by LC-MS. In one fraction, a dehydroferulic acid - tyrosine crosslink was detected and its structure was confirmed by comparison with a synthetic reference compound. The new crosslink was also identified in wheat and rye flour doughs, which had been prepared without addition of ferulic acid. The concentration of the dehydroferulic acid - tyrosine crosslink increased during wheat dough preparation.

3.3. Influence of the atmospheric carbon dioxide concentration on the quantitative protein composition of wheat grain

The concentration of atmospheric CO_2 in ambient air has continuously increased during the least decades combined with changes of the global climate, fauna and flora. A key question with respect to agricultural production is how agricultural crops will respond to future CO_2 concentrations. The aim of the present study was, therefore, to investigate the influence of CO_2 concentrations expected for the second half of this century on the quantitative protein composition of wheat grains, one of the worldwide most important sources for human nutrition. Within the frame of "The Braunschweig Carbon Project" and by means of a "free air carbon dioxide enrichment (FACE)" technique, winter wheat "Batis" was grown at an experimental field plot of the Federal Agricultural Research Centre in Braunschweig under different conditions. The FACE system consisted of six rings (20 m diameter each): 2 rings (FACE) with enriched CO_2 (550 µL/L) and blowers, 2 rings (BLOW) with ambient air (370 µL CO_2/L) and blowers, and 2 rings (FELD) with ambient air, but without blowers. One half of the rings (N_{100}) was supplied with an adequate amount of nitrogen (250 kg/ha) and the other half (N_{50}) with a reduced amount (110 kg/ha). The mature grains were harvested and the total amount of protein was determined by Kjeldahl (wholemeal flour) and Dumas (type 550 flour) methods. Total extractable proteins (albumins/globulins, gliadins, glutenins) were quantified by a combined extraction/HPLC procedure. The values of the three test series were highly correlated. Differences between BLOW and FELD samples could not be detected, thus blowers activated during day light time had no additional effect. As expected different N fertilization had a strong influence on the protein content, which was reduced in N50 samples by 23 % on an average. A further reduction at protein (-13 % on an average) was caused by the higher CO_2 concentration (FACE/N_{50} samples). In the case of N_{100} samples the influence of different CO_2 concentration was not clear. In one ring, the protein content of FACE samples was reduced by 14 % compared with BLOW samples, in the other ring differences could not be detected. HPLC analysis of extractable flour proteins indicated that increased CO_2 concentration reduced the amount of gluten proteins, but not that of albumins and globulins. Within gluten proteins gliadins were more affected than glutenins. Additionally, changes in the proportions of single gluten protein types could be observed.

Altogether the results indicated that the expected increase of atmospheric CO_2 will intensify the disadvantageous effect of low nitrogen fertilization on the gluten content of wheat grains.

3.4. Development and characterization of transgenic wheat without α-gliadins

α-Gliadins are the predominant protein group of wheat with respect to the amount and number of components. Their coeliac-toxicity is known since 1970 and attempts to eliminate them by breeding were without success. The utilization of an RNA interference (RNAi) approach, a relatively new method of gene engineering, was, therefore, chosen to investigate the potential of this technique to silence an entire gene family in wheat. A 313 bp target sequence, which was highly homologous in known DNA sequences of α-gliadins, was selected, isolated from wheat cultivar "Florida" and cloned into three different RNAi transformation vectors in sense and antisense orientation. The vectors were transformed into the scutellum of developing "Florida" embryos using the biolistic transformation. After selection by means of kanamycin resistance, transgenic plants were generated. Their phenotype and fertility were the same as those of the wildtype. Kernels of numerous transgenic lines were analyzed for their α-gliadin content by a combined extraction/HPLC procedure. The results demonstrated that wheat lines were created with strongly reduced or completely silenced α-gliadins. The loss of α-gliadins was compensated by an increase of albumins and globulins, ω- and γ-gliadins and HMW subunits of glutenin. A transgenic line with strongly reduced α-gliadin content was grown in a greenhouse. Mature kernels were harvested, milled into white flour and studied according to the rheological properties of dough and gluten and to the baking performance on a micro-scale. The results showed that dough resistance and extensibility were similar to those of wildtype dough, whereas gluten strength of the transgenic line drastically increased. Bread volume of the transgenic line was only slightly decreased and the crumb had a good appearance with regular pores showing that α-gliadins are not necessary for the baking performance of wheat.

3.5. Studies on the acid and enzymatic deamidation of glutamine residues in α-gliadins

Studies in the literature have shown that the specific deamidation of glutamine residues in gliadin peptides by tissue transglutaminase (tTG) is necessary for the stimulation of intestinal T cells of coeliac patients. The same effect can be achieved by unspecific deamidation induced by acids at high temperature. The aim of the present study was, firstly, to clarify whether deamidation occurs in the stomach and during HPLC separation under relatively mild acid conditions and, secondly, to identify those glutamine residues in stimulatory peptides that react with tTG. Peptides from α-gliadin sequence sections ((64-66), ((56-68), ((58-88) and variants with glutamic acid (E) instead of glutamine (Q) were synthesized on a peptide synthesizer and purified by RP-HPLC. Acid deamidation of glutamine was studied with tripeptide PQL ((64-66) by means of four different assays. Assay A (2 mol/L HCl, 50 °C, 360 min) was the positive control, assay B (0.01 mol/L HCL, 37 °C, 240 min) should mimic stomach conditions, assays C and D (acetonitrile/trifluoroacetic acid, pH 2.1) should mirror RP-HPLC conditions (50 °C, 60 min) and storage conditions after RP-HPLC separation (14 °C, 360 min), respectively. The putative transformation of PQL into PEL was quantified by RP-HPLC on C18 silica gel. The results demonstrated that PQL of assay A was almost completely deamidated after 6 h, whereas no deamidation could be detected in assays B, C and D. Consequently, gliadin peptides are not deamidated by the acid conditions of stomach and of HPLC separations. To identify glutamine residues reacting with tTG, peptide ((56-68) and three variants with E59, E63 and E65) were fluorescent-labeled at the N-terminal amino group and incubated with tTG. SDS-PAGE of the products demonstrated that the native peptide and its variants E59 and E63 were good substrates for tTG as visualized by deamidation products or crosslinks with tTG. However, substitution of Q65 by E65 prevented reactivity with tTG showing that the reaction of peptide ((56-68) is strictly restricted to Q65. In another assay, the incorporation of monodansyl cadaverin into glutamine containing peptides was used to determine the substrate specifity of tTG. Again ((56-68) and variants E59 and E63 turned out to be good substrates for tTG, whereas

variant E65 did not show any reaction, confirming the PQL motif as unique tTG recognition sequence in these gliadin peptides. This result was confirmed by the reactivity of the longer peptide ((58-88) having three copies of the recognition motif. Only the replacement of Q65, Q72 and Q79 within the PQL motifs by glutamic acid abolished the reactivity with tTG.

3.6. Quantitation of 3-aminopropionamide in potatoes - A minor but potent precursor in acrylamide formation

3-Aminopropionamide (3-APA) has recently been suggested as a transient intermediate in acrylamide (AA) formation during thermal degradation of asparagine initiated by reducing carbohydrates or aldehydes, respectively. 3-APA may also be formed in foods by an enzymatic decarboxylation of asparagine. Using a newly developed method to quantify 3-APA based on LC/MS/MS, it could be shown that the biogenic amine was present in several potato cultivars in different amounts. Further experiments indicated that 3-APA is formed during storage of either intact potatoes (20° C or 35 °C) or after crushing of the cells. Heating of 3-APA under aqueous or low water conditions at temperatures between 100 °C and 180 °C in model systems always generated more AA than in the same reaction of asparagine, thereby pointing to 3-APA as a very effective precursor of AA. While the highest yields measured were about 28 mol-% in the presence of carbohydrates (170 °C; aqueous buffer), in the absence of carbohydrates, 3-APA was even converted by about 63 mol-% into AA upon heating at 170 °C under aqueous conditions. Propanoic acid amides bearing an amino or hydroxy group in the (-position, such as 2-hydroxypropionamide and L-alaninamide were ineffective in AA generation indicating that elimination occurs only from the ß-position.

4. Physiology

4.1. Identification and functional expression of olfactory receptors specific for natural, odor-active alkylpyrazines

Certain members of the large group of alkylpyrazines such as 2,3-diethyl-5-methyl- and 2-isopropyl-3-methoxypyrazine are considered to be key food odorants based on their outstanding low odor thresholds in air. Using a FLIPR system, ninety-six mouse olfactory receptor chimeras expressed in a human cell line were screened for intracellular calcium signals after application of 2,3-diethyl-5-methylpyrazine. One responding receptor could unequivocally be identified. In silico analysis of the mouse and human genome data bases led to the identification of two further mouse genes and two human genes as the closest homologues. Cloning of their full-length coding regions and functional expression in HEK-293/15 cells revealed different efficacies of these olfactory receptors to induce intracellular calcium signals upon application of the differently smelling 2,3-diethyl-5-methylpyrazine (earthy) and 3-isobutyl-2-methoxypyrazine (peasy).

4.2. *In-vivo* studies on the metabolism of the monoterpene pulegone in humans explaining the different toxicological effects between (S)-(-)- and (R)-(+)-pulegone

The major *in-vivo* metabolites of (S)-(-)-pulegone in humans were newly identified as 2-(2-hydroxy-1-methylethyl)-5-methyl-cyclohexanone (8-hydroxy-menthone), 3-hydroxy-3-methyl-6-(1-methylethyl)-cyclohexanone (1-hydroxy-menthone), 3-methyl-6-(1-methylethyl)-cyclohexanol (menthol) and *E*-2-(2-hydroxy-1-methylethylidene)-5-methyl-cyclohexanone (10-hydroxy-pulegone) based on mass spectrometric analysis in combination with syntheses and NMR experiments. Menthofuran was not a major metabolite of pulegone and is most probably an artifact formed during work-up from known 10-hydroxy-pulegone and/or unknown precursors. The differences in toxicity between (S)-(-)- and (R)-(+)-pulegone can be explained by the strongly diminished ability for enzymatic reduction of the double bond in (R)-(+)-Pulegone. This might lead to further oxidative metabolism of 10-hydroxy-pulegone and the formation of further

currently undetected metabolites which might account for the observed hepatotoxic and pneumotoxic activity in humans.

4.3. Activity-guided identification of a chemopreventive compound in coffee beverage using in vitro and in vivo techniques

The aim of the present study was to apply an activity-guided screening procedure to coffee brew in order to identify a key chemopreventive compound by means of in vitro antioxidant tests as well as cell culture experiments, and to prove the in vivo activity of that compound by an animal feeding experiment. Solvent fractionation, followed by multiple-step ultrafiltration revealed that the polar coffee compounds with molecular weights below 1 kDa show the major inhibitory effect on the in vitro peroxidation of linoleic acid as well as the predominant chemopreventive enzyme modulating activity on the NADPH-cytochrome c-reductase (CCR) and glutathione-S-transferase (GST) in human intestinal Caco-2 cells. To identify the chemical structure of the most active antioxidants and chemopreventive compounds, the polar compounds were further separated by HPLC techniques, followed by the activity-guided screening of the individual HPLC-fraction. These experiments demonstrated the 5-chlorogenic acid as the most powerful antioxidant in vitro, whereas, in contrast, chemopreventive effects on the GST activity were found for N-methylpyridinium ions, the structure of which was elucidated by LC/MS and NMR experiments and confirmed by synthesis. The in vivo activity of coffee beverage and N-methylpyridinium ions were tested in a 15-day feeding experiment on rats. In the liver, feeding of 4.5 % coffee beverage resulted in an increase of GST and UDP-GT activity by 24 % and 40 % compared to animals fed the control diet ($p > 0.05$), respectively. Plasma total antioxidant capacity and plasma tocopherol were elevated in animals fed the coffee beverage and the N-methylpyridinium containing diet. In summary, the results demonstrating a strong in vitro antioxidant activity for coffee were confirmed by the feeding study. Surprisingly, feeding of N-methylpyridinium also resulted in an increased total antioxidant capacity in the plasma. The data indicate that the mode of action demonstrated for N-methylpyridinium in biological systems is different from that in foods.

4.4. Endogenous load of an advanced glycation end product is nutritionally determined - A study on a chemically-characterized AGE in rats

N(ε-Carboxymethyllysine (CML) is an advanced-glycation end-product (AGE) which has been implicated in micro- and macrovascular complications in diabetes. AGEs are formed by non-enzymatic glycation reactions between reducing carbohydrates and the exposed amino groups of amino acids or proteins not only in heat-treated foods, but also in the living organism. The extent to which AGEs present in the organism is due to the accumulation of food-derived AGEs or is endogenously formed, is still not known, mainly because chemically-characterized food-AGEs are lacking. In this study, casein-linked CML (CasCML), a chemically-characterized, food-derived AGE, was administered to male Wistar rats (n = 6 per group) at two different doses of 110 mg (CasCML-L) and 300 mg (CasCML-H) CML per kg body weight and day for 10 days. In relation to the ingested dose, the mean percentage of CML analyzed in urine, after acid hydrolysis followed by HPLC-fluorescence detection, was calculated at 26 % and 29 % for the CasCML-L and CasCML-H groups, respectively. For the plasma and the kidneys, the 3-fold dietary increase resulted in a 13- and 2.8-fold increase in total CML accumulation. Therefore, the endogenous load of AGEs is nutritionally determined, leading to strong implications for the dietary regime of diabetes patients.

4.5. Dietary bread crust AGEs bind to RAGE in HEK-293 kidney cells but are rapidly excreted after oral administration to healthy and subtotally nephrectomized rats

Investigating the cellular effects of dietary *Maillard* reaction compounds, p38-MAP kinase activation in renal HEK-293 cells is demonstrated for casein-linked N(-carboxymethyllysine

(CML), CML, bread crust (BC) and pronyl-glycine, a key compound formed in association with the process-induced heat impact applied to bread dough. Expression of the C-terminus truncated receptor for advanced glycation end products (RAGE) resulted in a reduced HEK-293-MAP kinase activation. As these findings unequivocally point to a RAGE-mediated activating effect of CML, BC and pronyl-glycine on kidney cellular signal transduction pathways, an animal study on rats was performed. Male Wistar rats were subjected to a sham operation (CTRL, n = 20), or to 5/6 nephrectomy (NX, n = 20). Both groups were randomized into two subgroups, fed 20 g of either a diet containing 25 weight-% BC or wheat starch (WS). The average daily intake of CML and pronyl-glycine was calculated at 11 and 1.1 mg per kg body weight and day, respectively. GC-MS analyses of CML, carboxyethyllysine (CEL) and pentosidine revealed increased levels of CML and CEL in the liver but decreased levels of both AGEs in the kidneys of CTRL and NX rats fed the BC diet compared to those on the WS diet. As the urine levels of CML were also elevated in those CTRL and 5/6NX rats, the results point to an enhanced excretion of AGEs after BC administration. Although renal insufficiency in the NX rats was reflected by proteinuria, the renal handling of AGEs was not impaired.

4.6. Genotoxicity and mutagenicity of melanoidins isolated from a roasted glucose-glycine model in human lymphocyte cultures, intestinal Caco-2 cells and in the Salmonella typhimurium strains TA98 and TA102 applying the AMES test

Melanoidins are formed during household cooking procedures and are part of our daily diet, but data on their toxicological potential are still scarce. Therefore, the mutagenic, cytotoxic and genotoxic activity of the water soluble total fraction (A), the water soluble high-molecular-weight fraction (HMW; molecular weight > 12,400 Da) and the remaining water soluble low-molecular-weight fraction (LMW) isolated from a glucose-glycine model system roasted at 125 °C was comprehensively studied in human lymphocytes (genetic end point: sister chromatid exchange (SCE)), Caco-2 cells (SCE, cell viability, cell proliferation) and in the Salmonella typhimurium strains TA98 and TA102 (Ames test). Tests were performed in a dose- and time-dependent manner. The results indicate a significant increase in SCE formation in human lymphocytes after the exposure to 0.05 % and 0.1 % of the melanoidin fractions. In Caco-2 cells, only the exposure to LMW increased the SCE formation as a matter of concentration. Cell's proliferation and viability decreased significantly after exposure to melanoidins. In the Ames test, melanoidins did not show a mutagenic potential, neither using the TA98 nor the TA102 strain. These results show that melanoidins isolated from the glucose-glycine mixture exhibited modest but significant genotoxic effects in human lymphocytes and, in particular the LMW, in Caco-2 cells, but they induce neither in low nor in very high concentrations mutagenicity in bacteria strains.

4.7. Coeliac disease-specific toxicological and immunological studies on HMW subunits of glutenin

In-vitro- and *in-vivo* toxicity tests described in literature indicate that the whole gliadin fraction and all gliadin types (α-, γ- and ω-gliadins) contain the precipitating factor in coeliac disease. The effect of the glutenin fraction, however, remains controversial, and the coeliac activity of HMW subunits of glutenin (HMW-GS) was investigated in one recent *in-vitro* study demonstrating the potential of these proteins to stimulate T-cells of coeliac patients. However, the only true test of coeliac toxicity is to show that these *in-vitro* reactions can be translated into *in-vivo* changes, using instillation into the small intestine. We, therefore, wished to investigate both T-cell immunogenicity and *in-vivo* toxicity of HMW-GS. A mixture of four HMW-GS (1Dx5, 1Dx7, 1Dy9, 1Dy10) was isolated from wheat cultivar Rektor by a specific extraction/precipitation procedure and was purified by preparative RP-HPLC. Analytical RP-HPLC, ELISA and SDS-PAGE indicated that the preparation contained only traces of other gluten protein (<0.18 %). HMW-GS were subsequently digested by pepsin and trypsin. Intestinal T-cell lines from 14

coeliac patients were then tested for their reaction to the digests of gluten (positive control) and of HMW-GS, in parts treated with tissue transglutaminase (tTG). 13 T-cell lines showed a positive reaction to gluten, 7 lines to HMW-GS and 8 lines to HMW-GS treated with tTG, showing that HMW-GS have the potential to stimulate T-cells of coeliac patients as well as gliadins. For the *in-vivo* studies, 500 mg each of the HMW-GS digest were instilled into the duodenum of three coeliac patients taking a gluten-free diet for a long period. Biopsies were taken at 0, 2, 4 and 5 or 6 hours after challenge and assessed for villous height to crypt depth ratio, entercyte cell height and intraepithelial lymphocyte count. All patients showed highly significant changes in the three parameters, maximal at 4, 5 or 6 hours. In conclusion it was shown for the first time that HMW-GS are toxic for coeliac patients.

5. Food Composition and Nutrition Tables

5.1. Vitamin and mineral composition of plant foods

Ausgangslage: Plant foods play an important role in human nutrition as deliverants of macro- and micronutrients. The recommendations of the German Nutrition Society (DGE) concerning the intake of fruits and vegetables are actually not achieved by the population. On the part of the industry rumours abounded that the content of vitamins and minerals in foods has decreased dramatically in the last decades, and following the supply of vitamins and minerals by plant foods would not be adequate any more. There is, however, no scientific proof for this assertion.

Plant growth and reproduction are depending on the absorption of minerals and a deficiency as well as the excess of mineral absorption leads to optically perceptible and characteristical changes of the plants. Minerals fulfill various functions in the plant metabolism: they are essentials of cell wall structures and constituents of a variety of enzymes and cofactors and are also involved in the regulation of the water balance. Vitamins are synthesized by the plants themselves and as constituents of enzymes, cofactors and antioxidants they are strongly involved in the plant metabolism. Plant growth and reproduction would not be possible in case of a significant decrease or a lack of these nutrients.

The mineral and vitamin content of plant foods is depending on a lot of internal and external factors, as there are the variety of the plant, the ripening stage of fruits and vegetables at harvest, climate, the soil, the kind of cultivation as well as transport and storage conditions. In the literature various studies could be found investigating these influences in selected fruits and vegetables exemplarily, showing significant effects for all parameters and varietal variations up to 300 - 400 % were found for the vitamin C concentration in specific fruits e.g.. When comparing food composition data, the representation of the data (e.g. concentration of the different vitamin derivatives and factors for calculating vitamin activity) and other quality parameters of the data as there are the analytical methodology and quality control procedures used have to be considered. Therefore it is evident that in the scientific discussion of the changes in food composition data the range of variation and the different influencing parameters has to be compared.
Regarding the food composition data exemplarily compiled for two selected plant foods from 10 different international food composition data tables, published over a time period of 50 years, we could find, that the vitamin and mineral content of foods remains remarkably stable.

5.2. Food composition and nutrition tables

The information about the composition of food adapted to the present scientific level is essential for administration, nutritional guidance and science. The "Souci-Fachmann-Kraut Food Composition and Nutrition Table" is actualized by evaluation of the international scientific publications available and by means of the PC database SFKDB. Selected data are transfered into the small table "Der kleine Souci-Fachmann-Kraut: Lebensmitteltabelle für die Praxis", which has been developed for the daily requirement of the consumer. The spectrum of food

constituents covered in the large SFK-nutrition table also addresses preventive-medical aspects by the group of the "special bioactive compounds".

The 3rd edition of the small Table "Der kleine Souci-Fachmann-Kraut: Lebensmitteltabelle für die Praxis" has been published in December 2003. The second update of the Online-Version of the "Souci-Fachmann-Kraut Food Composition and Nutrition Table" is available on the WWW since November 2004. The new version comprises the new foods spelt and spelt flour type 630, revised amino acid data of the food group cereal and cereal products, some new data of special carbohydrates and minerals. The preparation of the 7th edition has been continued. As an important aspect in the actual discussion about disease prevention and health, data of the group of the bioactive compounds will be extended. Due to the developed of the analytical methodology some data will be actualized in the future (e.g. iodine and folic acid data).