Leibniz Institut
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1.1.1. Unraveling the formation pathways of key Maillard-type aroma compounds using the carbon modul labeling technique

Due to the complex reaction cascades involved in the thermally induced carbohydrate degradation, different pathways involving different transient intermediates may exist in the formation of sensorially active Maillard-type target molecules. Based on model studies using defined mixtures of unlabeled and labeled $^{13}C_6$-glucose in the reaction with proline, a new method assigned as the carbon modul labeling, was developed to identify key transient intermediates involved in the formation of alkylpyrazines and 2,3-butandione as the examples.

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1.1.2. On the role of flours and sour dough fermentation as odorant sources of wheat breads

Investigations on the volatile fraction of wheat flour by aroma extract dilution analysis (AEDA) and stable isotope dilution assays (SIDA) demonstrated that wholemeal flour and white wheat flour (type 550) already consist of a number of odor-active compounds, e.g. (E)-2-nonenal, (E,Z)- and (E,E)-2,4-decadienal, (E)-4,5-epoxy-(E)-2-decenal, 3-hydroxy-4,5-dimethyl-2(5H)-furanone, and vanillin, which have already been identified as important contributors to the aroma of wheat bread crumb and crust. The flours differed significantly in

their aromas which could be put down only to distinct concentrations differences of flour odorants. Fermentation of the wholemeal flour with lactic acid bacteria does not lead - with the exception of acetic acid - to a generation of additional odorants but a disturbance of the flour odorant base was caused. Balancing the odorant concentrations before and after fermentation gave evidence that the microorganisms form and degrade specific odorants due to their metabolic properties. The results give a clear evidence that the type of flours and the use of selected lactic acid bacteria stems are important factors to modify and improve the aroma quality of wheat breads.

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1.1.3. Influence of the boiling process on the key aroma compounds of unhopped and hopped wort

The most important odorants in unhopped and hopped worts were evaluated by application of Aroma Extract Dilution Analyses. A decrease in hexanal and in Strecker aldehydes was found to be responsible for the flavor changes during boiling of unhopped wort. Further investigations showed that boiling of early hopped wort was accompanied by a considerable loss of hop odorants, such as myrcene or linalool without generating new odor-active compounds. Late addition of hops in the whirlpool led to higher yields of (R)-linalool and myrcene in the wort and, also, the final beer. Enantioselective analysis revealed that the enantiomeric distribution of linalool, originally found in hops, changed significantly during wort boiling.

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1.1.4. On the role of 3-methyl-2-butene-1-thiol in beer aroma

Application of a comparative Aroma Extract Dilution Analysis on aroma distillates obtained from a fresh beer bottled in green glass (FB) and from the same batch of beer, but exposed to daylight for 60 h (DB), revealed a total number of 37 odorants in FB and 39 odorants in DB. The most significant differences in the FD-factors of the odorants were found for 3-methyl-2-butene-1-thiol (MBT; sulfury), 3-(methylthio)propanal (potato-like) and phenylacetaldehyde (honey-like), which all were much increased in DB. Quantitation of MBT using a stable isotope dilution assay, showed a significant increase in the beer sample exposed to daylight. Interestingly, already in the FB significant amounts of MBT were measured indicating the contribution of MBT not only to the sunstruck off-flavor of light treated beer, but also to the overall, typical aroma of beers bottled in green glass. Preliminary studies on the precursors of MBT in beer confirmed a prominent role of isohumulone and cysteine, whereas the contribution of riboflavin was not as pronounced as described in the literature.

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1.1.5. The influence of human saliva on odorant concentration changes occurring in vivo

Intensity and persistence of retronasal aroma perception can differ significantly between substances. The reasons for this phenomenon could, up to now, not be fully elucidated. Therefore, the aim of the present work was to study the influence of saliva on the stability of potent aroma compounds. Incubation of selected odorants from different substance groups showed metabolic activity in varying extent depending on the structure of the odorant. Apart from oxidation of thiols to the corresponding disulfides, hydrolysis of esters and reduction of

aldehydes to their corresponding alcohols were observed while other compounds such as alkylmethoxypyrazines remained completely unmodified.

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1.1.6. Retronasal perception of Espresso aroma

Despite comprehensive studies in the field of coffee aroma little is known about Espresso aroma. Especially those compounds and factors which are involved in the longlasting aroma perception after Espresso consumption have not yet been clarified. For this reason the influence of physiological parameters as well as matrix composition on retronasal perception of Espresso aroma was in the focus of this work. First, the most potent odorants in freshly brewed Espresso were identified by means of gas chromatography-olfactometry. Then, using the EXOM approach, those odorants were identified which are present in exhaled air during Espresso consumption. Quantitation of these compounds in the breath with or without milk addition to the Espresso beverage exhibited significant differences. In correlation with sensory experiments, 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.

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1.1.7. Characterization of compounds contributing to the bitter off-taste of carrots and carrot products

Carrot containing foods are one of the most important products of the infant food industry. An intense bitter taste is, however, often the reason for the consumers rejection of such products. Inspite of extensive literature studies, for none of the compounds detected in carrots a correlation could be found between the sensory evaluation of the bitter taste and the results obtained by instrumental analysis. Sequential application of solvent extraction, gel permeation chromatography and HPLC in combination with taste dilution analyses revealed that not a sole compound but a multiplicity of bitter tastants contribute to the bitter off-taste of cold-stored carrots and commercial carrot puree, respectively. Among these bitter compounds, 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocoumarin (6 methoxymellein), 5-hydroxy-7-methoxy-2-methyl-chromone (eugenin), 2,4,5-trimethoxybenzaldehyde (gazarin), [Z]-heptadeca-1,9-dien-4,6-diin-3,8-diol (falcarindiol), [Z]-heptadeca-1,9-dien-4,6-diin-3-ol (falcarinol), and [Z]-3-acetoxy-heptadeca-1,9-dien-4,6-diin-8-ol (falcarindiol 3-acetate) could be identified on the basis of MS as well as 1D- and 2D-NMR experiments. Due to the low concentrations of < 0.1mg/kg and the high taste thresholds found for eugenin and gazarin, these compounds could be unequivocally excluded as important contributors to the bitter taste of carrots. Calculation of bitter activity values as the ratio of their concentration to their bitter detection threshold clearly demonstrated that neither in fresh and stored carrots, nor in commercial carrot puree, 6-methoxymellein contributed to the bitter off-taste. In contrast, the concentration of falcarindiol in stored carrots and even more pronounced in carrot puree was found to be 9- and 13-fold above its low bitter detection concentration of 0.04 mmol/kg, thus demonstrating that this acetylenic diol is significantly contributing to the bitter taste of the carrot products investigated.

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1.1.8. Identification of a sweetness enhancer formed by Maillard reactions by application of the comparative taste dilution analysis

Application of a novel screening procedure, the comparative taste dilution analysis (cTDA) on the nonsolvent-extractable reaction products formed in a thermally processed aqueous solution of glucose and L-alanine led to the discovery of the presence of a sweet enhancing Maillard reaction product. Isolation, followed by LC/MS, 1D- and 2D-NMR measurements, and synthesis led to its unequivocal identification as N-(1-carboxyethyl)-6-hydroxymethyl-pyridinium-3-olate. To the best of our knowledge, this so-called alapyridaine, although being taste-less itself, is the first nonvolatile, sweet enhancing Maillard reaction product reported in the literature. Depending on the pH value, the detection threshold of sweet sugars, amino acids and aspartame, respectively, was found to be significantly decreased when alapyridaine was present, e.g. the threshold of glucose decreased by a factor of 16 in an equimolar mixture of glucose and alapyridaine. Studies on the influence of the stereochemistry on taste enhancing activity revealed that the (+)-(S)-alapyridaine is the physiologically active enantiomer, whereas the (-)-(R)-enantiomer did not affect sweetness perception at all.

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1.2. Techno-Functional Properties

1.2.1. Study of the effect of PGPR in chocolate: Influence on the flow properties of chocolate

The effect of PGPR on the yield value of chocolate mass was demonstrated by means of a dynamic stress rheometer with a plate/plate measurement system. It has been shown that mixtures of PGPR and lecithin are more active than the two emulsifiers alone. To isolate fractions and components of PGPR that were sufficiently high for rheological measurements, a commercial PGPR sample was fractionated by extraction with n-hexane and methanol and by column chromatography. The rheological measurements gave an interesting result: PGPR contains non-polar as well as polar components which both are able to strongly decrease the yield value of chocolate mass. The simple PGPR components 1-monoricinolein, 1-diricinolein, and 1-triricinolein were synthesized to study the influence of the ricinoleic acid chain length on the flow properties of chocolate mass. To investigate the influence of the polar site of PGPR, glycerol, diglycerol, and triglycerol were esterified with polycondensated ricinoleic acid. In some cases stearic acid was used as a chain terminator. It was found that the ricinoleic acid chain length as well as the degree of polymerization of the glycerol portion has a positive influence on the flow properties of chocolate. Elongation of the fatty acid site from one to three ricinoleic acid units led to a decrease of the yield value. The same effect was observed when diglycerol or triglycerol instead of glycerol were used as a basis for PGPR. A substantial influence can be attributed to the minor fatty acids of castor oil, which act as terminators for the ricinoleic acid chain and lead to an increased hydrophobicity of the entire molecule. For these classes of compounds a positive effect on the flow properties of the chocolate mass was established.

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1.2.2. Functional properties of pentosans from rye and wheat

To get evidence for structure-function relationships of pentosans in breadmaking, pentosans were isolated from four wheat and rye cultivars, respectively. They were characterized by the

determination of the carbohydrate composition. No significant differences were found within the wheat and rye pentosans. The determination of the content of ester-bound ferulic acid in the pentosans was carried out by alkaline hydrolysis and HPLC with UV detection at 310 nm. In addition, six diferulic acids were synthesized as reference compounds and analyzed in the polysaccharide samples. All of them were present in the samples. The degree of dimerization was calculated and monitored in the course of the breadmaking process. Neither for the wheat nor for the rye breadmaking process an increase of the degree of dimerization was found. Furthermore, the molecular weight pattern of the pentosans in relation to the breadmaking process was investigated by gel permeation chromatography. Strong differences between samples from flour, dough, and bread were found. In all samples four characteristic fractions with identical peak maxima and retention times were present. However, only two of them showed UV-absorbance at 310 nm. From this it can be assumed that only these two fractions might contain ferulic acid or diferulic acids. Since the degree of dimerization of ferulic acid is not correlated with the breadmaking process, the formation of high-molecular-weight pentosan fractions, as they were identified in the bread samples, is caused by other effects than ferulic acid dimerization.

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2. DEVELOPMENT OF SPECIAL ANALYTICAL TECHNIQUES

2.1. A rapid method for the simultaneous quantitation of (R)- and (S)-linalool in beer using solid phase microextraction (SPME) in combination with a stable isotope dilution assay (SIDA)

Application to monitor changes during storage of beer A stable isotope dilution assay (SIDA) was developed for the quantitation of both linalool enantiomers using synthesized [2H2]-R/S-linalool as the internal standard. For enrichment of the target compound from beer a solid phase microextraction method (SMPE) was developed. In comparison to the more time-consuming extraction/ distillation clean-up of the beer samples, the results obtained by SPME/SIDA were identical, even under non-equilibration conditions. The SPME/SIDA yielded exact data independently from headspace sampling parameters, such as exposition time or ionic strength of the solution. Analysis of five different types of beer showed significant differences in the linalool concentrations which were clearly correlated with the intensity of the hoppy aroma note as evaluated by a sensory panel. In addition, significant differences in the R/S ratios were measured in the beers. Storage of the beers over 12 months did not influence the total amounts of linalool but led to partial racemization resulting in a loss of the (R)-isomer in favor of the 80 times less aroma-active (S)-isomer. Model experiments showed that the pH-value is an important factor for the racemization rate of linalool during beer storage.

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2.2. A new, selective LC/MS-method for the quantitation of acrylamide based on a stable isotope dilution assay

Acrylamide (AA) was found to form a stable thioether in reasonable yields when reacted with 2-mercaptobenzoic acid (40 to 50 % after 3 h at 20€ÊC). Based on this finding and using [13C3]-AA as the internal standard, a sensitive, and selective new stable isotope dilution analysis (SIDA) for AA quantitation in food samples was developed based on single stage LC/MS. Comparison of the quantitative results obtained by applying the new method to

potato chips, crispbread or butter cookies with data obtained by two SIDAs, using direct measurement of AA by GC/MS, but differing in the work-up procedure, revealed detection limits in the same order of magnitude (6.6 εg/kg). Quantitative data obtained by application of the three methods on the same samples of potato chips or cookies, respectively, were also in very good agreement. Quantitation of AA in crispbreads treated with an amylase/protease mixture did not show increased AA levels, thereby indicating that inclusion of AA in starch/protein gels is not very probable during breadmaking.

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2.3. Specific and sensitive quantitation of folate vitamers in foods by stable isotope dilution assays using high-performance liquid chromatography-tandem mass spectrometry

Stable isotope dilution assays were developed for the quantitation of the folate vitamers 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, 10-formylfolate and pteroylglutamic acid in food samples by using deuterated isotopomers as internal standards. Vitamers and their labeled analogues were analyzed simultaneously by HPLC/MS/MS using selected reaction monitoring (SRM), which allowed a higher specifity than other methods published previously. Sample preparation involved treatment by protease in sequence with α-amylase and rat serum deconjugase followed by anion exchange chromatography. The detection limits for 5-methyltetrahydrofolate, 5-formyltetrahydrofolate, tetrahydrofolate, 10-formylfolate and pteroylglutamic acid were found to be 0.5, 1.2, 1.5, 0.6 and 2.6 εg/100 g fresh weight, respectively. Using the new method, folate contents were determined in meat, cereals, and vegetables. Data were in good agreement with literature data, except results for broccoli, which were much lower than reported in previous studies.

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2.4. Pantothenic acid quantification by a stable isotope dilution assay based on liquid chromatography-tandem mass spectrometry

A stable isotope dilution assay for the quantification of free and total pantothenic acid has been developed by using [13C3,15N]-pantothenic acid as the internal standard. The three-dimensional specifity of liquid chromatography-tandem mass spectrometry enabled the unequivocal determination of the vitamin. Due to the very simple extraction and clean-up procedure, free pantothenic acid could be analyzed within two hours, which is much faster than by microbiological or gas chromatographic assays. For quantification of total pantothenic acid, the vitamin was liberated from its conjugates by an overnight incubation with pigeon liver pantotheinase and alkaline phosphatase. In analyses of corn flour, the intra-assay coefficient of variation was 8.5 % (n=5) and 15.3 % (n=4) for free and total pantothenic acid, respectively. When pantothenic acid was added to corn starch at a level of 6 mg/kg, a recovery of 97.5 % was found. Application of the stable isotope dilution assay to whole egg powder, hazel nuts, corn revealed similar data compared to those listed in nutrition data bases, whereas the content in mushrooms and porcine liver determined by the newly developed assay appeared to be lower and that of cocoa higher than reported in the literature.

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2.5. Comparison of methods for the quantitative determination of phospholipids in lecithins and flour improvers

Phospholipid classes were determined qualitatively and quantitatively in 8 commercial lecithins and 3 flour improvers by thin layer chromatography (TLC), high-performance liquid chromatography (HPLC) and 31P nuclear magnetic resonance spectroscopy (31P NMR). The total amounts of phospholipids as well as the amounts of phospholipid classes in the samples were comparable, but depended on the method used for quantification. Highest selectivity was provided by 31P-NMR as all phospholipids and lysophospholipids could easily be quantified. By TLC only lysophosphatidyl choline could not be quantified, whereas HPLC was the method with the lowest selectivity, because lysophospholipids, except lysophosphatidyl ethanolamine, could not be determined. Sensitivity was best for HPLC and TLC with detection limits of 20 to 170 εg/mL. By means of 31P-NMR these figures increased by a factor of 10 to 70. The coefficients of variation were 5.5, 6.8, and 12.8 % for quantification by TLC, HPLC, and 31P-NMR, respectively, showing that TLC was the method with the best reproducibility. Altogether, 31P-NMR can be recommended for the quantification of phospholipids, because it is easy to perform and results can be quickly obtained. As it requires minimum instrumental equipment, TLC is a good alternative to 31P-NMR. If high sensitivity is required, HPLC is the best method.

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3. RELATIONSHIP BETWEEN THE STRUCTURE OF BIOPOLYMERS AND THEIR TECHNOLOGICAL PROPERTIES

3.1. Effect of high pressure on the rheological and chemical properties of gluten

Structure and properties of proteins can be irreversibly changed by treatment with high pressure. For example, proteins of egg, milk, meat and fish are denatured by a hydrostatic pressure of 300 - 400 MPa even at room temperature and form gels. It is also known that high pressure influences gluten properties, but only poor detailed information is available in literature. The aim of the present work was, therefore, to characterize the effect of high pressure on the rheological and chemical properties of gluten in dependence on temperature and incubation time. Gluten of the wheat cultivars Astron (good baking quality, strong gluten) and Contra (poor baking quality, weak gluten) was isolated by pasting and washing with diluted NaCl solution (wet gluten). A portion of the gluten obtained was freeze-dried (dry gluten). Wet gluten or rehydrated dry gluten were treated in a high-pressure instrument, whereupon pressure (0.1 - 800 MPa), temperature (30 - 80€ÊC) and incubation time (5 - 30 min) were modified. Rheological changes were measured by extension tests (maximum resistance, extensibility) and chemical changes were determined by quantitation of gliadin and glutenin fractions using an extraction and RP-HPLC procedure. Treatment of wet gluten with 200 MPa at 30 38€ÊC for 30 min increased extensibility and decreased resistance; at higher pressure and temperature wet gluten became stronger and less extensible. The effects on Contra gluten were more intense then on Astron gluten and extensilibity was more sensitive to pressure than maximum resistance. Extension tests of gluten treated with very high pressure and temperature (e.g. 600 MPa, 60€ÊC) were not possible, because cohesivity of gluten was lost. Altogether, changes in pressure had much stronger effects on rheological properties than changes in temperature. The effect of pressure on gluten properties was dependent on the kind of salts added to gluten before treatment; for example, CaCl2 showed stronger effects than NaCl. Quantitation of gluten proteins indicated that the ratio of gliadin to glutenin was increased by relatively low pressure (200 MPa) and was decreased by high pressure and temperature. These results were in agreement with changes in rheological properties. Within gliadin types cysteine-free ‰-gliadins were not sensitive to pressure. In contrast, extractability of cysteine containing α- and γ-gliadins was strongly decreased after treatment

with high pressure (e.g. 800 MPa, 60€ÊC). Because these proteins were completely recovered in the glutenin fraction after reduction of disulfide bonds, it could be assumed that cleavage and restructuring of disulfide bonds were involved in pressure-induced chemical reactions.

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3.2. Studies on high-molecular-weight glutelin aggregates from transgenic rye stably expressing HMW subunits of wheat

Numerous studies described in literature indicate that the amount of SDS-insoluble glutenin aggregates, the so-called glutenin macropolymer (GMP), is highly correlated with dough strength and bread volume of wheat. Corresponding studies on rye flour have not been done until now. The aim of the present investigation was to determine amount and protein composition of GMP of non-transgenic rye (L22) in comparison with two transgenic rye lines (L26, L8) stably expressing HMW subunit 10 and HMW subunits 5+10 of wheat, respectively. Flours were extracted with a buffered SDS containing solvent (1 %, pH 6,9) at room temperature. Dried extracts and residues were dissolved in 50 % 2-propanol under reducing conditions and analyzed by means of RP-HPLC on C8 silica gel. The results demonstrated that the non-transgenic flour L22 contained only a very small amount of GMP (about 6 % of total protein corresponding to about 5 mg per g flour). GMP of L22 majorly consisted of γ-75k-secalins (64 %). After introduction of HMW subunit 10 (L26), the proportion of GMP was increased to about 22 % and its amount to about 20 mg per g, a value corresponding to the lower range of GMP found in different wheat cultivars. Quantitation of protein types revealed similar proportions of γ-75k-secalins (48 %) and HMW subunits (42 %) present in GMP of L26. HMW subunits 5+10 (L8) caused a drastic increase of GMP to 63 % based on total protein corresponding to about 60 mg per g flour. This amount was much higher than the range shown for wheat cultivars (20 37 mg). The proportion of HMW subunits (49 %) was mugh higher than that of γ-75k-secalins (34 %). Altogether, the results indicated that HMW subunits of wheat, in particular, HMW subunit 5 provoked a drastic shift of monomeric and oligomeric flour proteins into SDS-insoluble aggregates by the formation of additional intermolecular disulfide bonds. The high proportion of GMP in relation to oligomeric and monomeric proteins might be responsible for the poor baking performance of L8 flour shown by a previous study.

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3.3. Studies of partial sequences of γ-40k-secalins of rye

Though γ-40k-secalins are a major protein type within rye storage proteins, total amino acid sequences are not known in contrast to gluten proteins of wheat. Well-reputed structural features such as amino acid compositions and molecular masses indicated a close relationship between γ-40k-secalins and γ-gliadins of wheat, but the degree of homology of amino acid sequences and the positions of intramolecular disulfide bonds are unknown. Therefore, two major components of γ-40k-secalins (R1, R2) were analyzed for partial amino acid sequences. R1 and R2 derivatized with 4-vinylpyridine were isolated from the prolamin fraction of rye cultivar Danko by means of a two-step RP-HPLC on C18 silica gel. The proteins were digested in parallel with trypsin and thermolysin and the partial hydrolysates were separated by RP-HPLC. Simultaneous measurement of UV absorbance at 210 and 254 nm allowed the detection of all peptides eluted as well as the specific detection of pyridylethylated cysteine peptides. Isolated peptides were characterized by sequence analysis and, in parts, by mass spectrometry, and assigned to known sequences of γ-gliadins. The results demonstrated that

the N-terminal domain of R1 and R2 remained undigested after tryptic hydrolysis; they agreed with γ-gliadins in their molecular masses and in the absence of cysteine residues. Most of the isolated peptides were originated from the C-terminal domains, they covered 83 % (R1) and 77 % (R2), respectively, of the C-terminal domain of a known γ-gliadin (clone pW1020). The comparison of R1 and R2 revealed differences only in a few sequence positions. The degree of homology between the C-terminal domains present in γ-40k-secalins and γ gliadins pW1020 was about 85 %. All eight cysteine residues of γ-gliadins were found in R1 and R2 sequences, remarkably, sequences close to corresponding cysteine residues were identical for γ-40k-secalins and γ-gliadins. Therefore, it can be assumed that the positions of disulfide links are homologous.

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3.4. Study on the heat-induced aggregation of milk proteins by thiol-disulfide exchange reactions

Application of heat to milk proteins leads to aggregates that are stabilized by intermolecular disulfide bonds. The formation of the aggregates is caused by thiol/disulfide exchange reactions provoked by the free thiol group of β-lactoglobulin. Aim of the present study was to detect free thiol and disulfide groups in native and heated solutions of milk proteins and to describe the course of the thiol/disulfide exchange reactions induced by heat. An online detection system for disulfide bonds has been developed and has successfully been applied for the determination of native and heat-induced disulfide bonds of milk proteins. It has been demonstrated, that aggregation of β-lactoglobulin follows a two-step process. Firstly the native thiol group is shifted to a more hydrophilic part of the molecule (positions 66 and 160) corresponding to an activation of β-lactoglobulin for intermolecular reactions. In a second step the newly formed thiol groups can react intermolecularily with another β-lactoglobulin molecule, with α-lactalbumin or with κ casein. This type of reaction induces a free thiol group in α-lactalbumin and κ-casein, respectively, which is then able to promote polymerization by incorporating β-lactoglobulin, α-lactalbumin, and κ-casein molecules into the aggregates. In this study, heat induced disulfide bonds between whey proteins and caseins have been proven for the first time. Furthermore, it has been shown that αS2-casein does probably not participate in thiol/disulfide exchange reactions because of steric hindrance.

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4. PHYSIOLOGY

4.1. In vitro studies on the effect of malt molecular weight fractions on biotransformation enzymes in intestinal caco-2 cells

In the present study, water-soluble non-enzymatic browning products (melanoidins) formed in roasted malt were separated, quantified and investigated for their effects on detoxifying mechanisms in intestinal Caco-2 cells. The melanoidins were prepared from roasted malt by hot water extraction and the water-soluble compounds were separated into different molecular weight (MW) fractions by gel filtration chromatography. By monitoring the effluent at 300 nm, seven molecular fractions I-VII were consecutively collected, revealing that about 2.3 % of the water-soluble compounds had mean MWs between 10 and 30 kDa. Thus, the bulk of water-soluble malt melanoidins consisted of MW > 30 kDa, amongst which about 58 % showed mean MWs between 60 and 100€kDa, whereas about 32 % exhibited mean MWs of 200 kDa. Biotransformation enzyme activities of NADPH-cytochrome c-reductase (CCR) and

glutathione-S-transferase (GST) were analyzed in Caco-2 cells after 48 h of exposure to the different MW fractions. The low MW fraction of 10 kDa was most effective in activating the CCR and the GST activity (+122 % and +33 % vs control, resp.). The majority of the mid MW compounds tested showed an activating effect on CCR and an inhibitory effect on GST activities. These effects were most pronounced for compounds of up to 70 kDa and > 200 kDa, but less distinct for fractions of an average MW of 100 kDa.

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4.2. Pronyl-lysine as an antioxidant, chemopreventively active site of bread crust melanoidins in vitro

Application of an in vitro antioxidant assay to solvent fractions isolated from bread crust, crumb and flour, respectively, revealed the highest antioxidative potential for the dark brown, ethanol solubles of the crust, whereas corresponding crumb and flour fractions showed only minor activity. In order to investigate whether these browning products may also act as antioxidants in biological systems, their modulating activity on detoxification enzymes was investigated as a functional parameter in intestinal Caco-2 cells. The bread crust and, in particular, the intensely brown, ethanolic crust fraction induced a significantly elevated glutathione-S-transferase (GST) activity and a decreased Phase I NADPH-cytochrome c-reductase (CCR) activity compared to crumb-exposed cells. Antioxidant screening of Maillard-type model mixtures, followed by structure determination revealed the pyrrolinone reductones 1 and 2 as the key antioxidants formed from the hexose-derived acetylformoin and Nα-acetyl-L-lysine methyl ester or glycine methyl ester, chosen as model substances to mimic non-enzymatic browning reactions between the lysine side chain or the N-terminus of proteins, respectively. Quantitation of protein-bound pyrrolinone reductonyl-lysine, abbreviated as pronyl-lysine, revealed high amounts in the bread crust (62.2 mg/kg), low amounts in the crumb (8.0 mg/kg), and the absence of this compound in untreated flour. Exposing Caco-2 cells for 48 h to either synthetically pronylated albumin or to purified pronyl-glycine (3) significantly increased Phase-II GST activity by 12 % or 34€%, respectively, thus demonstrating for the first time that pronylated proteins as part of bread crust melanoidins act as monofunctional inducers of GST, serving as a functional parameter of an antioxidant, chemopreventive activity in vitro.

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4.3. RAGE-mediated MAPK activation by food-derived Maillard reaction products and by N-methylpyridine, a non-Maillard reaction product formed in roasted coffee

Investigating the cellular effects of food compounds formed by heat treatment during processing, we recently demonstrated the expression of the receptor for advanced glycation end products (RAGE) and the p44/42 MAP kinase activation by casein-Nε-(carboxymethyl)lysine (casein-CML), a food-derived AGE, in the intestinal cell line Caco-2. In this work, we report a Caco-2 p44/42 MAP kinase activation by bread crust and coffee extract. After identification, quantification and synthesis of two key compounds formed in association with the process-induced heat impact applied to bread dough and coffee beans, those compounds, namely the AGE pronyl-glycin and the non-AGE N methylpyridinium, were also demonstrated for the first time to activate the p44/42 MAP kinase through binding to RAGE in Caco-2 cells. Blocking of RAGE by an antagonistic antibody and expression of C-terminally truncated RAGE resulted in a reduced Caco-2- and HEK-293-MAP kinase activation. These findings unequivocally point to a RAGE-mediated activating effect of

chemically defined food-derived, thermally generated products, both, AGEs and non-AGEs, on cellular signal transduction pathways involved in inflammatory response and cellular proliferation.

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4.4. Expression of the biotransformation enzyme GST by dietary Nε-carboxymethyllysine in the rat

Nε-Carboxymethyllysine (CML) is an advanced glycation end product, found in both heat treated foods and in living organisms. In the experiments described below, we have evaluated the effects of CML on the expression of the detoxification enzyme, glutathione-S-transferase(GST), in the rat and in intestinal cells in cell culture. In the first animal experiment, casein-linked CML was administered to Wistar rats at two pharmacological doses (110 and 300 mg CML x kg body weight-1 x d-1) for 10 days. Phase-II GST enzyme activity was analyzed in the kidneys. In a second study, CML was administered in a diet supplemented with bread crust (25 % w/w), such that rats received a moderate dose of 11 mg CML x kg body weight-1 x d-1. The induction of GST isoenzymes was analyzed in the kidneys. In vitro experiments were also performed to study the effects of casein-CML and bread crust on GST induction in the intestinal Caco-2 cell line. Both animal experiments revealed an inductive effect of casein-linked CML and of bread crust on GST activity in the kidneys. The results obtained from the cell culture experiments by enzyme activity analysis and Western Blotting confirmed these inductive effects on the GST. In conclusion, dietary advanced glycation products containing CML were shown to enhance the expression of GST in both animal models and in cell culture.

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4.5. Coeliac diesease-specific immunological studies on peptides from α-gliadins

Previous studies have shown that a peptide corresponding to amino acid residues 56 - 75 of α-gliadins exacerbates coeliac disease in vivo and that fragment peptides corresponding to residues 57 - 68 and 62 - 75 stimulate T cells from coeliac patients. In order to find out which residues are essential for T cell stimulation, different synthetic peptides were produced and studied using T cell stimulation assays. Because deamidation of the glutamine residue at position 65 (Q65) has been shown to be important for T cell activation, peptides were modified with glutamic acid at that position (E65). The peptides were synthesized by means of a peptide synthesizer and purified by two steps of reversed-phase liquid chromatography. All peptides were chromatographically pure and their masses determined by mass spectrometry corresponded to the theoretical values. T cells were isolated from small intestinal biopsies obtained from coeliac patients and cloned. T cell lines and clones were incubated with antigen-presenting cells, which had been pre-incubated with peptic-tryptic digests of gliadin or gluten or the peptide to be investigated, and 3H-thymidine. The stimulatory effect was determined by the measurement of radioactivity incorporated and cytokine concentration. Firstly, the stimulatory effects of peptide G9 (α56 - 75/E65) and the overlapping fragment peptides G5 (α56 - 68/E65) and G4 (α62 - 75/E65) were studied. The results demonstrated that stimulation index and γ-interferon production were highest for G9, followed by G4; the values for G5 were significantly lower. At various concentrations of peptide, stimulation of T cells with the non-deamidated peptide G8 (Q65) was consistently "H50 % less than that with G9 (E65). For further studies, the sequence of peptide G4 was modified by an alanine residue in each position except E65 (G4-1A - G4-14A).

Immunoassays demonstrated that while substitutions G4-11A through G4-14A had no effect on T cell stimulation, other substitutions had a profound influence. Substitutions at position G4-3A through G4-10A abolished stimulation with the exception of position 9 (G4-9A), where there was residual γ interferon production. Substitutions at positions 1 and 2 led to a partial downregulation of T cell stimulation. Thus, the results indicated that sequences including residues 62 - 71/ E65 (PQPELPYPQP) were important for an immunological effect. Peptide G9 was shortened stepwise by one residue of the N- and C-terminus. The stimulatory effects of peptides G9-2 through G9-8 did not significantly differ from that of peptide G9. Shortening to peptide G9-10 (FPQPELPYPQ) caused a partial downregulation of stimulation, whereas peptides G9-12 (PQPELPYP) and G9-14 (QPELPY) had no significant stimulatory effect.

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5. 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 för 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 work on the 3rd edition of the small Table Der kleine Souci-Fachmann-Kraut: Lebensmitteltabelle für die Praxis has been finished, the publication is planed for autumn this year. The preparation of the 7th edition has been continued. A detailed evaluation of the database showed data gaps and old data especially in the case of the amino acids. Therefore the data of this group partly has been revised and as an important aspect in the actual discussion about disease prevention and health, data of the group of the bioactive compounds has been extended. Due to the development of the analytical methodology some data should be actualized in the future (e.g. iodine and folic acid data). The Online-Version of the Souci-Fachmann-Kraut Food Composition and Nutrition Table is availiable on the WWW since January 2001. The concept of the database with its different search and calculation tools enables the user to search for the specific food items, defined concentration and energy values, respectively. In order to document the data quality and to give the user informations about the scientific background of the SFK-database, the publication of the following parameters: source of literature, analytical methodology and number of samples has been realized with the last update of the online-version.

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