Leibniz Institut
Deutsche Forschungsanstalt für Lebensmittelchemie

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1. STUDIES ON THE HEDONIC VALUE OF FOOD

1.1. Aroma and Taste (Flavour) as Parameters of Food Quality

1.1.1. Sensory Study on the Character Impact Odorants of Roasted Arabica Coffee

The potent odorants were quantified in a sample of roasted Arabica coffee originating from Colombia. On the basis of the results 27 odorants showing high odor activity values were dissolved in an oil/water mixture (1:20, v/v). The flavor profile of the model obtained was very close to that of the real sample. In duo and triangle tests the model was compared with models missing one or several odorants. The sensory analyses were performed by 10 assessors who evaluated 18 different models. These experiments indicated that 2-furfurylthiol, 4-vinylguaiacol, several alkyl pyrazines, furanones, acetaldehyde, propanal, methylpropanal, 2- and 3-methylbutanal had caused the coffee flavor.

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1.1.2. Flavour and off-flavour compounds of black and white pepper (Piper nigrum L.)

Dilution and concentration experiments as well as enantioselective analysis of optically active monoterpenes indicated (()-linalool, (+)-(-phellandrene, (-)-limonene, myrcene, (-)-(-pinene, 3-methylbutanal and methylpropanal as the most potent odorants of black pepper. Additionally, 3-isopropyl-2-methoxypyrazine and 2,3-diethyl-5-methylpyrazine were detected as important odorants of a black pepper sample with a moldy, musty off-flavour. Quantification of fourteen odorants and calculation of the odour activity values were the basis of an aroma model reflecting most of the odour notes of black pepper. Omission tests indicated (- and ß-pinene, myrcene, (-phellandrene, limonene, linalool, methylpropanal, 2- and 3-methylbutanal, butyric acid and 3-methylbutyric acid as key odorants. The musty/moldy off-flavour of a sample of black pepper was caused by a mixture consisting of 2,3-diethyl-5-methylpyrazine (2.9 µg/kg) and 3-isopropyl-2-methoxypyrazine (0.2 µg/kg). Quantification of 19 odorants and calculation of their odour activity values were the basis for an aroma model which reflected the odour profile of a white pepper sample showing a faecal off-flavour. Omission tests indicated limonene, linalool, (-pinene, 1,8-cineole, piperonal, butyric acid, 3-methylbutyric acid, methylpropanal, 2- and 3-methylbutanal as key odorants of white pepper. The faecal off-flavour was caused by skatole and was enhanced by the presence of p-cresol. In six samples of white pepper the intensity of the faecal off-flavour paralleled the concentration of both, skatole and p-cresol. In the sample with the strongest off-flavour the concentrations amounted to 2.6 mg/kg (skatole) and 12.4 mg/kg (p-cresol).

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1.1.3. Identification of the key odorants in barley malt (caramalt)

Application of the Aroma Extract Dilution Analysis on a distillate prepared from ground caramalt kernels followed by identification experiments revealed 3-methylbutanal (malty), 1-octen-3-one (mushroom-like), methional (cooked potato), (E,E)-2,4-decadienal (fatty, waxy), vanillin (vanilla-like), 2- and 3-methylbutanoic acid (sweaty) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like) as the most odor-active compounds. Using static headspace/olfactometry, the very volatile odorants dimethyl sulfide (cooked vegetable-like) and 2-methylpropanal (malty) were identified as additional odorants contributing to the overall rye-bread crust-like odor of the caramalt.

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1.1.4. Fatty acid tryptamides as shell indicators in cocoa products

Tetracosanoyl-2-(3-indolyl)ethane amide (lignocerinic acid tryptamide; LAT) and docosanoyl-2-(3-indolyl)ethane amide (behenic acid tryptamide; BAT) were identified as the most prominent tryptamides in cocoa shells based on electrospray-mass spectrometry and 1H-NMR-measurements. The structure of LAT, which is reported for the first time in cocoa shells, and, also, that of BAT was confirmed by synthesis. By using synthesised heptadecanoyl-2-(3-indolyl)ethane amide as the internal standard, a sensitive and reproducible method was developed for the quantification of LAT and BAT in the pg-range by means of HPLC/ fluorescence detection. The detection limit was determined to be 30 pg/run. In authentic shell samples 50 fold higher concentrations of both tryptamides were determined compared to the cocoa endosperm. In 15 commercial chocolate samples, concentrations of 23.1 µg to 63.0 µg of the tryptamides (sum of both) per g of fat were found. A first experiment attempted to correlate the tryptamide content with the amounts of shells in a model chocolate showed that the method is a promising tool to determine the shell content in the quality assessment of cocoa products.

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1.1.5. Solvent Assisted Flavour Evaporation (SAFE) - a new and versatile technique for the careful and direct isolation of aroma compounds from complex food matrices

A compact and versatile distillation unit was developed for the fast and careful isolation of volatiles from complex food matrices. In connection to a high vacuum pump (5 x 10-3 Pa), the new technique, assigned as Solvent Assisted Flavour Evaporation (SAFE), allows the isolation of volatiles from either solvent extracts, aqueous foods, e.g. milk or beer, aqueous food suspensions, such as fruit pulps, or even matrices with a high oil content. Application of SAFE to model solutions of selected aroma compounds resulted in higher yields from either solvent extracts or a fatty matrix (50 % fat), respectively compared to previously used techniques, such as the high vacuum transfer. Direct distillation of aqueous fruit pulps in combination with a stable isotope dilution analysis enabled the fast quantification (60 min including MS analysis) of e.g., the very polar and unstable 4-hydroxy-2,5-dimethyl-3(2H)furanone in e.g. strawberries (3.2 mg/kg) or tomatoes (340 µg/kg). Furthermore, the direct distillation of aqueous foods, such as beer or orange juice, gave flavourful aqueous distillates free from non-volatile matrix compounds.

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1.2. Colour as Parameter of Food Quality

1.2.1. Comparison of the effectivity of amino acids and proteins in melanoidin formation

In heated aqueous solutions of glucose and alanine the majority of colored compounds formed were shown to have molecular weights below 1000 Da. Compounds with molecular weights above 3000 Da were found in only trace amounts, whereas high-molecular colorants could not be observed under the conditions applied, which are typical for cooking processes of foods. Contrary, the reaction between casein and glucose leads to a drastic increase in the molecular weights by a carbohydrate-induced oligomerization of the protein. More than 43 % of the reaction mixture was shown to be pentamers or even higher oligomers of casein exhibiting molecular weights of more than 100 000 Da. This cross-linking of casein was found to run in parallel with the color intensity of the products formed, indicating that chromophoric substructures, derived from carbohydrates, are incorporated into these oligomers. The data indicate that the formation of melanoidins during cooking of foods by polymerization of reactive low-molecular weight compounds should be very unlikely in the Maillard reaction between carbohydrates and amino acids.

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1.2.2. Characterization of a colour precursor in the early stage of the Maillard reaction of carbohydrates and primary amino acids

The Maillard reaction between carbohydrates and amino acids are chiefly responsible for the browning formation during thermal food processing. Studies on the effectivity of certain carbohydrate degradation products in browning development revealed furan-2-aldehyde and glycolaldehyde as by far the most effective colour precursors when reacted in the presence of L-alanine. ESR studies demonstrated that furan-2-aldehyde generated coloured compounds exclusively via ionic mechanisms, whereas glycolaldehyde led to colour development accompanied by intense radical formation. In corroboration with literature data, these radicals were also detected in heated mixtures of L-alanine and pentoses or hexoses, respectively, and were identified as 1,4-dialkylpyrazinium radical cations by ESR as well as LC/MS measurements. Studies on the mechanisms of radical formation revealed that, in contrast to the literature, glyoxal is formed as an early product in hexose/L-alanine mixtures prior to radical formation. Reductones then initiate radical formation upon reduction of glyoxal and/or glyoxal imine, formed upon reaction with the amino acid, into the radical precursors glycolaldehyde and alkylaminoacetaldehyde, respectively. Aiming at the clarification of the role of these radical cations in colour formation, model experiments revealed that not the radical cations itself, but hydroxylated 1,4-dialkyl-1,4-dihydropyrazines formed upon oxidation and hydrolysis are the penultimate colour precursors in the early stage browning reaction of carbohydrates and amino acids.

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1.2.3. "CROSSPY" - A novel radical intermediate in the melanoidin formation in bread crust and roast coffee

Colour generating reactions of protein-bound lysine with carbohydrates were studied under thermal conditions in order to gain insights into the role of protein/carbohydrate reactions in the formation of food melanoidins. ESR spectroscopy of melanoidins, which were isolated from heated aqueous solutions of bovine serum albumine (BSA) and glycolaldehyde, and synthetic experiments with N(-acetyl-L-lysine revealed protein-bound 1,4-bis-(5-amino-5-carboxy-1-pentyl)-pyrazinium radical cations (CROSSPY) as a previously unknown type of

cross-linking amino acid leading to protein dimerization and melanoidin formation. To verify their formation in foods, wheat bread crust, and roasted coffee beans, showing elevated non-enzymatic browning, were investigated by ESR spectroscopy. An intense radical was detected, which, by comparison with the radical identified in the BSA/glycolaldehyde model experiment, was proposed as the protein-bound CROSSPY.

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1.2.4. "BISARG" - a Novel Colored protein modification crosslinking two arginine moieties

When N(-acetyl-L-arginine was heated with glyoxal in aqueous solution at pH 7.0 in the presence of furan-2-aldehyde, an intense red-brown colour was developed. The compound mainly evoking this colour was identified as (S,S)-1-(4-acetylamino-4-carboxy-1-butyl)-2-imino-4-[(Z)-(2-furyl)methylidene]-5-(2-[1-(4-acetylamino-4-carboxy-1-butyl)-4-[(E)-(2-furyl)methylidene]-5-oxo-1,3-imidazol-2-inyl](azamethylidene-1,3-imidazolidine BiSARG by application of several one- and two-dimensional NMR experiments and, in addition, by LC/MSn measurements and UV/VIS spectroscopy. This is the first time that a chromophoric compound comprising four linked rings with two arginine moieties incorporated was identified in a Maillard reaction system. This novel type of chromophore indicates the possibility that food melanoidins might be generated by protein oligomerization via colored cross-linking structures, e.g. between two arginine residues.

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2. STUDIES ON THE RELATIONSHIP BETWEEN THE STRUCTURE OF BIOPOLYMERS AND THEIR TECHNOLOGICAL PROPERTIES

2.1. Study of the effect of DATEM

DATEM was fractionated by gel permeation chromatography and HPLC to isolate individual components with good baking performance. Their activity was checked by micro-scale methods on the basis of 10 g of flour. Three major components were isolated and their structures were determined by mass spectrometry and NMR spectroscopy. The major component of DATEM is a diacylglycerol with stearic acid in position 1 and diacetyl tartaric acid in position 3 of glycerol. The major components were synthesised and characterised by micro-scale (10 g of flour) and normal-scale (300 g of flour) baking tests and by micro-scale rheological methods. From this, models for the mechanism of the action of DATEM were deduced. Finally, DATEM synthesis was optimised in order to produce high contents of the threee major components. Low temperatures during synthesis (100°C) were favorable for the production of the major DATEM components. The application of sodium acetate at a temperature of 100°C increased the content of active components up to 88 %. Commercial DATEM samples contained only 35 to 57 % of active components. With these optimised DATEM samples, the quality of bread can be maintained by using lower amounts of additive.

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2.2. Fractionation and reconstitution of wheat flour - Effect on dough rheology and baking

A method for the fractionation and reconstitution of wheat flour was developed, which makes it possible to investigate the contribution of flour fractions to baking performance and dough rheology. Micro-scale rheological methods and a micro-scale baking test on the basis of 10 g of flour were used. Two wheat varieties with different baking performance were studied. Fractionation involved defatting of flour by dichloromethane, formation of a dough, isolation of gluten by washing the dough with distilled water, collection of the aqueous suspension and separation into a soluble fraction and two starch fractions (upper and lower layer) by centrifugation. Reconstitution of flour gave reproducible results. The baking volumes of the reconstituted flours decreased by approximately 20 %, the respective doughs and glutens had higher resistances than the native flours. Mixing of reconstituted flour in the presence of reduced glutathione (0-150 nmol/g) decreased the dough resistance, but the values of the native flour could not be fully established. An increase of the total protein content of the reconstituted flour (8-16 %) was proportional to the baking volume. Increasing amounts of the soluble fraction led to more extensible, weaker doughs. Gliadin and glutenin had a weakening and strengthening effect on the dough, respectively. The addition of high molecular weight (HMW) subunits of glutenin caused an increase in dough extensibility and resistance, whereas with low molecular weight (LMW) subunits of glutenin only the dough resistance was increased. Micro-scale baking tests showed that HMW subunits increased the loaf volume strongly, while the opposite effect was observed on addition of LMW subunits.

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2.3. Characterisation of ω-gliadins from different wheat species

(-Gliadins are minor components of gluten proteins and characterised by high amounts of Glx and Pro. In contrast to the other gluten protein types, total amino acid sequences have not been described up to now and molecular masses were derived from SDS-PAGE mobility ranging from 55,000-79,000. Relevant studies on (-gliadins were performed only with common (bread) wheat; according to differences in amino acid compositions and molecular masses, (-gliadins were classified into the (5-type and the (1,2-type. Other wheat species have not yet been investigated. Therefore, (-gliadins were isolated by RP-HPLC from spelt, durum wheat, emmer and einkorn. In the case of common wheat, three different forms (winter wheat, spring wheat, wheat rye hybrid) were compared. The HPLC patterns of (-gliadins were typically different amongst wheats. Amino acid compositions of all (-gliadins analysed revealed significantly higher values for Glx, Pro and Phe compared with the other gluten proteins. Differences in the proportions of these amino acids allowed a clear differentation into (5- and (1,2-types. (5-Type gliadins had 51-57 % Glx, 18-21 % Pro and 9-10 % Phe, and (1,2-type had 39-45 % Gly, 22-31 % Pro and 6-8 % Phe. Furthermore, the proportions of Leu and Ile were typically different. Remarkably, gliadin fractions of emmer and einkorn did not contain any (1,2-type. The determination of molecular masses by MALDI-TOF mass spectrometry revealed a range of 44,000-55,000 for the (5-type and of 34,000-44,000 for the (1,2-type. Thus, real masses were, by far, lower than those derived from SDS-PAGE mobility.

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2.4. The influence of extreme sulfur fertilisation on mineral content, protein composition and gluten properties of wheat grown under ecological conditions

Deficiency of sulfur (S) in soil is known to decrease crop yield and wheat quality caused by very strong doughs and reduced bread volume. This can be prevented by a moderate S fertilisation. The influence of increased or extreme S fertilisation on wheat properties,

however, is not known. Therefore, wheat variety Bussard was grown under ecological conditions and different S fertilisation (0, 50, 100, 200, 400 kg S/ha). Analyses of kernels and whole-meal indicated that S fertilisation had no effect on N, S, K and Mg contents and on amounts of total gliadins, glutenins and HMW subunits. In contrast, S and K contents of straw were significantly increased by higher S fertilisation. HPLC analyses of endosperm meal did not reveal any difference in qualitative and quantitative gluten protein composition. Extension tests with gluten isolated from corresponding doughs, however, demonstrated a remarkable decrease of maximum resistance parallel with increasing S fertilisation. It can be assumed that S fertilisation influences the amount of low-molecular-weight thiol compounds, e.g., glutathione, in flour, which are known to weaken dough and gluten.

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2.5. Quantitative determination of thiol groups in wheat flour with Ellman's reagent

The presence of thiol groups and their differing contents in wheat flour have important effects on the rheological properties of dough and gluten, but only few quantitative data are available in the literature. Therefore, the classical method of Ellman was optimised for the application to wheat flour. The results of the quantitative determination of thiol groups in flour was strongly influenced by extraction procedure and solvent. The highest values were obtained with an SDS containing buffer using a method, which included 60 min for stirring flour suspension, before Ellman's reagent was added, and further 30 min for colour development. For the photometric determination of the coloured extract, measurement against a blank derived from the same amount of flour is recommended, because flour extract itself is coloured slightly yellow. Enzymatic digestion with thermolysin did not significantly improve the accessibility of thiol groups. During storage under air, the content of thiol groups in non-defatted flour decreased more than that in defatted flour. Flour milled under nitrogen had a higher amount of thiol groups than flour milled under oxygen. Rheological properties of dough and gluten were strongly dependent on the thiol content in flour. Prepared under nitrogen, they showed a lower maximum resistance and a greater extensibility than those prepared under air.

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2.6. Studies on the quality of dried gluten

Dry wheat gluten is applied by millers and bakers to improve the properties of baked products. The quality of commercial gluten, however, varies to a great extent and significantly depends on different processing parameters. The influence of such parameters like raw material (wheat variety) and processing conditions (e.g. drying temperature) are not know in detail. Moreover, a simple test of gluten quality is not available. Therefore, different dry glutens were characterised after rehydration by kneading and extension tests, oscillation measurements and baking tests on a micro-scale. Rheological properties of rehydrated gluten depended on the following parameters: quality of wheat variety, duration of the washing process, presence of salt, drying temperature and stress imput during drying. Drying temperatures above 60°C caused a strong increase in gluten hardness and a decrease in extensibility and were detrimental for gluten quality. Dough models based on starch and gelatine or based on rye flour were shown to be useful to judge the quality of gluten according to kneading properties and baking performance, respectively. Studies on freeze-dried gluten from nine wheat cultivars using these models demonstrated a good agreement with the kneading and baking qualities of corresponding flours.

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2.7. Microscopic structures in wheat dough and their influence on the porperties of dough and bread

When optimally mixed wheat doughs are reshaped after a short resting time, gluten and starch separate: gluten particles aggregate and develop a thicker and more coherent network, whereas starch accumulates in the meshes of this network. This demixing of protein and starch is accompanied by dough-hardening resulting from the more coherent network and from the rheopex behaviour of accumulated starch. Dough-hardening is observed in uniaxial extension tests and can be used as an indicator of the degree of demixing. Microscopic investigations of dough and bread crumb show that demixing of dough gives rise to an amelioration of loaf volume and shape and to larger crumb pores. The degree of demixing depends on gluten quality and, therefore, on wheat cultivar and can be influenced by additives hindering the aggregation of gluten, i.e. oil, pentosans or urea.

3. CHARACTERISATION OF TOXIC STRUCTURES IN PROTEINS

3.1. Binding of prolamin peptides to small intestinal brush border membranes of coeliac patients and control persons

According to the pathogenesis of coeliac disease, significance of prolamin peptide interactions with enterocyte membranes is controversially discussed. For binding studies, two peptides (GXI: FPGQQQPFPPQQ, GXIV: LQPQNPSQQQPQ) from the coeliac active N-terminal region of (-gliadins and a peptide (ZI: LAPSAIIPQFLPPV) from the repetitive domain of non-toxic zein were, therefore, synthesised by using an automatic synthesizer. The peptides were biotinylated and purified by reversed-phase HPLC and gel permeation HPLC. Purity and composition were confirmed by automatic sequencing and electrospray ionisation MS. Additionally, a peptic tryptic digest of gliadin (PT-GLI) was produced. Brush border membranes were isolated from small intestinal biopsies of untreated coeliac patients (n = 49), treated coeliac patients (n = 30) and control persons (n = 43). Binding capacities of membranes were measured with a dot blot chemiluminiscence assay (streptavidin conjugated with peroxidase, luminol). Comparing treated patients with controls, significantly enhanced membrane binding of PT-GLI and of ZI could be observed, but only slightly increased binding of the synthetic gliadin peptides GXI and GXIV. The values for untreated patients were between those of the other groups. Independent of groups, membrane binding capacities for coeliac-active gliadin peptides exceeded those for the zein peptide.

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4. FOOD COMPOSITION AND NUTRITION TABLES

4.1. Actualisation of the 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 whole scientific data material and by means of the PC-data bank 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. In future, the spectrum of food constituents covered in the large SFK-nutrition table will also adress preventive-medical

aspects. At the period of this report the following tasks have been carried out: - Finishing of the actualisation of the data; establishing of the printing sheets by the SFKDB data bank; submitting of the corrected printing sheets to the members of the BML-working group for the SFK-table for critical review; then submitting of the whole tables as final printing sheets to the publisher (Wissenschaftliche Verlagsgesellschaft, Stuttgart). The whole table should be ready at the end of this year. - Preparation of the 3rd edition of the "Kleiner Souci Fachmann Kraut: Lebensmitteltabelle für die Praxis".

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4.2. Comparison of nutrition data from old and new food tables

At the publicity today the question is discussed whether the content of nutritions in the present foods are lower than those in the past caused by the environment and new production conditions. As a first step of answering a comparison is made of the quoted amounts of calcium, magnesium, phosphor, vitamin B1 and vitamin C in old and in new food tables for a couple of important foods to estimate whether such trend can be observed. This tendency could not be established significantly in any case.

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