Leibniz Institut
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Contents

Summaries

1. STUDIES ON THE HEDONIC VALUE OF FOOD - AROMA AND TASTE (FLAVOUR) AS PARAMETERS FOR FOOD QUALITY

1.1. Studies on Character Impact Odorants of Roasted Coffee

Twenty-two compounds, which had been revealed by dilution experiments as potent odorants, were quantified by stable isotope dilution assays in ground roasted Arabica (*Coffea arabica*) and Robusta coffees (*Coffea canephora var. Robusta*) as well as in brews prepared from these materials.

Calculation of odour activity values (OAVs, ratio of concentration to odour threshold) indicated beta-damascenone (I), 2-furfurylthiol (II), 3-mercapto-3-methylbutyl-formate (III) and 3-methyl-2-buten-1-thiol (IV) as the most potent odorants of the roasted coffees. In addition, guaiacol (V) in particular in the Robusta coffee, as well as 3-isobutyl-2-methoxy-pyrazine (VI) and 4-hydroxy-2,5-dimethyl-2(2H)-furanone (VII) in the Arabica coffee belong to the key odorants on the basis of their high OAVs.

A shift in the concentrations of the odorants took place during preparation of a brew. The thiols II and III, methanethiol (VIII), beta-damascenone (I) as well as the Strecker aldehydes methylpropanal (IX) and 3-methylbutanal (X) showed the highest OAV. However, the ranking of the potent odorants was different. The sequence in the Arabica coffee was II followed by I, III, X, IX and VIII, whereas in the Robusta coffee brew II was followed by VIII, X, I, IX and III.

The extraction yields obtained during the preparation of the brews were determined for 17 odorants. Polar compounds (e.g. V, VII) were extracted with higher yields (75-100 %), nonpolar compounds (e.g. I, VI) only with yields of 10-25 %.

The overall odour of the models containing the odorants in the concentration levels which had been found in the two brews, was clearly coffee-like. The models reproduce the differences in the odour profiles of the two brews.

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1.2. Roast-smelling odorants in cereal products

Stable isotope dilution assays were developed for the quantification of the roast-smelling popcorn odorants, 2-acetyl-tetrahydro-pyridine (ACTPY) and 2-propionyl-1-pyrroline (PPY). Both and, in addition, the two further roast-aroma compounds, 2-acetyl-1-pyrroline (ACPY) and acetylpyrazine, were quantified in different popcorn samples. In fresh hot-air popped corn, ACTPY showed the highest concentration (437 µg/kg), followed by ACPY (24 µg/kg) which were established as the key contributors to the roast popcorn odour. During storage of a popcorn sample for seven days in a sealed polyethylene bag, the concentration of ACTPY, ACPY, and PPY decreased to about one third. Model studies using aqueous maize extracts and distinct precursor compounds revealed the pair proline/fructose as the effective precursor system in ACTPY formation, while the pair 1-pyrroline/2-oxopropanal was most effective in the generation of ACPY. A reaction scheme suggesting that ACPY is formed by an "acylation" of the intermediate 1-pyrroline by 2-oxopropanal is discussed.

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1.3. Key odorants in processed flavours - Key odorants in a thermally treated solution of ribose and cysteine

Application of the Aroma Extract Dilution Analysis (AEDA) on a solvent extract isolated from a thermally treated solution (145°C; 20 min) of cysteine/ribose led to the identification of 2-furfurylthiol, 3-mercapto-2-pentanone, 2-methyl-3-furanthiol, 5-acetyl-2,3-dihydro-1,4-thiazine, 3-mercapto-2-butanone and bis-(2-methyl-3-furyl)disulfide showing the highest flavour dilution (FD) factors among the 29 odour-active volatiles. HRGC/olfactometry of decreasing headspace volumes (SHO) established especially 2-furfurylthiol and 2-methyl-3-

furanthiol as important odorants and revealed 2-thenylthiol and ethyl thiol as further key contributors to the overall roasty, meat-like, sulphury odour of the model mixture.

5-Acetyl-2,3-dihydro-1,4-thiazine, identified for the first time among the volatiles of Maillard model reactions or foods, exhibited an intense roasty, popcorn-like odour at the low odour threshold of 0.06 ng/L air which was in the same order of magnitude as those reported in the literature for the roasty-smelling odorants 2-acetyl-1-pyrroline and 2-acetyl-2-thiazoline.

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1.4. Key odorants in processed flavours - Studies on the formation and stability of the roast-flavour compound 2-acetyl-2-thiazoline

By ^{1}H- and ^{13}C-NMR measurements, 2-(1-hydroxyethyl)-4,5-dihydro-thiazol (HDT) was identified as the main volatile product formed during storage of an aqueous solution of cysteamine and 2-oxo-propanal at low temperatures (6°C). The structure of HDT was confirmed by synthesis. Systematic studies on the thermal stability of the HDT under different conditions either in aqueous solution or during high resolution gas chromatography revealed that the HDT is an important intermediate in the formation of the intensely roasty smelling food flavour compound 2-acetyl-2-thiazoline (AT). Model studies showed that more than 10 % of the precursor HDT were converted into AT simply by heating for 10 min in water. The activation energy of this reaction was 57.4 KJ/Mol. Experiments on the thermal stability of AT itself revealed that heating in aqueous solution also led to a degradation of AT, whereas heating in an oil significantly stabilized the flavour compound.

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1.5. Model reactions on the stability of disulphides in heated foods

Bis-(2-methyl-3-furyl)-disulphide (MFT-MFT) and bis-(2-furfuryl)-disulphide (FFT-FFT) dissolved either in benzene or in water were heated at 100°C for 2 hours. In benzene, 2-methyl-3-furanthiol and 2-furfurylthiol were formed when the hydrogen donors 1,4-cyclohexadiene, 1,4-hexadiene or the antioxidant BHT were present. In water, MFT-MFT, FFT-FFT, and also cystine were hydrolysed with formation of the corresponding thiols which were trapped by the reaction with 4-vinylpyridine. Labeling experiments, and the measurement of EPR spectra gave an insight into the mechanism of the disulphide cleavage.

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1.6. Changes in the odorants of boiled fish as affected by the storage of the raw material

The study on the flavour of boiled fish (Annual Reports 1993, 1995) was continued. Homogenates of salmon (A, B) and of cod (C, D) were stored at -60°C (A, C) and at -13°C (B, D). After boiling A and C exhibited the mild flavour of the fresh fish, whereas B smelled fatty, train-oily and D showed a malty odour defect. The potent odorants of the four samples were screened by dilution experiments and then quantified by stable isotope dilution assays. Calculation of odour activity values (OAVs, ratio of concentration to odour threshold) revealed (Z)-1,5-octadien-3-one (I), (E,Z)-2,6-nonadienal (II), propionaldehyde (III), acetaldehyde (IV) and methional (V) as the character impact odorants of A as well as I, II, IV, V and (E,E)-2,4-decadienal as those of C. The off-flavours, which were formed when the raw

material had been stored at the higher temperature, were mainly caused by an increase of II, (Z)-3-hexenal and (Z,Z)-3,6-nonadienal in B and in 3-methylbutanal in D.

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1.7. Metallic off-flavour in buttermilk

The odour-active volatiles in a fresh fermented sweet cream buttermilk (FB) and a sour cream buttermilk (SCB), in which a metallic odour note had been formed after a storage period of 4 days at 8°C, were compared on the basis of aroma extract dilution analyses. Among the 13 odorants detected in the FB sample, the coconut-like smelling gamma-and delta-decalactone and delta-octalactone showed the highest Flavour Dilution (FD)-factors. In the SCB sample, 9 of the 13 odorants present in the FB sample appeared with significantly higher FD-factors. Among them, 4,5-epoxy(E)-2-decenal, 3-methyl indol and (E)-2-undecenal showed the most significant increase. Furthermore, ten odorants were additionally detected in the SCB sample among which the metallic smelling (E,Z)-2,6-nonadienol predominated with the highest FD-factor. On the basis of sensory experiments, the dienol, showing the low odour threshold of 0.07 ng/L air and 1.3 µg/L in buttermilk, respectively, is suggested as the key flavour compound causing the metallic off-odour in stored sour cream buttermilk.

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1.8. Studies on the flavour of Swiss cheese (Emmentaler)

Flavour models were developed for two samples (A and B) of Swiss cheese differing in the ripening stage and the flavour profile. The models were based on an unripened, freeze-dried cheese of the Mozzarella type. Compounds which, in previous studies, had been screened as contributors to the odour and taste of Swiss cheese were added to the base in various combinations and in concentration levels equal to those found in Swiss cheeses A and B. Models composed of methional, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone, acetic acid, propionic acid, lactic acid, succinic acid, glutamic acid, sodium, potas-sium, calcium and magnesium salts, ammonium, phosphate and chloride were judged to meet the flavour of Swiss cheese very well.

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1.9. Key odorants in strawberry flavour

By a calculation of odour activity values (ratio of concentration to odour threshold) on the basis of quantitative measurements obtained from stable isotope dilution assays, eleven odorants were elucidated as important odorants in a fresh strawberry juice. Among them, (Z)-3-hexenal, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, methyl butanoate, ethyl butanoate, ethyl 2-methylpropionate and 2,3-butandione were established as the key flavour compounds responsible for the typical green, caramel-like, fruity overall odour of the juice. A mixture of the eleven most potent odorants added to a model juice matrix in concentrations equal to those in the fresh juice, resulted in an odour profile very similar to that of the fresh juice indicating the strong flavour impact of the compounds identified.

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2. STUDIES ON THE RELATIONSHIP BETWEEN THE STRUCTURE OF BIOPOLYMERS AND THEIR TECHNOLOGICAL PROPERTIES. TEXTURE AS QUALITY PARAMETER OF DOUGH AND BREAD

2.1 A turbidimetric determination of gluten protein types in wheat flour

The technological properties of wheat flour are strongly dependent on the amounts and proportions of the different gluten protein types. For the quantitative determination of gliadins, total glutenin subunits and HMW and LMW subunits, a turbidimetric method was developed. The standard procedure comprised the subsequent extraction of wheat flour with a salt solution, with 50 % 2-propanol (gliadins) and 50 % 2-propanol under reducing conditions and increased temperature (glutenin subunits). Aliquots of the gliadin and glutenin extracts were mixed with 2-propanol to a final alcohol concentration of 83 % and the turbidity of the precipitates was measured photometrically at 450 nm and 20°C. Another aliquot of the glutenin extract was mixed with acetone to a final concentration of 40 % acetone and precipitated HMW subunits were determined turbidimetrically. The sample was then filtered and mixed with 2-propanol to a final concentration of 83 % in order to precipitate and determine the LMW subunits. Control analyses with reversed-phase HPLC indicated that the precipitation of the different protein types was quantitative and specific, and absorbance areas of HPLC were highly correlated to turbidity absorbances. The turbidimetric measurements were reproducible, linear over a wide absorbance range and sensitive.

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2.2 Further studies on disulphide bonds in wheat gluten

Computer modeling of the N-terminal sequences (residues 1-50 with three cysteine residues) of the homologous HMW subunits 5 and 7 revealed different sterical positions of the cysteines. In the structure of subunit 7, the first two cysteines approach one another closely (0.43 nm), which favours an intramolecular disulphide bond. Experimental evidence for this bond was found previously. In contrast, the distance of the first two cysteines in subunit 5 is 2.21 nm and makes an intramolecular bond unlikely. The cysteine residues of the N-terminal regions of subunit 5 are, therefore, involved in disulphide bonds different from those of subunit 7.

Adjacent cysteine residues are important elements of the disulphide structure of LMW subunits, alpha-gliadins and gamma-gliadins. Partial reduction of cystine peptides containing adjacent cysteines with tris-(2-carboxyethyl-)phosphine hydrochloride, subsequent separation of fragments by RP-HPLC, alkylation with iodoacetamide and sequence analysis were used to identify the arrangement of disulphide bonds formed by the adjacent cysteines. It was shown that the first of the adjacent cysteines is linked with a cysteine residue of division III, while the second is linked with a cysteine residue of division V.

The addition of ^{35}S-labelled glutathione as a tracer to flour before dough mixing and the isolation and sequence analysis of radioactive peptides from an enzymatic digest of glutenin enabled the identification of the binding sites of glutenin proteins for endogenous glutathione.

The results demonstrated that glutathione is almost exclusively bound to those cysteine residues that have been proposed to form intermolecular disulphide bonds. In particular, cysteine residues, which are typical of LMW subunits and responsible for their aggregative nature, are affected.

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2.3. Relations between the amount of gluten protein types and technological properties of wheat flours determined by micro tests

Fourteen wheat flours characterised by micro versions of extension and baking tests were analysed for the relative amounts of the gluten protein types using the extraction/HPLC procedure previously developed. Relations between wheat properties and protein amounts were determined by regression analysis. The results indicated that the maximum resistance of dough and gluten and the values of gluten index are strongly dependent on the amount of glutenin subunits; additionally, they are influenced by the ratio gliadins/glutenins. With respect to the glutenin types, HMW subunits are more effective than LMW subunits. The extensibility of dough and gluten is mostly dependent on the ratio gliadins/glutenins. Bread volumes are determined to a higher degree by the total amount of gluten proteins than by the amounts of single protein types. The correlations between the micro baking tests and gluten proteins are higher, when cultivar specific, variable mixing times are used.

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2.4. Characterization of toxic structures in proteins - Investigations into coeliac disease

Immunochemical methods are currently preferred to determine gluten in food. With respect to heat processed food, however, loss of antigenicity and reduced extractability of gliadin are major problems. In the present study, RP-HPLC was used to determine extractability of gliadin from wheat bread in comparison with flour. The results indicated that the extractability of gliadin from bread is strongly reduced using standard conditions (extraction with 60 % ethanol); alpha- and gamma-gliadins are affected much more than x-gliadins. For a complete extraction, increased temperature and reducing conditions are required, by which glutenin subunits are coextracted with gliadins. This extract can be used for an accurate determination of gluten (gliadins + glutenin subunits) by RP-HPLC.

Studies on coeliac toxicity have been focussed to the hexaploid bread wheat, other cultivated wheat species (tetraploid durum wheat and emmer, diploid einkorn), however, have not yet been tested. For comparison with potential toxic amino acid sequences of alpha-gliadins from bread wheat, the gliadin fractions of the different wheat species were separated by RP-HPLC and dominant alpha-gliadins were analysed for amino acid sequences. The results did not reveal any significant differences between bread wheat and the other wheat species. All wheat species investigated contain alpha-type gliadins with potential toxic sequences and, therefore, should be avoided by coeliac patients.

Fourteen peptides with overlapping amino acid sequences from the N-terminal part of alpha-gliadins (residues 3-56) were synthesised, purified and tested for coeliac activity in the fetal-chicken-test. The results obtained until now indicated that several regions of the N-terminal part cause activity. Further tests will be necessary to define the sequences of highest toxicity.

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3. FOOD COMPOSITION AND NUTRITION TABLES

Information about the composition of foods is essential for administration, nutritional guidance, economy and science. The large scientific work "Souci, Fachmann, Kraut: Food

Composition and Nutrition Tables" is kept up to date by a continuous survey of scientific literature and with the aid of the PC-data bank SFKDB. It is concerning also the related small table "Der kleine Souci, Fachmann, Kraut: Lebensmitteltabelle für die Praxis" which had been developed for the daily requirements of the consumer.

In the future the spectrum of the food-constituents of the large nutrition table will be adjusted to the preventive-medical point of view. For this mind, the following preparation for the 6th edition of the book were carried out:

- Uptake of unsaturated trans-fatty acids into the SFKDB-data bank; evaluation of the related data from the original literature.
- Flavones, flavonols and the regarding dihydro derivatives; evaluation of the literature; preparation of the data for their uptake into the table.
- Essential trace elements; nutritive-physiological valuation of nickel as essential trace element and as allergen.
- Actualisation of the data for minerals, trace elements and vitamins.

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