Leibniz Institut
Deutsche Forschungsanstalt für Lebensmittelchemie

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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

In continuation of our work on the character-impact odorants of ground roasted coffee the chemical structure of two earthy smelling compounds, which were found in dilution experiments, were established. In Robusta coffee they were identified as 2-ethenyl-3,5-dimethylpyrazine and 2-ethenyl-3-ethyl-5-methylpyrazine by comparison of GC and MS data with those of the corresponding reference substances. The odour threshold values (0.014 ng/L, air) of the new pyrazines were as low as those of 2-ethyl-3,5-dimethyl- and 2,3-diethyl-5-methylpyrazine. 3-Ethenyl-2-ethyl-5-methylpyrazine was also detected in coffee. Its odour threshold value was 8000 times higher than that of the 2-ethenyl-3-ethyl-5-methylpyrazine. After addition of HBr the two ethenylethylmethylpyrazine isomers were separated by capillary GC. The results indicated the presence of the two isomers in a ratio of 1 to 1 in coffee.

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1.2. Apparatus for quantitative headspace analysis of the characteristic odorants of baguettes

An apparatus which based on the principle of dynamic headspace analysis was developed for the determination of the release of potent odorants from two types of baguettes (INT and ART) differing in their odour profiles. Model mixtures containing the odorants in the concentrations found by the headspace analyses were evaluated using the apparatus as an olfactometer and comparing the odour profiles of the models with those of the baguettes. The results indicated that the losses of methylpropanal (I), 2- and 3-methylbutanal (II, III) and, on the other hand, an increase of hexanal and (E)-2-nonenal led to a stale off-flavour. As the concentrations of I-III were larger in the ART crust than in the INT, the change of the pleasant baguette odour to a stale off-odour was slower in the ART baguette. It was suggested that the apparatus designed for quantitative headspace analysis was also suitable for a determination of the release of flavour compounds from other foods.

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1.3. Key odorants in processed flavours

1.3.1. Thermally treated mixtures of glucose/cysteine and rhamnose/cysteine

Application of an aroma extract dilution analysis on extracts prepared from either thermally treated solutions (20 min, 145° C) of glucose/cysteine (I) or rhamnose/cysteine (II) led to the identification of 2-furfurylthiol (roasty, coffee-like), 5-acetyl-2,3-dihydro-1,4-thiazine

(roasty), 3-mercapto-2-butanone (sulfury, rotten), 3-mercapto-2-pentanone (catty) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (caramel-like) with the highest odor activities among the 34 odor-active volatiles detected in I. In II, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 3-hydroxy-6-methyl-2(2H)-pyranone (seasoning-like), 5-methyl-2-furfurylthiol (roasty, coffee-like), 2-furfurylthiol and 5-acetyl-2,3-dihydro-1,4-thiazine appeared with the highest Flavor Dilution (FD)-factors among the 18 compounds detected by HRGC/O. Among the flavor compounds identified, 2-propionyl-2-thiazoline is reported for the first time among the flavors of Maillard model reactions or foods, respectively. The odorant elicited an intense roasty, popcorn-like odor at the low odor threshold of 0.07 ng/L in air.

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1.3.2. Studies on the formation of the intense flavour compounds 2-methyl-3-furanthiol, 2-acetyl-2-thiazoline and sotolon in cysteine/carbohydrate mixtures

On the basis of data obtained from a number of model studies and, based on the yields of the intense odorants 2-methyl-2-furanethiol (MFT), 2-acetyl-2-thiazoline (AT) and 3-hydroxy-4,5-dimethyl-2(5H)-furanone (SOT) formed, 2,3-butandione, 2-oxopropanal and hydroxyacetaldehyde were identified as important intermediates in the formation of the three odorants from sugar/cysteine reactions. I.e., MFT was shown to result in a 1.4 % molar yield by reacting hydroxyacetaldehyde with mercaptoacetone. The latter was shown to be formed from 2-oxopropanal and H2S in significant amounts. 2-Acetylthiazolidine which easily forms from 2-oxopropanal and cysteamine upon standing at room temperature, was identified, besides its tautomer 2-(1-hydroxyethyl)-dihydrothiazol as key intermediate in AT formation. The oxidation step needed to generate the AT was elucidated to be a metal-induced autoxidation. The seasoning-like smelling SOT is generated from 2,3-butandione and hydroxyacetaldehyde. From these results reaction pathways were deduced explaining the formation of the three odorants.

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1.4. Key odorants generated by thermal treatment of yeast extracts - Correlation with precursor amino acids

Application of an Aroma Extract Dilution Analysis (AEDA) on a flavor concentrate isolated from a heat-processed (145°C, 20 min) aqueous solution of a commercial yeast extract (CYE) revealed 2-furanmethanethiol, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, 2-methoxyphenol and 3- and 2-methylbutanoic acid as the key odorants among the 16 odor-active compounds of the intensely roasty, sweet smelling solution. Compared with CYE, in a thermally treated autolyzate prepared under laboratory conditions from baker's yeast (SPYA) several odorants, e.g., methional, 2-acetyl-2-thiazoline, 3-hydroxy-4,5-dimethyl-2(5H)-furanone (Sotolon), phenylacetic acid and 2,3-diethyl-5-methylpyrazine showed higher flavor dilution (FD) factors, whereas the reverse was found for 2-furanmethanethiol (FMT). The amounts of its precursor amino acid cysteine in the CYE and the SPYA were well correlated with the different odor activities of the FMT in both solutions. Detailed model studies on the formation pathways of FMT indicated the binary mixtures 2-furaldehyde/cysteine as well as mercapto-2-propanone/hydroxyacetaldehyde as important intermediates in FMT formation. Heat-treatment of a water-soluble, low molecular weight fraction isolated from baker's yeast cells predominantly generated the roast odorant 2-acetyl-1-pyrroline (ACPY). Under certain fermentation conditions, the amounts of its precursor ornithine in the yeast were increased, leading to higher odor activities of ACPY after thermal treatment of the extract.

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1.5. Evaluation of the key odorants in milk chocolate and cocoa mass by aroma extract dilution analyses

Application of an Aroma Extract Dilution Analysis (AEDA) on the volatiles of a commercial milk chocolate revealed 51 odor-active compounds in the Flavor Dilution (FD) factor range of 8 to 1024, 44 of which could be identified. The following 13 odorants contributed with the highest FD-factors to the overall chocolate flavor: 3-methylbutanal (malty); 2-ethyl-3,5-dimethylpyrazine (potato chip-like); 2- and 3-methylbutanoic acid (sweaty); 5-methyl-(E)-2-hepten-4-one (hazelnut-like); 1-octen-3-one (mushroom-like); 2-ethyl-3,6-dimethylpyrazine (nutty, earthy); 2,3-diethyl-5-methylpyrazine (potato chip-like); (Z)-2-nonenal (green, tallowy); (E,E)-2,4-decadienal (fatty, waxy); (E,E)-2,4-nonadienal (fatty); R-delta-decalactone (sweet, peach-like) and 2-methyl-3-(methyldithio)furane. Application of the AEDA on the cocoa mass used in the production of the milk chocolate led to the identification of 37 odorants, seven of which were sensorially not detected in the chocolate. By contrast, 11 odorants were present in the milk chocolate, but were not sensorially relevant in cocoa mass, e.g. R-delta-decalactone and 5-methyl-(E)-2-hepten-4-one.

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1.6. Studies on the formation of a metallic off-odour in buttermilk

Quantitative measurements and sensory experiments had revealed the intensely metallic smelling (E,Z)-2,6-nonadienol (NDOH) as the key odorant responsible for this type of off-odour developing during storage of sour cream buttermilk. A series of experiments in which synthesised precursors were added to fresh buttermilk and the formation of NDOH was followed, suggested the following formation pathway during manufacturing: glyceryl alpha-linolenate is peroxidized to glyceryl 9-hydroperoxy-10,12,15-octadecatrienoate (9-HPOT) by oxygenases of the starter cultures. The 9-HPOT is then cleaved by acid catalysis into (E,Z)-2,6-nonadienal which is in turn reduced into the (E,Z)-2,6-nonadienol by enzymes of the lactic acid bacteria.

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1.7. Ripening of Emmental cheese wrapped in foil with and without addition of lactobacillus casei subsp. casei

The study reported here was part of a collaboration with the Federal Dairy Reseach Institute of Bern.

Altogether 15 odorants and 18 taste substances, including volatile acids and minerals, were quantified in 4 loaves of Emmental manufactured with (n = 2) and without (n = 2) the addition of L. casei subsp. casei. 2-Methylbutanal, 3 esters, 2-heptanone, 5-ethyl-4-hydroxy-2-methyl-3(2H)-furanone, acetic acid, propionic acid, glutamic acid and succinic acid increased between the 3rd and 12th month of ripening, whereas delta-decalactone decreased. The solubility of magnesium, calcium, chloride and phosphate ions in aqueous extracts increased during ripening. The concentrations of other compounds, e.g. 3-methylbutanal, methional, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, lactic acid, showed no clear development. Models prepared on the basis of data found for 9 months old cheeses indicated

that the flavour profiles were mostly affected by the concentration differences of acetic acid and propionic acid.

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1.8. Comparative evaluation of potent odorants of boiled beef by aroma extract dilution and concentration analysis

After boiling, beef was extracted with dichloromethane, and the volatile fraction including the solvent was distilled off from the non-volatile material. The distillate was divided in two portions. One half was subjected to aroma extract dilution analysis (AEDA), and the other to aroma extract concentration analysis (AECA). In the latter, the concentration of the extract was accompanied by a series of gas chromatography-olfactometry (GCO) analyses, whereas with regard to the first portion the extract was at first concentrated to a small volume and then diluted stepwise for GCO. Both screening procedures confirmed the presence of 32 odorants which were all identified after a 250-fold concentration of the extracts. However, the ranking of the compounds in order of odour potency was different due to losses of the odorants in AEDA. 2-Furfurylthiol (I) and 4-hydroxy-2,5-dimethyl-3(2H)-furanone (II) followed by 2-methyl-3-furanthiol (III) and a group containing 3-mercapto-2-pentanone (IV), 1-octen-3-one (V) and (E)-2-nonenal (VI) were indicated by AECA as the most potent odorants of boiled beef. In AEDA, on the other hand, the ranking was I, II, III followed by V and VI; thiol IV was of minor importance.

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1.9. Stability of furanoid fatty acids in soybean oil

Analysis of the positional distribution of the furanoid fatty acids, 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (F20) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F22) in soybean oil (SBO) indicated that they were preferentially esterified with the primary OH-groups of glycerol molecules. Hydrogenation of SBO partly reduced the concentrations of F20 and F22. During exposure of SBO to daylight, F20 and F22 were completely degraded within 2 days, whereas linoleic acid and linolenic acid were not affected. ß-Carotene inhibited both the degradation of the furanoid fatty acids and their oxidation to the odorant 3-methylnonane-2,4-dione (MND), which contributes strongly to the light-induced off-flavor of SBO. A model experiment indicated that light exposure of SBO, before silica gel chromatography and refining, prevented the formation of MND during storage of the oil.

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1.10. High resolution gas chromatography / selective odorant measurement by multisensor array (HRGC/SOMMSA) - a useful approach to standardise multisensor arrays for use in the detection of key food odorants

The typical odour of several foods have been shown to be represented by only a small portion of their volatile ingredients. In order to control food quality, monitoring of these key food odorants is indispensable. In the case of butter, the odour can be simulated by composing diacetyl, butanoic acid and (-decalactone in an appropriate ratio. A sensory panel consisting of five persons evaluated a strong similarity of the recombinant to natural butter. The off-odour of aged (rancid) butter was shown to be caused by an increased concentration of, especially, butanoic acid. In the experimental setup presented here different kinds of sensors were

attached, besides a flame ionisation detector (FID), at the exit of a gas chromatographic column (GC). The sensors involved were different types of metal oxide semiconductors and a surface acoustic wave (SAW) device coated with poly(isobutylene). In different GC runs, recombinants of fresh and rancid butter were analysed by recording the FID signal and the signals of the different sensors in parallel. The quantities applied were 0.35 µg diacetyl, 3.7 µg butanoic acid (rancid: 37 µg) and 4.9 µg delta-decalactone in each GC run. The SAW sensor and a ZnO sensor mounted at the port of the GC responded with high signals to butanoic acid and delta-decalactone. Both sensors are, however, insensitive to diacetyl. The different relative sensitivities allow discrimination of butanoic acid and delta-decalactone without GC-separation. A palladium doped ZnO sensor was sensitive to diacetyl. Using the HRGC/SOMMSA, the characterisation of sensors can be done by replacing natural foodstuff samples by the described recombinants.

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

2.1. Studies on disulphide bonds in cereal proteins

2.1.1. Disulphide bonds of gamma46-gliadin

In comparison with previous studies on gamma-gliadins from the wheat variety Rektor, the investigation of the disulphide structure of gamma46-gliadin should show whether a gamma-type gliadin from another wheat variety and obtained by differing preparation and separation procedures has an analogous structure. gamma46-Gliadin was isolated by Popineau and Pineau (INRA, Nantes) from the wheat variety Hardi using Martin process for the preparation of gluten, 50 % aqueous dioxane for gliadin extraction, and ion exchange chromatography for gliadin separation. gamma46-Gliadin was digested with thermolysin, and 5 cystine peptides were isolated by RP-HPLC, sequenced by Edman degradation and assigned to known amino acid sequences of gamma-gliadins. The results demonstrate that the eight cysteine residues of gamma46-gliadin are connected by the same four intramolecular disulphide bonds as those of gamma16- and gamma17-gliadin from variety Rektor.

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2.1.2. Distribution of ^{35}S-labelled glutathione on the Osborne fractions of dough and gluten

Though rheological properties of wheat dough and gluten are affected by endogenous glutathione (GSH), little is known about the distribution of GSH on the different protein fractions of wheat dough and gluten. 35S-labelled GSH was added as a tracer to the flours of three wheat varieties different in dough and gluten properties. After mixing, the dough obtained and the gluten washed out from the dough were fractionated into water solubles, globulins, gliadins and glutenins. The distribution of radioactivity was, independent on the variety, about 65 % in water solubles (mainly non-protein bound GSH), 6-7 % in each of globulins and gliadins, and 21 % in glutenins. An influence of variety was detected only in the distribution of labelled GSH on acid-soluble and acid-insoluble glutenins. A dough resting time of 35 min did not show any effect, and oxidised labelled GSSG was distributed on the protein fractions of dough in the same way as reduced GSH. In gluten washed from dough, the proportion of labelled GSH was significantly increased in gliadin (11 %) and glutenin (35

%) and decreased in water solubles. All experiments demonstrated that within gluten proteins, GSH was bound much more to glutenin than to gliadin. This reflects very well the weakening effect of GSH on dough and gluten by depolymerisation of glutenin aggregates.

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2.1.3. Isolation and characterisation of cysteine peptides from HMW subunits of rye glutelin

Cysteine residues of HMW subunits are important for the aggregation of wheat glutenin and thus, for the rheological properties of dough and gluten. Whereas number and positions of cysteine residues in wheat HMW subunits are well known, only a few informations are available for corresponding rye subunits. For the investigation of cysteine containing sequences, HMW subunits were isolated from rye flour Danko by a specific extraction/precipitation procedure and digested with alpha-chymotrypsin. The cysteine peptides of the partial hydrolysate were enriched by covalent chromatography on Thiopropyl Sepharose, separated by RP-HPLC, alkylated with 4-vinylpyridine and purified by RP-HPLC. Altogether, 51 cysteine peptides were obtained and sequenced by automatic Edman degradation. 24 Peptides could be assigned to HMW subunits of wheat, most of the other peptides to inhibitors or chymotrypsin. The cysteine peptides derived from HMW subunits represented 6 different cysteine residues: Ca, Cb and Cd of domain A, Cy of domain B (y-type) and Cz of domain C. One additional cysteine residue located in domain C was found in rye subunits; this residue does not occur in wheat subunits. The adjacent cysteine residues Cc1Cc2 typical for y-type subunits of wheat could not be detected in rye subunits.

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2.2. Investigations of technological properties and gluten protein compositions of different spelt wheat varieties

The flours of twenty spelt wheat varieties and two bread wheat varieties were investigated for the rheological dough properties and baking behaviour by means of micro-scale tests and for the qualitative and quantitative compositions of the different types of gliadins and glutenin subunits by means of RP-HPLC. The results demonstrated that the spelt wheat varieties differed strongly in dough and gluten extensibility, maximum resistance and extensigram areas. Loaf volumes varied in a broad range and were correlated with extension area of dough and maximum resistance of gluten. For the characterisation of gluten protein types, flours were extracted stepwise with a salt solution, 60 % ethanol and a glutenin extraction solvent. Gliadins and glutenin subunits were separated and quantified by RP-HPLC on C8 silica gel. The chromatograms showed typical patterns, which allowed the identification of most varieties and their classification according to the relationship with bread wheat. The quantitative data revealed that spelt wheats have significantly higher amounts of total gliadins and a higher ratio of gliadins to glutenin subunits in comparison with bread wheat. The ratio gliadins/glutenin subunits and that of single gliadin and glutenin subunit types were highly correlated with maximum resistance of dough and gluten, with extension area and with loaf volume.

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2.3. Investigations on dough hardening during the processing of wheat doughs

Standard extensions tests as well as tests on a micro-scale show that kneaded dough gets weaker and more extensible during a resting period. When the dough is homogenised and reshaped during resting, however, resistance against uniaxial extension is increased and extensibility is decreased. This effect can be observed, in particular, for wheat varieties with strong dough and gluten. The effect of dough hardening is diminished by addition of more water during mixing and increased by addition of starch to flour. When doughs are measured with methods that are not based on uniaxial extension, the effect cannot be observed just as little with gluten that was obtained from reshaped dough. Thus, oxidative cross-linking of gluten proteins proposed by literature cannot be the reason for dough-hardening. An explanation is given by visual observation of doughs during capillary viscosimetry and by microscopy. Directly after kneading starch and gluten are thoroughly mixed. The subsequent shaping procedure causes an aggregation of gluten protein and an accumulation of starch granules. This demixing of starch and protein accounts for the observed dough-hardening.

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2.4. Study on the effect of DATEM

8 Commercial DATEM samples from 2 producers of DATEM and 13 synthesised DATEM samples in which the fatty acid was varied from caproic (C6:0) to behenic acid (C22:0) were investigated by a micro-scale baking test and by micro-scale rheological methods based on 10 g of flour. Commercial DATEMs proved the suitability of the baking test to study the effect of emulsifiers by increasing the volumes of the breads up to 60 % at a concentration of 0.3 %. Synthesised DATEMs were comparable to commercial products. The increase of the loaf volume after the addition of synthesised DATEMs was dependent on the chain length of the fatty acid of the DATEM with an optimum for DATEM on the basis of glycerol monostearate (increase of loaf volume 62 %). The optimal concentration with respect to the quantity of flour was 0.3 %. The effect of DATEM on the rheology of dough was small. However, much greater was the effect on the glutens isolated from doughs prepared with DATEM. The resistance of gluten increased after the addition of increasing amounts of DATEM.

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3. CHARACTERISATION OF TOXIC STRUCTURES IN PROTEINS

3.1. Collaborative studies on the quantitative determination of gliadin in wheat starch

According to Codex Alimentarius Standard for "Gluten-free Foods", wheat starch can be used in a gluten-free diet provided that the protein content determined by N analysis (F = 5.7) is not greater than 0.3 %. The proposal for a revised text submitted in 1996 included a limit of 100 ppm gliadin determined by an immunochemical method. The relationship between N and gliadin content would be of great interest, but is uncertain. For this reason, 16 starch samples with different protein contents were analysed by eight groups from seven European countries using commercial immunochemical kits, own immunoassays, mass spectrometry and reversed-phase HPLC. The gliadin contents determined with the commercial kit were between 1 and 822 ppm and four starches were above the limit of 100 ppm. Even when outliers were removed, repeatability and reproducibility of the determinations were poor. Regression analysis indicated that zero gliadin corresponded to 0.19 % protein (N x 5.7) and 100 ppm gliadin to 0.24 % protein; the correlation coefficient was 0.76. When a gliadin standard isolated from the wheat variety Rektor was used instead of the kit standard, the gliadins contents were significantly higher. Obviously, the reactivity of the monoclonal antibodies of

the kit against single gliadin components is different; thus, the trueness of values is still questionable. The other immunochemical methods used (own polyclonal or monoclonal assays, SDS-immunoblotting) could not clarify the situation, because the variance of values was even higher than with the commercial kit. Mass spectrometry and reversed-phase HPLC introduced into the collaborative tests could be alternatives for immunochemical methods, but have to be tested or developed by further studies. Altogether, however, all results agreed that the gliadin limit of the revised Codex Standard is much lower than that of the old version. This decrease of limit could be important for highly sensitive coeliac patients.

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4. FOOD COMPOSITION AND NUTRITION TABLES

Information about the composition of foods is essential for administration, nutritional guidance, economy and science.

The "Souci, Fachmann, Kraut: Food Composition and Nutrition Table" is kept up to date by a continuous survey of scientific literature and by means of the PC-databank SFKDB. It is concerning also the related small table "Der kleine Souci, Fachmann, Kraut: Lebensmitteltabelle für die Praxis" which has been developed for the daily requirements of the consumer.

In the future the spectrum of the food constituents covered in the large nutrition table will also address preventive-medical aspects.

For the 6th edition the following preparation were carried out:

- Uptake of several new leafy vegetables which belong to the group of so called "wild vegetables". Uptake of the data of their main constituents, important mineral- and trace elements, carbohydrates, amino acids and fatty acids.
- Flavones and flavonoles: evaluation of the comprehensive original literature and preparation for their uptake.
- Main constituents, mineral- and trace elements, carbohydrates and vitamins: actualisation of the data.
- Trace elements: comparison of the data for Zn, Cu and Ni of food raw products with their origins at different regions of the world.
- Evaluation of a new shape of the nutrition table for the 6th edition and the writing of the respective software.
- Preparation of the 3rd edition of the "kleine Souci-Fachmann-Kraut, Lebensmitteltabelle für die Praxis".

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