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Summaries

1. FOOD ANALYSIS

1.1. Gaschromatography/olfactometry of static headspace samples

The following study on the flavours of green and black tea demonstrates that most of the compounds causing the odour of a food can be identified in the static headspace sample on the basis of the results of a preceding aroma extract dilution analysis (AEDA).

AEDA of the volatile fractions of green and black tea samples revealed 28 odorants of which 27 were identified. As the odour quality and the chromatographic properties of most of the odorants occurring in the air above the tea powders agreed with those which were identified by the preceding AEDA, these compounds were detectable by gas chromatography-olfactometry (GC-O) in static headspace samples. (Z)-Hex-3-enal, linalool, (Z)-octa-1,5-dien-3-one, oct-1-en-3-one, (Z)-hept-4-enal, butane-2,3-dione, 2-methylpropanal, 3-methylbutanal, 3-methylnonane-2,4-dione, (E)-non-2-enal, octanal, (E,Z)-nona-2,6-dienal and hexanal were found in the headspace volume of 40 mL which was drawn at 40°C from both kinds of tea. (E,E)-Nona-2,4-dienal and α-pinene were detected as additional odorants of black tea. The most potent odorants occurring in the air above the tea powders were evaluated by the analysis of decreasing headspace volumes, e.g. (Z)-hex-3-enal was the sole odorant in 2 mL of the headspace of green tea and linalool in 0.5 mL of that of black tea. This procedure indicated that the difference in the odours of green and black tea was mainly due to a higher concentration of (Z)-hex-3-enal, (Z)-octa-1,5-dien-3-one and butane-2,3-dione and the much lower concentration of linalool in the air above the former.

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1.2. Indicator substances for the butter flavour

Sensory evaluation of five different kinds of butter revealed an Irish sour cream butter (ISC) and a farmer sour cream butter (FSC) with the highest overall odour intensities. Nineteen odour-active compounds were detected by aroma extract dilution analysis in a distillate of the ISC butter. The highest FD-factors were found for delta-decalactone, skatole, (Z)-6-dodeceno-γ-lactone and diacetyl followed by (E)-2-nonenal, (Z,Z)-3,6-nonadienal, (Z)-2-nonenal and 1-octen-3-one. Odour activity values (OAV; ratio of concentration to odour threshold) were calculated from quantitative data determined by means of stable isotope dilution assays and from odour thresholds in oil. Diacetyl followed by delta-decalactone and butanoic acid showed the highest OAVs in the ISC butter and a cultured butter with creamy, sweet odours. In contrast, a sweaty, rancid odour predominated in the FSC butter in which butanoic acid showed the highest OAV. The odour of a solution of diacetyl (0.34 mg/kg), delta-decalactone (4.9 mg/kg) and butanoic acid (3.6 mg/kg) in sunflower oil was in very good agreement with the odour of the cultured butter containing the same amounts of these odorants. The data suggest that the three odorants should be useful as indicator substances for the objective assessment of the buttery odour note in milkfat containing products.

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1.3. Quantification of flavour compounds by a stable isotope dilution assay

The quantification of cyclic enolones like 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon) and 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (EHMF) with other substances as internal standards may lead to incorrect results, because sotolon and EHMF are difficult to isolate due to their high solubility in water. Furthermore, they are unstable under normal gas chromatographic conditions. Therefore a stable isotope dilution assay was developed for the quantification of sotolon and EHMF. The method was applied to fenugreek seeds, lovage, seasonings and soya sauce revealing that sotolon contributes more to the ±hydrolyzed vegetable protein-like± flavour note than does EHMF. Boiling of the aqueous extracts of fenugreek or lovage under weakly acidic conditions significantly increased the amounts of sotolon.

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1.4. Quantification of low molecular SH-compounds in flours and doughs

An isotope dilution assay was developed for reduced glutathione (GSH), total glutathione (GSH, oxidized glutathione and protein-glutathione mixed disulfides), free cysteine (Cys) and total cysteine (Cys, cystine and protein-Cys mixed disulfides). The new method was checked by the determination of these compounds in some flours and in crude glutenins. It was applied to flours of two wheat cultivars (DNS and K, Kanzler) and to their doughs which had been mixed for 3 min at 30°C. In the doughs without additions, GSH (values in nmol/g) decreased from 100 to 44 (DNS) and from 35 to 17 (K), while Cys increased from 13 to 42 (DNS) and from 8 to 18 (K). Addition of L-threo-ascorbic acid (AA) accelerated the disappearance of GSH and inhibited partially the increase of Cys. In contrast, D-erythro-AA was inactive. The results confirmed the hypothesis that the improver action of L-threo-AA is caused by a rapid oxidation of GSH to its rheologically inactive disulfide.

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1.5. Glycoproteins in wheat gluten: comparison of detection methods

Recent studies have shown that the HMW subunits of glutenins are, in fact, glycoproteins. Because the proportion of carbohydrates is low, effective and sensitive separation and detection methods are necessary for their analysis. The comparison of SDS-PAGE using small-sized commercial Excel Gel SDS-plates or self-made SDS-PG-PAGE-plates revealed that the HMW subunits are completely separated only on the self-made plates. The detection of glycoproteins with Schiffs reagent is only suitable as screening method because of unspecific reactions with nonglycosylated proteins. For immunochemical detection methods, the transfer of the glycoproteins to a membrane is essential. Both electroblotting and diffusion blotting are suitable. In contrast to glycoproteins from soya, the HMW subunits did not react with concanavalin A. The method using digoxigenin and an anti-digoxigenin enzyme linked immunoassay, however, proved to be a very specific and sensitive procedure to detect glycoproteins in wheat gluten: all of the HMW subunits and some of the LMW subunits showed a positive reaction; the detection limit was comparable with that of the very sensitive silver staining.

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1.6. Quantification of gluten protein subgroups in wheat flour

The technological properties of wheat cultivars are strongly dependent on the amounts and proportions of gluten proteins. For these reasons, an extraction and separation procedure was developed for the quantitative determination of the different gliadin (ω5, ω1,2-, α-, γ-) and glutenin (LMW-, HMW-) subgroups. The extraction of gliadins from flour was achieved at room temperature with 60 % aqueous ethanol after pre-extraction with a salt solution. Subsequently, the glutenin subunits were extracted under nitrogen and at 60°C with 50 % aqueous 1-propanol containing Tris/HCl (pH 7.5), urea and dithioerythritol. The separation and quantitative determination of the different subgroups were then performed by RP-HPLC on C_8 silica gel.

The analytical method developed allows a sensitive, reproducible and relatively fast quantitative determination of all gluten protein subgroups. It is easy-to-apply to other

materials, e.g. commercial flours, vital gluten or flours from other cereals. The results obtained could provide informations highly useful for the evaluation of cereal quality and for breeding, selection and genetic improvement.

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1.7. Nutrition tables

Food composition and nutrition tables are essential for administration, nutritional guidance, economy and science. The large scientific work ±Souci, Fachman, Kraut: Food Composition and Nutrition Tables± is kept up to date by a continuous survey of the scientific literature with the aid of the data bank SFKDAT. The same is true for the related small table ±Der kleine Souci, Fachmann, Kraut: Lebensmitteltabelle für die Praxis±, which was developed for the daily requirements of the consumer.

The manuscript for the 5th edition of the large table was: finished. Focal points of activities in this period of time were the supplement and revision of data of the vitamins B12, D, K, folic acid and the establishment of tables for some new foods.

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2. FOOD CHEMISTRY, BIOCHEMISTRY AND MICROBIOLOGY

2.1. Aroma of heated meat

The study on the aroma of heated meat was continued with

(a) Quantification of character-impact odour compounds of roasted beef

Beef was roasted for 7 min in frying pans made of glass (A) or of stainless steel (B). The lower temperature in A yielded a more roasty-sweet flavour and the higher temperature in B a roasty-harsh, more caramel-like flavour. Using stable isotope dilution assays the concentration levels of the odour compounds 4-hydroxy-2,5-dimethyl-3(2H)-furanone (I), 2-acetyl-2-thiazoline (II), 2-ethyl-3,5-dimethylpyrazine (III), 2,3-diethyl-5-methylpyrazine (IV), guaiacol (V) and methional (VI) were determined in samples A and B. The data obtained were divided by the corresponding odour thresholds which were evaluated nasally and retronasally. The results indicated that the flavour differences of A and B were preferentially caused by differences in the concentration levels of odorants I, II, IV, and VI.

(b) Identification of a species-specific odorant of stewed beef

Aroma extract dilution analysis of the volatile fraction of stewed beef revealed a compound that smelled tallowy, beef-like. The compound was identified as 12-methyltridecanal (MT) and had a sensitivity odour threshold of 0.1 µg/kg in water. Using a stable isotope dilution assay 431 µg/kg of MT was found in stewed beef. The lipid fraction was isolated from the lean meat of different animal species and then hydrolysed. High amounts (µg/g lipid) were liberated from beef (44 to 149), lower from veal, lamb, springbuck and red deer (5 to 19) and very low amounts from chicken, turkey and pork (0.3 to 2.7). Plasmalogens were detected as the precursors of MT.

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2.2. Furanoid fatty acids as precursors of an aroma compound of green and black tea

The occurrence of 3-methyl-2,4-nonanedione in green and black tea (cf. 3.1.1.1) suggested that furanoid fatty acids (F-acids), which had been earlier identified as precursors of this dione, are present in tea. Therefore, the fraction of F-acids was analysed, both, in fresh leaves of the plant and in samples of processed green tea. 10,13-Epoxy-11,12-dimethyloctadeca-10,12-dienoic acid (F-I) and 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid (F-II) were detected as precursors of MND, and their amounts were quantified in the tea samples by using a stable isotope dilution assay. During processing of green and black tea most of the F-acids, whose concentrations in the freshly harvested leaves amounted to 50 mg/kg (dry matter) and 712 mg/kg for F-I and F-II, respectively, are degraded.

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2.3. Changes in the odorants of boiled fish in dependence on the storage of the raw material

Aroma extract dilution analysis of freshly harvested and boiled trouts (A) resulted in 24 odorants of which 19 were identified. Twelve compounds were quantified by isotope dilution assays in A, in boiled trouts of which the raw material had been stored for 17 weeks at -13°C (B) and in homogenates which were freshly prepared and boiled (C) or stored for 14 weeks at -13°C and then boiled (D). Calculation of odor activity values (OAVs, ratio of concentration to odor threshold) revealed (Z)-1,5-octadien-3-one, (E,Z)-2,6-nonadienal and methional as the most potent odorants of A and C. After storage of the raw material the OAVs of (Z)-3-hexenal and (Z,Z)-3,6-nonadienal were strongly enhanced. Consequently, both aldehydes contributed substantially to the fatty, fishy off-flavor of B and D. (Z)-3-Hexenal is proposed as indicator substance to objectify the fatty, fishy off-flavour of boiled trouts.

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2.4. Flavour of roasted white sesame seeds

The most odour active compounds in roasted (30 min; 180°C) white sesame seeds were elucidated by an aroma extract dilution analysis and their contribution to the overall flavour assessed by a determination of odour activity values (OAV; ratio of concentration to odour threshold). The results revealed 2-furfurylthiol, 2-methoxyphenol, 2-phenylethylthiol and 4-hydroxy-2,5-dimethyl-3(2H)-furanone followed by 4-vinyl-2-methoxyphenol, 2-pentylpyridine, acetylpyrazine and 2-ethyl-3,5-dimethylpyrazine as the most important odorants in the sesame flavour. Decreasing the roasting time (10 min; 180°C) significantly changed the overall flavour. This change was mainly due to a comparatively higher OAV of the roasty smelling 2-acetyl-1-pyrroline and a lower OAV of 2-methoxyphenol in the short-roasted compared to the long-roasted sample. To identify the precursors of the key odorants, a method for the fractionation of unroasted seeds was developed. The formation of 2-pentylpyridine and 2-methoxyphenol from their precursors (E,E)-2,4-decadienal/ammonia and ferulic acid, respectively, was studied in detail.

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2.5. Investigations into the ripening of Cheddar cheese

Cheddar cheeses from two different factories were ripened over 24 weeks at 10°C and then analyzed for peptides soluble in citrate buffer at pH 4,6 by RP-HPLC. Thirteen peptides with molecular masses between 3800 and 7400 were isolated and assigned to the corresponding amino acid sequences of the casein fractions via Edman degradation and amino acid analysis. All peptides were fragments of the sequence regions K29-S96 of beta-caseins A1 and A2. The amounts and proportions of these peptides from both cheeses were differing and varied differently during ripening. Thus, they may be suitable markers for the characterization of cheese processing and stage of ripening.

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2.6. Relationship between loaf volume and rheological properties of wheat dough and gluten

The relations between loaf volume and rheological properties of wheat dough and gluten have not been clarified in detail until now. The reason for that could be that differently composed and produced doughs have been used for the baking tests and the rheological studies of dough. Therefore, three different microvariants of baking tests (M1-M3) were compared and the resulting loaf volumes were related to the dough properties.

The variants M1 and M2 were performed without and with addition of sugars, fat and ascorbic acid, respectively. Dough mixing corresponded to that of the rheological studies. M3 was the micro variant of the rapid-mix-test using the same formula as M2, but strongly different dough mixing conditions.

Nine different wheat cultivars of known rheological properties were compared. The resulting loaf volumes of M1 were small, in comparison to M2 and M3, and within the cultivars, the differences were less pronounced. The correlations between loaf volume and dough properties were in a medium range ($r > 0.7$). The loaf volumes of M2 were significantly higher and strongly correlated ($r = 0.87$-0.97) to the maximum resistance of dough and gluten. In the case, of M3, however, relations between loaf volume and the strength of dough and gluten properties could not be established ($r = 0.09$-0.36).

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2.7. Relationship between the amount of gluten protein types and the technological properties of different wheat cultivars

Eight wheat cultivars originating from different countries and characterised by different technological properties and a commercial wheat flour were analysed for the amount of the gluten protein types (ω5-, ω1,2-, α- and γ-gliadins, HMW gliadin, LMW, x-HMW and y-HMW subunits of glutenin). The correlations between the amounts and cultivar properties (protein content, SDS-sedimentation volume, dough development time, resistance and extensibility of dough and gluten, bread volume) revealed significant differences between gliadins and glutenin subunits. Whereas the amount of gliadins was strongly correlated only to the protein content, the amount of glutenin subunits showed good correlations to most of the properties. The ratios of gliadins, in particular of α-gliadins, to the HMW subunits were also strongly correlated, either negatively (dough development time, maximum resistance, loaf volume) or positively (extensibility).

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2.8. Partial sequences from HMW subunits of rye glutelins

Previous studies have shown that the HMW subunits of rye and wheat glutelins are closely related in amino acid composition, molecular weight and N-terminal amino acid sequence. For further comparative studies on typical partial sequences, the precipates of HMW subunits from the rye cultivars Danko and Halo and from wheat cultivar Rektor were digested with trypsin. The partial hydrolysates were pre-separated into the fraction I (Mr <10,000) and II (Mr >10,000) by membrane centrifugation and then separated into single peptides by RP-HPLC. Dominating peptides were rechromatographed preparatively and characterised by amino acid analysis and N-terminal sequence determination: The resulting sequences were then compared with known sequences from wheat HMW-subunits.

Even if the sequences of the rye peptide are similar to those of wheat, they are not identical in any case. On an average, the variations were about 20 %. Most of the rye peptides could be assigned to the x-type subunits of wheat. Within the most characteristic repeating sequences, significant differences between rye and wheat were not detected.

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2.9. Fragmentation of gliadin and casein peptides by the intestinal mucosa

The pathogenesis of coeliac disease is unknown; immunological reactions, lectin-like reactions and enzyme deficiencies are discussed as mechanism. In order to investigate differences in proteolytic activities, the intestinal mucosa of four different groups of patients (coeliac patients with flat and recovered mucosas, patients with cow milk allergy and flat mucosa, control persons with normal mucosa) were incubated with the coeliac active gliadin peptide B3144, the casein peptide Cas-P and without addition of peptides. The fragmentation of the peptides was followed up by RP-HPLC.

Generally, the casein peptide was hydrolysed easierly by both normal and flat mucosas than the gliadin peptide. Differences between coeliac patients with recovered mucosa and control persons were not evident; thus, a primary enzyme deficiency could not be demonstrated. A severe secondary peptidase deficiency, however, was observed with flat mucosas. Obviously, the degree of deficiency caused by coeliac disease is higher than that of cow milk allergy.

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2.10. Bitter taste of enzymic hydrolysates of casein

The bitter tasting tryptic hydrolysate of beta-casein A2 was separated by RP-HPLC into 18 peptides, which represented 97 % of the protein sequence. Only three peptides had a bitter taste; their contribution to the overall bitterness of the hydrolysate was about 11, 21 and 60 %, respectively. The threshold values indicate that in the case of larger peptides neither hydrophobicity nor size are responsible alone for bitter potency, but that conformational parameters must be of great importance. Furthermore, it can be concluded that only a part of the structure is responsible for the contact with the receptor.

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