

Annual Report 1994

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Summaries

1. FOOD ANALYSIS

1.1. Dependence of 12-methyltridecanal concentration in beef meat from the age of the animal

12-Methyltridecanal (12-MT), which contributes strongly to the characteristic aroma of stewed beef, was determined by a stable isotope dilution assay in meat samples from 9 bovine animals of different age. The results, which were related to the amount of phospholipids (PL), indicate that 12-MT increases linearly with the age of beef (meat), e.g. from 36 µg/g PL in a 4

months old calf to 810 µg/g PL in an 8 years old cow. The increase of 12-MT in the PL per month varied between 8.4 and 10.9 µg/g (mean: 9.3 ± 0.78 µg/g). Possibly, 12-MT is suitable as an indicator for the estimation of the age of a beef sample.

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1.2. Quantitative analysis of caffeic and ferulic acids in oat meal. - Comparison of a conventional method with a stable isotope dilution assay

[13C]-Caffeic and [13C]-ferulic acid were synthesized and then used as internal standards for the determination of these acids (free and esterified) in oat meal. A comparative study indicated that 84 % of the ferulic acid, but only 32 % of the caffeic acid, which is more susceptible to oxidation than the former, could be found by a conventional analytical approach.

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1.3. Gas chromatography/olfactometry of static headspace samples (GCO-H)

On the basis of a preceding aroma extract dilution analysis hexanal and trans-2,3-epoxyoctanal followed by pentanal and (E,E)-2,4-nonadienal were identified by GCO-H as the odorants causing the rancid off-flavour of oat extrudates stored for one year at room temperature. Addition of alpha-tocopherol to the oat meal before extrusion inhibited the formation of the off-flavour in the extrudate. GCO-H and the quantification of hexanal as indicator substance confirmed the aroma stability of the extrudate containing the alpha-tocopherol.

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1.4. Quantification of HMW gliadin in flours of different wheat varieties

Only a few studies have characterized the structure of HMW gliadin, an alcohol-soluble protein aggregate of wheat flour, and only few quantitative data are available. The methods used for quantification are time-consuming and insensitive. For this reason, a relatively fast, reproducible and sensitive procedure, using gel permeation chromatography on Superdex 200 and UV-measurement, was developed to determine the proportion of HMW gliadin present in total gliadin. Investigations of 12 different wheat varieties revealed that the proportions of HMW gliadin vary from 17-25 % depending on the variety. The corresponding alcohol-soluble protein aggregates of rye had significantly higher proportions (35 %). According to the extractibility with aqueous alcohols and the effect on the rheological properties of dough and gluten, HMW gliadin is closely related to the monomeric gliadins.

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1.5. Rapid determination of reduced glutathione and cysteine in wheat flours

The cyclo voltammetric (CV) determination of cysteine (Cys) and reduced glutathione (GSH) in wheat flour extracts includes the following steps: Derivatization of Cys and GSH at pH 4.5 with 10 µmol of N-phenylmaleimide (NPMI). Backtitration with 10 µmol Cys and determination of the amount of Cys (equal to the sum of Cys and GSH), which was not converted into the NPMI derivative, by CV. This amount of Cys was equal to the sum of Cys

and GSH occurring in the flour extract. In a parallel experiment GSH was selectively removed from the flour extract by addition of ascorbic acid. The remaining Cys was determined by CV after addition of NPMI and backtitration. GSH was calculated by subtraction of the amount of Cys from that of the sum of Cys and GSH. The results obtained for three flours were in good agreement with those found by a reference method; only in one case the values for GSH were different.

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1.6. Nutrition tables

Food composition and nutrition tables are 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 the scientific literature with the aid of the data bank SFKDB. 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. The book has been published in the autumn 1994.

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2. FOOD CHEMISTRY, BIOCHEMISTRY AND MICROBIOLOGY

2.1. Aroma of heated meat - Precursors of ethyldimethylpyrazine isomers and 2,3-diethyl-5-methylpyrazine

Reaction systems (pH 5.6) containing water-soluble substances of beet meat in various combinations were heated for 7 min at 180°C. Lacking of alanine in a mixture consisting of free amino acids, monosaccharides, lactic acid, carnosine and creatine inhibited strongly the formation of 2-ethyl-3,6-dimethylpyrazine (I), 2-ethyl-3,5-dimethylpyrazine (II), 2-ethyl-5,6-dimethylpyrazine (III) and 2,3-diethyl-5-methylpyrazine (IV). Carnosine as well as lactic acid stimulated the formation of the four pyrazines in the reaction system fructose/alanine, but the most effective model was obtained when, in the latter binary mixture, fructose was replaced by 2-oxopropanal. In all reactions pyrazine I was the major product but, on the basis of its much lower odour threshold, pyrazine II was the most important odorant of the ethyldimethylpyrazines.

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2.2. Changes in the most odour-active volatiles of strawberries induced by processing

Application of an aroma extract dilution analysis on extracts from fresh and heat-treated (100°C; 30 min) strawberries revealed 4-hydroxy-2,5-dimethyl-3(2H)-furanone, butanoic acid, (Z)-3-hexenal and acetic acid followed by ethyl butanoate, 2- and 3-methylbutanoic acid, methyl butanoate, 2,3-butandione and methyl 2- and 3-methylbutanoate as the most odour-active compounds in the fresh fruits. The heat-treatment led to a significant decrease in the FD-factors of especially (Z)-3-hexenal and the three esters. In addition, nine compounds appeared as new odorants in the heated fruit material among which (E,E)-2,4-decadienal, (E)-beta-damascenone and an unknown compound with a geranium-like flavour were the most

odour-active. Quantification of fourteen odorants by stable isotope dilution analyses implied that the flavour differences induced by the fruit processing are mainly caused by higher concentrations of the sweet, honey-like smelling (E)-beta-damascenone and lower concentrations of the green-smelling (Z)-3-hexenal and three ester, exhibiting fruity odors, in the heated strawberries. Further studies revealed that by freezing and thawing of fresh fruits a strong decrease in (Z)-3-hexenal is induced, too.

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2.3. Cardboard Off-Flavour in Butter Oil

The increase of (Z)-4-heptenal (I), (Z)-2-nonenal (II), (E)-2-nonenal (III) and 1-octen-3-one (IV), which may contribute to the off-flavours in butter oil, was quantified by stable isotope dilution assays in samples stored at 35°C. At a lower peroxidation level BHA, BHT, alpha-tocopherol and gamma-tocopherol (additions of 0.47 mmol/kg each) inhibited the formation of I to III by 40-50 % and that of IV by 20-30 %. Acceleration of the autoxidation by the addition of copper ions (1 and 8.5 mg/kg) enhanced the inhibitory effect of the antioxidants. Sensory evaluations revealed that the cardboard off-flavour in butter oil was caused by a mixture of II (>1.5 µg/kg) and III (>23 µg/kg).

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2.4. A comparative study on the potent odorants of different virgin olive oils

The potent odorants of two olive oils differing in the flavour were evaluated by aroma extract dilution analysis, static headspace analysis and calculation of odour activity values. The changes of nine potent odorants and of (Z)-3-hexenol were determined at different ripeness stages of the olives. The level of the green smelling odorant (Z)-3-hexenal was high in the unripe fruits and that of the fruity esters increased slowly during the maturation of the olives.

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

By screening experiments 3-methylbutanal (I), ethyl butanoate (II), ethyl 3-methylbutanoate (III), methional (IV), ethyl hexanoate (V), 4-hydroxy-2,5-dimethyl-3(2H)-furanone (VI), 2-ethyl-4-hydroxy-5-methyl-3(2H)-furanone (VII), acetic acid (VIII), propionic acid (IX), lactic acid (X), ammonia (XI), glutamic acid (XII), potassium dihydrogen phosphate (XIII), calcium hydroxide (XIV) and magnesium hydroxide (XV) were found to be potent odorants and taste compounds of Emmentaler.

Mixtures of the compounds I to XV in various combinations and in concentration levels equal to those found in Emmentaler were added to an unripened and freeze-dried cheese (Mozzarella) and then the water content of Emmentaler was adjusted. The sensory study indicated that the combination of the compounds IV, VI to XV resulted in a model which was very similar to grated Emmentaler regarding to the odour and taste impressions.

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2.6. Aroma of bread

Aroma extract dilution analyses revealed twenty-eight odorants in fresh rye bread crust and twenty in the crumb. On the basis of high flavour dilution factors, methional (boiled potato), 3-methylbutanal (malty), (E)-2-nonenal (green, tallowy), (E,E)-2,4-decadienal (fatty) and acetic acid (sour, pungent) belonged to the potent odorants of the crust, and phenylacetaldehyde, (E)-2-nonenal and (E,E)-2,4-decadienal to those of the crumb. Compared to the crust, especially the odour activities of methional, 3-methylbutanal, 2-ethyl-3,5-dimethylpyrazine and 4-hydroxy-2,5-dimethyl-3(2H)-furanone were significantly lower in the crumb.

Calculation of odour activity values (OAV; ratio of concentration to odour threshold) indicated that the higher OAV of methional in the rye crust and the higher OAV of 2-acetyl-1-pyrroline in the wheat bread crust mainly contributed to the flavour difference of the two kinds of bread.

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2.7. Disulfide bonds in wheat gluten

Though the disulfide bonds of gluten are considered to be important for the viscoelastic properties of wheat doughs, they had not been localized and characterized until now. In continuation of previous studies, 19 further cystine peptides from enzymatic hydrolysates of glutenin were isolated, analyzed and assigned to known sequences of gluten proteins. For the first time, an intermolecular disulfide bond between a γ -type HMW subunit and a LMW subunit was proven. Other cystine peptides confirmed the positions of disulfide bonds present in LMW subunits and γ -gliadins, found previously. Furthermore, two monomeric α -gliadins were isolated by RP-HPLC and analyzed for disulfide bonds. The cystine peptides of both proteins reflected one intramolecular disulfide bond within domain III and two intramolecular disulfide bonds between domain III and V. Altogether, the results indicate that intramolecular disulfide bonds of gluten proteins are not randomly linked, but strongly directed. For intermolecular disulfide bonds, however, different combinations of linkages were found.

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2.8 Effect of nitrogen fertilization on the composition of wheat flour proteins

Although different supplies of nitrogen during wheat growth strongly influence the protein composition of flours and, consequently, their dough property and baking quality, the effect on the amounts and proportions of the different flour protein types have still not been determined. The flours of 16 wheat varieties, grown at two different levels of nitrogen, were therefore characterized by a detailed protein analysis using the extraction/HPLC procedure developed previously. The results demonstrated that the amounts of albumins and globulins are not influenced, whereas those of gluten proteins are strongly influenced by different N-fertilization. The effect on gliadin was more pronounced than on glutenin, as well as the effect on major protein types (α -, γ -gliadins, LMW subunits of glutenin) in comparison with minor types (χ -gliadins, HMW subunits of glutenin). The proportions of hydrophilic proteins (χ -gliadins, HMW subunits of glutenin) were increased by high levels of N-fertilization and those of hydrophobic proteins (γ -gliadin, LMW subunits of glutenin) were decreased. The degree of the effects on both amount and proportions of single protein types was strongly dependent on the variety.

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2.9. Effects of HMW subunits of glutenin on the rheological properties of wheat gluten

Previous investigations of different wheat varieties have revealed a high correlation between the amount of HMW subunits of glutenin and the maximum resistance of dough and gluten and also between the ratio gliadin/HMW subunits and extensibility. In order to confirm such relationships, gliadin and HMW subunits of different compositions were added to standard flours, and the corresponding glutes were then characterized by extension tests. The extensibility of gluten was increased by monomeric proteins (gliadin, reduced HMW subunits) and was decreased by aggregated proteins (reoxidized HMW subunits). The maximum resistance of gluten was increased by reoxidized HMW subunits, when the major proportion was in an aggregated state (reoxidation with BKrO_3). Beside molecular-weight distribution, the thiol content of HMW subunits appeared to influence those effects. The amount of aggregated HMW subunits was much more important for the rheological properties of gluten than different combinations of HMW subunits.

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2.10. Microscopic studies on gluten structure in bread crumb

Microscopic studies on bread crumb described in the literature give informations on pore size and wall thickness, but because of the low magnification used the differentiation of gluten structure is not possible. Utilizing a washing procedure, critical point drying and scanning electron microscopy, the spatial structure of gluten protein was visualized both on the surface and within the pore walls of bread crumb. On the surface, film-like gluten structures were extended between starch granules. Highly extended films were frequently disrupted to form a fibrillar network. Also within pore walls, porous gluten films were observed. Partially, they were oriented parallel to the surface, partially they were crosslinked forming a network of porous sheets. The formation of the gluten structure in bread crumb can be traced back to the gluten structure in flour particles demonstrated previously.

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2.11. Spelt wheat and coeliac disease

Spelt wheat (*Triticum spelta* L.) has not been investigated for the toxicity on coeliac disease patients until now. Because clinical studies are out of considerations for ethical reasons, spelt wheat and coeliac-active bread wheat (*Triticum aestivum* L.) were compared by the analysis of N-terminal sequences of alpha-gliadins, which have been proposed to be responsible for the toxic effect. The gliadin fractions of the spelt wheats 'Roquin' and 'Schwabenkorn' and of the bread wheat 'Rektor' were preparatively separated by RP-HPLC and major alpha-gliadin components were then compared by N-terminal sequence analysis. The results did not reveal any significant difference between spelt and bread wheats within the first 25 positions. For the determination of sequences further from the N-terminus, the gliadin fractions of the spelt wheats were hydrolyzed with pepsin and trypsin. The resulting peptides were successively separated by gel permeation chromatography and RP-HPLC. Those peptides derived from the N-terminal part of alpha-gliadins were identified by reference peptides isolated previously from bread wheat. Retention times upon RP-HPLC and amino acid compositions of corresponding peptides confirmed the identity of spelt and bread wheat concerning the N-

terminal sequences of alpha-gliadins from position 3 to 56. For these reasons, it can be concluded that spelt wheat is a coeliac-toxic cereal and has to be avoided by coeliac patients.

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