

## Annual Report 1992

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### Summaries

#### 1. FOOD ANALYSIS

##### 1.1. Furan fatty acids in butter and butter oil

Nine furan fatty acids (F-acids), among which 12,15-epoxy-13,14-dimethyleicosa-12,14-dienoic acid and 10,13-epoxy-11,12-dimethyloctadeca-10,12-dienoic acid predominated, were

detected in butter and butter oil. The total amount of the F-acids in four butter samples varied between 116 and 476 mg/kg.

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### **1.2. A comparative study on the potent odorants of different virgin olive oils**

The potent odorants of four olive oil samples differing in the flavour were quantified and their odour activity values (OAVs) were calculated by dividing the concentrations in the oil samples by the flavour threshold values in an oil. The odorants having higher OAVs were contrasted with the different notes of the flavour profiles of the olive oils. It was derived that the following compounds contributed mainly to the flavour notes given in brackets: (Z)-3-hexenal (green), ethyl 2-methylbutyrate, ethyl isobutyrate, ethyl cyclohexanoate (fruity), (Z)-2-nonenal (fatty) and 4-methoxy-2-methyl-2-butanethiol (black-currant like). The results show that the calculation of OAVs is an approach to objectify the flavour differences of olive oil samples.

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### **1.3. Aroma of coffee**

Aroma extract dilution analysis (AEDA) revealed 13 compounds as important contributors to the aroma of the roasted coffee (powder): 2-methyl-3-furanthiol (I), 2-furfurylthiol (II), methional (III), 3-mercapto-3-methylbutylformate (IV), 3-isopropyl-2-methoxypyrazine (V), 2-ethyl-3,5-dimethylpyrazine (VI), 2,3-diethyl-5-methylpyrazine (VII), 3-isobutyl-2-methoxypyrazine (VIII), 3-hydroxy-4,5-dimethyl-2(5H)-furanone (sotolon, IX), 4-ethylguaiacol (X), 5-ethyl-3-hydroxy-4-methyl-2(5H)-furanone (XI), 4-vinylguaiacol (XII) and (E)-beta-damascenone (XIII). A comparative AEDA of the coffee powder and brew showed in the brew an increase of III, IX, vanillin and 4-hydroxy-2,5-dimethyl-3(2H)-furanone and a decrease of I, II, IV, V, VII and VIII.

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

An isotope dilution assay (IDA) for free reduced glutathione (GSH) and total glutathione (GSH, GSSG and protein bound glutathione) was developed and its accuracy and sensitivity were established.

The new method for GSH requires extraction of the flour sample with a buffer at pH 4.5 containing N-ethyl maleimide (NEMI) and [<sup>14</sup>C]-GS-NEMI, purification of the labeled and unlabeled GS-NEMI by three chromatographic steps and assay of the specific radioactivity of the GS-NEMI isolated. Total glutathione is assayed after reduction with dithioerythritol.

Applications of the IDA indicated that the levels of GSH (16 to 41 nmol/g) and total glutathione (170 to 185 nmol/g) were relatively low in flours with low ash content, but increased with increasing extraction grade. The level of GSH was higher in a flour obtained from kernels which were ground in the absence of gaseous oxygen. Storage of flours reduced the GSH concentration. IDA of fractions obtained from flour showed that the extraction residue, mainly consisting of starch and glutelins, contained most of the bound glutathione.

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### **1.5. Capillary electrophoresis of carbohydrates**

The capillary electrophoresis is a relatively new method, which seems to be suitable for the analysis of low molecular weight components in foodstuffs. Complex mixtures of mono- and oligosaccharides including acid carbohydrates were separated without derivatization, detected by indirect UV photometry, and quantitatively determined via the peak areas. In this way, glucose, fructose and sucrose were determined in orange juice.

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### **1.6. Detection of soya in meat products. Comparison of different blotting- and detection techniques after electrophoresis on ExcelGel SDS**

Different electrophoretic techniques were compared with regard to the detection of soyproteins in foodstuffs, especially in meat products. In combination with specific staining techniques commercial ExcelGel SDS-plates may be used as well as self-made SDS-PAGE-plates. The advantages of the commercial plates are availability, with the analytical procedure the disadvantage is the higher price and saving of time.

Both, semidry blotting (electroblotting) and diffusion blotting are suitable for the protein transfer to Immobilon membranes. Staining of the gels after blotting showed that electroblotting is more effective.

The investigated detection methods (immunogold/silverstain and lectin blotting) are both well suitable for many applications. IGSS needs more time, but is easier to perform. A further advantage is the higher sensitivity (5 times for emulsion-types ausages and at least 10 times for sterilized meat products) in comparison to the lectin blotting. The reason is that the anti-soyprotein serum contains antibodies against all protein fractions of the soybean, while the latter method responds only to the 7S-fraction, which is the minor one in soy beans.

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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, 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 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 preparation of the manuscript for the 5th edition of the large table, which should be published in autumn 1993, has been continued. Focal points of activity were the composition of slaughtered animals, available carbohydrates, trace elements (aluminium), vitamins (B6, A, and A-active carotinoids), carotinoids without vitamin A activity and glutathion.

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## **2. FOOD CHEMISTRY, BIOCHEMISTRY AND MICROBIOLOGY**

## **2.1. Aroma compounds of heated meat**

Aroma extract dilution analysis of the volatile fraction isolated from roasted beef resulted in 25 odour compounds of which 22 were identified. 2-Acetyl-2-thiazoline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, guaiacol, 2-ethyl-3,5-dimethylpyrazine and 2,3-diethyl-5-methylpyrazine were detected as the character impact compounds for the roasty, caramel-like, burnt and earthy odour notes. (E)-2-Nonenal, (E,E)-2,4-decadienal and gamma-octalactone originated, at least in part, from the fat used for the roasting of the meat.

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## **2.2. Studies on the formation of meat-like flavor compounds**

The formation of 2-methyl-3-furanthiol (MF-SH), its oxidation product bis(2-methyl-3-furyl)disulfide (MF-S<sub>2</sub>) and 2-furfurylthiol (FF-SH) were determined in model systems containing the precursors in concentration levels approximated to those occurring in meat. The degradation of thiamine was more effective in the production of MF-SH than the reaction of ribose and cysteine. Addition of cysteine, and, to a lesser extent, of H<sub>2</sub>S enhanced the formation of MF-SH from thiamine. In contrast to thiamine its pyrophosphate derivative was inactive as precursor of MF-SH. The amounts of FF-SH and MF-S<sub>2</sub> formed were very low in all models investigated.

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## **2.3. Studies on the formation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone in wheat bread crust and popcorn**

4-Hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) occurs in many processed foods and was recently established by us as important odorant in wheat bread crust and popcorn. To gain an insight into its origin, the precursors in wheat dough and corn were elucidated on the basis of quantitative measurements. Baking experiments revealed yeast as the most important source of HDMF in wheat bread crust. Furthermore, boiling of an aqueous solution of the low molecular weight compounds from disrupted yeast cells yielded high amounts of the odorant. Analysis of the free sugars present in this fraction and results of model experiments indicated fructose-1,6-diphosphate as the predominant precursor of HDMF. In contrast, dry-heating was necessary to liberate HDMF from its precursors in corn. Model experiments revealed that these conditions, simulating the popping process, caused the formation of HDMF from glucose and fructose which predominated in corn and were little active, when heated in aqueous solution. Further model experiments showed that acetylformoine is an important intermediate in the formation of HDMF from hexoses.

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## **2.4 Stale off-flavour of wheat bread**

The headspace volatiles of the crust from both, a freshly-baked and a stored (96 h) white bread, were evaluated by aroma extract dilution analyses (AEDA). In the fresh crust, 3-methylbutanal (malty), 2-acetyl-1-pyrroline (roasty) and 2,3-butandione (buttery) appeared with the highest FD-factors, followed by 2-methylpropanal, 1-octen-3-one, 2-ethyl-3,5-dimethylpyrazine and (E)-2-nonenal with lower FD-factors. During storage a stale off-odour was formed in the crust. This was reflected by the AEDA: The FD-factors of 3-methylbutanal,

2-acetyl-1-pyrroline, 2,3-butandione, 2,3-pentandione and methional were significantly lowered in the crust of the stored white bread, while the FD-factors of 1-octen-3-one, 2-ethyl-3,5-dimethylpyrazine and (E)-2-nonenal remained unchanged. Quantitative measurements of five odorants by stable isotope dilution assays established that the stale off-odour formed during storage of white bread was caused by the rapid loss of key odorants of the fresh bread, e.g. 2-acetyl-1-pyrroline and 3-methylbutanal, and the retention of those formed by a lipid peroxidation, e.g. (E)-2-nonenal.

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### **2.5. Primary odorants of pale lager beer - Differences to other beers and changes during storage**

Application of an aroma extract dilution analysis to the flavour concentrate of a fresh pale lager beer revealed 33 potent odorants (FD-factor ranging from 16 to 1024) among which 3-methylbutanol, 2-phenylethanol, 4-vinyl-2-methoxyphenol, 3- and 2-methylbutanoic acid, 4-hydroxy-2,5-dimethyl-3(2H)-furanone (HDMF) and butanoic acid showed the highest FD-factors. Compared to the pale lager beer the odour activity value (OAV; ratio of concentration to odour threshold) of HDMF in a dark lager beer and of 4-vinyl-2-methoxyphenol in a wheat beer, was significantly higher. Sensory experiments corroborated that these odorants were important contributors to the overall flavour of the dark lager and the wheat beer, respectively. In two alcohol-free beers the OAVs of eight of the most important odorants were markedly lower.

After storage of a pale lager beer (14 d; 40°C), which had been spiked with oxygen, the FD-factors of most of the primary odorants of the fresh beer remained unchanged. On the other hand, phenylacetaldehyde (sweet, honey-like), 3-methyl-3-mercaptopbutylformiate (catty, ribes odour) and an unknown compound with a sweet, aniseed-like note appeared as additional important odorants in the stored sample.

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### **2.6. Disulfide bonds in wheat gluten**

Disulfide bonds of glutenin are considered to be important for the viscoelastic properties of wheat doughs. However, until now they had not been localized and characterized. Therefore, cystine peptides were isolated from enzymatic hydrolyzates of glutenin, analyzed for their amino acid sequences and attributed to known sequences of glutenin proteins. The structures of 36 cystine peptides were solved. It could be shown that HMW subunits of glutenin are involved in inter- and intramolecular disulfide bonds. Several cystine peptides came from LMW subunits of glutenin and from gamma-gliadins. There was no indication for intermolecular disulfide bonds between HMW and LMW subunits or HMW subunits and gamma-gliadins.

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### **2.7. Glutenin fractions and rheological properties of different wheat varieties**

In the literature are many indications for relations between HMW subunits of glutenin and wheat quality. Nine wheat varieties of different quality were quantitatively analyzed for glutenin fractions (HMW subunits, total HMW glutenin, total LMW glutenin, total glutenin)

and for rheological properties of dough and gluten (maximum resistance, extensibility, mixing time). The results showed a strong correlation between the total amount of HMW subunits, especially of those of the x-type, and maximum resistance as well as mixing time. The pattern of the y-type subunits seemed to be less important.

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### **2.8. Quantitative determination of $\alpha$ -, $\gamma$ -, and $\omega$ -gliadins in different wheat varieties**

The influence of gliadin components on wheat quality is occasionally discussed in the literature. However, quantitative data are lacking. Therefore, a representative selection of wheat varieties with known rheological properties has been quantitatively analyzed for gliadin. The comparison of different isolation procedures showed that the direct extraction from flour with 50 % aqueous ethanol and the use of ultrasound delivered the best results. The isolated amounts of gliadin were proportional to the total protein content of the flours. The gliadin patterns, obtained by HPLC, have been typical for the varieties, but exhibited no relations to the rheological data of the corresponding doughs. The alpha-gliadins were throughout the main fraction with the lowest range of variations. Wheat/rye hybrids with 1B/1R-substitutions or 1BL/1RS-translocations showed the highest amounts of x-gliadins (x-secalins), which were balanced throughout by lower amounts of gamma-gliadins.

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### **2.9. Specificities of monoclonal antibodies raised against the gliadin peptide B3144**

To develop a sensitive and specific immunochemical assay for the determination of gliadin in food, the gliadin peptide B3144 was used as immunogen to raise monoclonal antibodies. The specificities of five antibodies to different antigens were characterized by ELISA. Three antibodies cross-reacted with one or more of the coeliac non-toxic prolamins of rice, maize, millet and sorghum. Two antibodies bound specifically to the coeliac toxic prolamins of wheat, rye, barley and oats. Their specificities to the gliadin peptides B3142, B3143 and B3144 and the fragment peptides CT-1 and CT-2 indicated that one antibody binds in the region of the proline residue 36 of alpha-gliadins.

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### **2.10. The course of the SDS- and zeleny sedimentation test and related phenomena studied at the light microscope**

The course of the sedimentation test for the evaluation of gluten quality was investigated by light microscopy. Flour particles swelled up reversibly in the sedimentation solutions. Single strands of the gluten network did the same, the swelling up being stronger in the SDS-sedimentation solution than in the Zeleny reagent. The investigation of gliadin and glutenin showed only glutenin to be capable of swelling, whereas gliadin completely dissolved.

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### **2.11. Changes in wheat properties by heating**

The properties of wheat are changed by heating. The background of these changes, especially the role of different flour components, is not well known. Therefore, two classes of wheat,

CWRS and DNS were investigated in the temperature range of 50-65°C. The extensograms of doughs from heated kernels, and of heated doughs showed that the samples responded differently: DNS was relatively insensitive below 65°C, while the doughs of CWRS became strong and less extensible already between 50 and 55°C.

Flours were separated into starch, gluten and water-soluble components, which were remixed in the native and the heated state. Starch and water-soluble fraction exhibited no heat-dependent differences. Contrary, gluten showed the same changes after heating, as dough did. Altogether, gluten seems to be mostly responsible for the changes caused in wheat by heating.

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