DFA
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Summaries

1. Food Analysis

1.1. The flavour of bouillon

Bouillon was analyzed for volatile and nonvolatile components. The volatile fraction was obtained by vacuum distillation and extraction with pentane/diethyl ether (2:1, v/v). The volatiles showing high odour units were evaluated by flavour dilution analysis and then identified. Nonvolatile components were obtained from bouillon lyophilisates by gel permeation chromatography and RP-HPLC. The fractions were analysed for proteins, peptides, amino acids, nucleotides and nucleosides. The lyophilisate was analyzed further for organic acids and minerals. Taste qualities and taste thresholds of the components were estimated.

The results of the chemical and sensory analyses were used for a stepwise simulation of bouillon. A system composed of the vacuum distillate and gelatine, aspartic acid, glutamic acid, 5'-AMP, 5'-IMP, carnosine, anserine, carnitine, chloride and phosphate was judged to meat the original bouillon very well.

1.2. Aroma extract dilution analysis of commercial meat flavourings

Eight commercial meat flavourings were compared by aroma extract dilution analysis (AEDA). The number of odorants appearing with significant FD-factors varied greatly (8-33). Some

odorants with a high FD-factor in the aromagrams of broths prepared from beef and chicken meat also appeared in the aromagrams of the flavourings, e.g. bis(2-methyl-3-furyl)disulphide, the character impact compound for the "meaty" flavour note. Other compounds, e.g. 2-methyl-3-(methyldithio)-furan appeared with a significant FD-factor only in the aromagrams of the flavourings. The results indicate that AEDA is a suitable technique for characterizing commercial food flavourings.

1.3. Flavour compounds as indicatiors of the oxidative fat deterioration

A stable isotope dilution assay was developed for the quantification of hexanal, (Z)-3-hexenal, 1-octen-3-one, (Z)-1,5-octadien-3-one, 1-octen-3-hydroperoxide, (E)-2-nonenal, (Z)-2-nonenal, (E,Z)-2,5-nonadienal, (E,E)-2,4-decadienal, trans-4,5-epoxy-(E)-2-decenal, and 3-methyl-2,4-nonandione. The oil sample was spiked with the synthesized deuteriated internal standards, and the volatile fraction was distilled off from the oil and then separated by HPLC. Each of two HPLC fractions isolated was analysed by capillary gas chromatography/mass spectrometry (chemical ionization mode). Mass chromatograms were recorded for selected ions to differentiate between the unlabeled analytes and their deuteriated analogues. The procedure was applied to soya-bean oil samples which were stored in the presence and absence of light at room temperature. A comparison of the data obtained with the taste threshold data indicated that 3-methyl-2,4-nonandione was the most flavour-active compound after 48 hours of storage in daylight.

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

The work was continued with the development of a stable isotope dilution assay for beta-damascenone, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DHF; Furaneol) and its methyl ether, 4-methoxy-2,5-dimethyl-3(2H)-furanone (MDF).

Application of the method for beta-damascenone showed that, during the preparation of a coffe brew, about 18 % of the beta-damascenone occurring in the ground coffee (293 µg/kg) was extracted by the hot water.

The method developed for DHF was quite sensitive and DHF could be analysed in sections of one strawberry fruit. A relative high amount of MDF was found only in a sample of commercially obtained strawberry juice. Obviously this juice had been prepared from overripe fruits, in which higher amounts of MDF had been detected by Ito et al. [1990].

1.5. Investigation into the ripening of cheddar

Analytical methods, which give insight into the ripening state of cheese are important for processing. A control is possible via the protein degradation, e.g. very simple by measuring the nitrogen soluble at pH 4.6 (extent of ripening) or in 12 % trichloroacetic acid (depth of ripening). A more detailed differentiation should be possible by following degradation products with chromatographic and electrophoretic methods.

Cheddar of German and English provenience was available, which was ripened at 4ÊC and 10ÊC. Samples were taken between 3 and 36 weeks. The soluble nitrogen, determined by the Kjeldahl and the o-phthaldialdehyde methods, increased rather linear with the ripening time. Rising the ripening temperature from 4ÊC to 10ÊC resulted in an increase of the soluble nitrogen by a factor of 1,3. The values for the English Cheddar were about 20 % lower as those for the German cheese during the whole ripening interval. Different average chain lengths of peptides were observed for German and English cheeses in both the pH 4.6-soluble and the TCA-soluble fractions, which points to different peptide patterns.

The separation of the peptides soluble at pH 4.6 by RP-HPLC delivered characteristic peptide patterns and a significant increase of peptide peaks with increasing ripening time. Some peptides reached a plateau, others went through a maximum. The peptide patterns of English and German Cheddar were quite different and seem to allow a distinction.

The isolation of some prominent peptides and their attribution to the casein sequences is in progress.

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 LINDAS. The same is true for the related small table "Der kleine 'Souci, Fachmann, Kraut': Lebensmitteltabelle för die Praxis", which was developed for the daily requirements of the consumer.

The 4th edition of the large table appeared in November 1989. The preparation of the manuscript for the 5th edition is in progress. It is focussed on data about dietary fibre, fatty acids, cholesterol, tocopherols, iodine, sodium and potassium.
The manuscript for the 2nd edition of the small table was finished in May 1990 and is in press. An Italian version of the small table appeared in April 1990.

2. **Food Chemistry, Biochemistry, and Microbiology**

2.1. Primary Flavour Compounds in Dill Seed and Dill Herb (*Anethum graveolens* L.)

The volatile components of dill seed and herb were analysed by gas chromatography-olfactometry which reveals the odorants having the highest odour-activity value (ratio of the concentration to the odour threshold). (+)-(S)-Carvone was the predominent odorant of dill seed. (+)-3R,4S,8R)-3,9-Epoxy-1-p-menthene, methyl 2-methylbutanoate, (+)-(S)-alpha-phellandrene and myristicin were the most important odorants of dill herb. Calculation of the odor-activity values on the basis of quantitative and odour threshold data confirmed the results of the aroma extract dilution analysis.

2.2. Studies on the formation of wheat bread flavour

The primary odorants of a model bread crust prepared from a chemically leavened wheat dough were compared to those earlier identified in a crust from a yeasted dough. The most striking difference was that in the model crust the odour-activity value of 2-acetyl-1-pyrroline (ACPY), the flavour impact for the roasty crust note, was decreased by a factor of more than thirty. Further studies showed that heat treatment of a suspension of disrupted yeast cells in the presence of sucrose led to ACPY as one of the most important odorants indicating that its precursors are located in the yeast cells. Quantitative studies on different yeast/carbohydrate homogenates revealed that carbohydrate degradation by yeast enzymes prior to heating increased the amounts of ACPY. The yeast contained 890 mg/kg dry weight of proline. Model studies, in which aqueous solutions of proline and sugars or phosphorylated sugar metabolites

were heated, revealed that proline and dihydroxyacetone phosphate are important precursors of ACPY formation during baking.

2.3. Primary flavour compounds of the wheat bread crumb

Characterization of the crumb flavour of wheat bread by means of an aroma extract dilution analysis indicated diacetyl, methional, 1-octen-3-one, (Z)- and (E)-2-nonenal, (E,E)-2,4-decadienal and trans-4,5-epoxy-(E)-2-decenal as the most potent odorants. The epoxydecenal had the lowest odour threshold (1.5 pg/l, air) among the homologous series of trans-4,5-epoxy-(E)-2-alkenals C7-C11. Prolongation of the dough fermentation resulted in a change of the crumb flavour which was mainly due to an increase in the concentrations of 3-methylbutanol and 2-phenylethanol. Stable isotope dilution assays indicated that the concentrations of the "roasty" smelling flavour compounds 2-acetyl-1-pyrroline and 2-acetyltetrahydropyridine were 30-fold lower in the crumb than in the crust. (E)-2-Nonenal, which already occurred in the flour, increased strongly during the baking process.

2.4. Wheat during maturation: analysis of gliadins and glutenins by RP-HPLC

In continuation of a study of changes in the chemical composition of the wheat variety Schirokko during ripening, the gliadins and glutenins were investigated in more detail by RP-HPLC. The chromatograms of the gliadin fraction show very clearly that the x-gliadins appear first and the gamma-gliadins last of all in course of the ripening process. A relatively long period of about four weeks after flowering is necessary for the development of the "ripe", strongly differentiated alpha-gliadin pattern. The chromatograms of the glutenins exhibit for the HMW subunits only qualitative changes in course of the maturation. On the other hand the LMW subunits show an increasing differentiating, similar to the gliadins. Related to whole glutenin, the HMW subunits increase from about 15 % to 30 %, while the LMW subunits decrease from about 75 % to 60 %. The MMW glutenins remain relatively constant in the range of 10 %. Remarkable quantitative shifts were observed within the HMW glutenins during ripening. While the subunits 3 and 10 [nomenclature according to Moonen] dominate the pattern in early stages, high amounts of subunit 5, followed by decreasing amounts of subunits 3 and 10 and, with larger distance, subunits 1 and 9 are characteristic for ripe Schirokko.

2.5. Separation and quantitative determination of HMW subunits of glutenin from different wheat varieties

From the literature, it is well known for a larger number of wheat varieties that the HMW subunit patterns of glutenin, obtained by SDS-PAGE, are qualitatively correlated with functional properties. In previous investigations on the separation of whole glutenin by RP-HPLC with an urea containing elution system, HMW subunits of several varieties were resolved into three subrractions x-z. The proportion of these subfractions was correlated with the bread-making quality. In order to obtain further data about the relation between HMW subunit patterns and the functional properties of wheat varieties, methods with a higher resolution for HMW subunits were required. An urea-containing system with an extremely flat gradient was developed and delivered the best quantitative results, because of its high resolution power. 24 wheat varieties with different functional properties were quantitatively analyzed for their HMW subunits. The obtained values were correlated with physical parameters, namely SDS-sedimentation volumen and maximum resistance. The results clearly demonstrated the significance of the type as well as of the amount of HMW subunits relatively to the functional properties.

2.6. Fractionation and characterization of reduced glutenin from the wheat variety rektor

Wheat flour of the variety Rektor was defatted and extracted stepwise with buffered 0.4 M NaCl and 70 % (v/v) aqueous ethanol. The glutenin containing residue was further extracted with buffered 70 % (v/v) ethanol at 4Êc under reducing conditions. The extract (E1) was fractionated by preparative reversed-phase high-performance liquid chromatography on C18 silica gel into twentyfive components. Based on amino acid compositions and the hydrophobicities, most proteins were classified into five groups. The first two groups (peaks 2-4 and 5-7), which had similar compositions and were characterized by high contents of Glx, Pro and Phe, corresponded to the x5- and x1,2-gliadins. The third group (peaks 8-17) was the major group, and contained low-molecular-weight (LMW subunits of glutenin characterized by compositions that were similar to alpha- and gamma-gliadins, but with a higher content of Ser and lower contents of Asx and Ala. The last two minor groups (peak 20-21 and 22-25) were either identical with or closely related to the c-gliadins. N-terminal sequence analysis of the four major components from the LMW subunit group revealed unique sequences, not present in other types of gluten proteins.

The four high-molecular-weight (HMW) subunits were isolated from the residue of extract E1 by extraction with 70 % EtOH/DTE at pH 3.5 and 60ÊC. The extract (E4) was separated by preparative reversed-phase HPLC on C8 silica gel, and the components characterized by amino acid analysis and by determination of N-terminal amino acid sequences. These subunits had similar amino acid compositions, with the highest contents of Gly (18.2-19.8 mol-%), Tyr (5.1-6.4 mol-%) and Thr (3.2-3.8 mol-%) of gluten proteins. Differences were observed in the content of single amino acids. Nine steps of Edman degradation revealed identical N-terminal amino acid sequences expect for position 6 (Glu, Gly or Arg). The results agree with data for corresponding HMW subunits of other varieties.

2.7. Classification of the protein components of gluten

On the basis of data from literature, and previous results with the variety Rektor, the gluten proteins can be classified into six groups or subgroups: The high-molecular-weight group contains the HMW subunits of glutenin, the medium-molecular-weight group consists of two subgroups (x5-type and x-1,2-type gliadins) and three subgroups (LMW subunits of gluten, alpha-type and gamma-type gliadins) are present in the low-molecular-weight group.

This classification follow the system of Shewry et al. ("HMW prolamins, S-poor prolamins, S-rich prolamins"), but the term "prolamins" is avoided, because, according to the classical nomenclature of Osborne, it is restricted to proteins of cereal endosperm, which are soluble in aqueous alcohols at neutral pH in the cold and in the absence of reductants.

From the known data it can be concluded that the protein components within one group or subgroup are very similar. The observed differences can be attributed to substitution, deletion and/or insertion of individual amino acids and/or oligopeptides.

2.8. Information on the shape of sweet receptors by computer modelling

General models for sweeteners have been developed using a molecule building program by superimposition of the e/n-systems of a large number of sweet and nonsweet compounds from diverse structural classes. The models are based on psychophysical data (sweet thresholds) and clearly show the possible dimensions of the molecules compatible with sweet taste. The sweet potency depends strongly on the location of hydrophobic groups within the given sweet space.

The models include amino acids, oxathiazione dioxides, benziso-thiazolone dioxides, carboxyalkylbenzamides, naphthoimidazoles and related compounds.