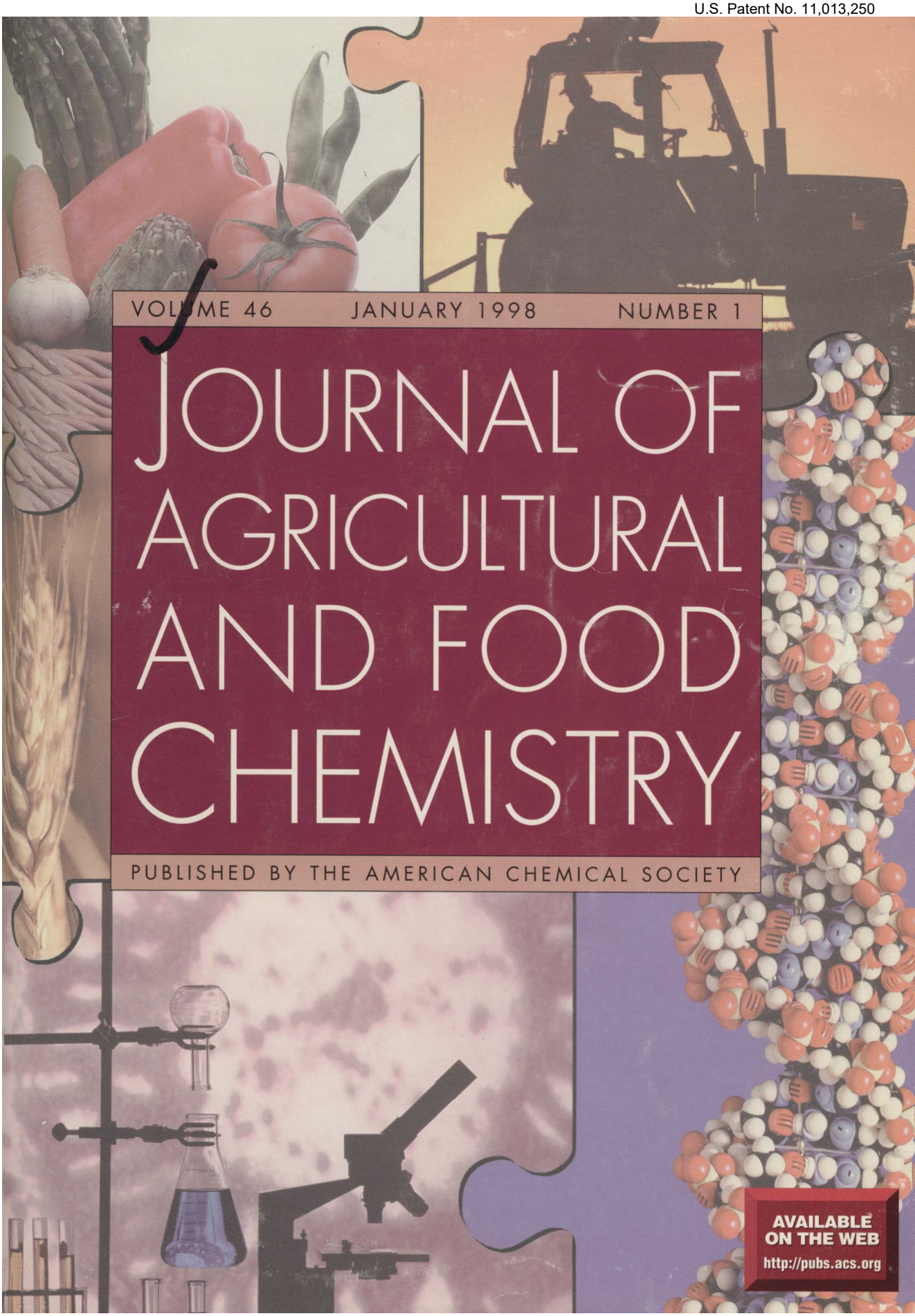
VOLUME 46 JANUARY 1998 NUMBER 1
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
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# JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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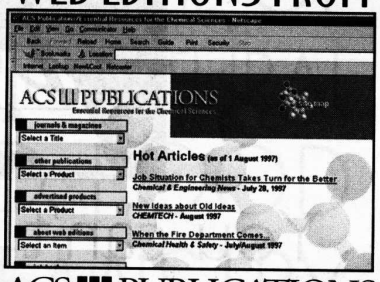


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*Journal of Agricultural and Food Chemistry* (ISSN 0021-8561) is published monthly by the American Chemical Society at 1155 16th St., N.W., Washington, DC 20036. Periodicals postage paid at Washington, DC, and additional mailing offices. POSTMASTER: Send address changes to *Journal of Agricultural and Food Chemistry*, Member & Subscriber Services, P.O. Box 3337, Columbus, OH 43210.

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JAFCAU 46(1) 1−360 (1998)
ISSN 0021-8561

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**JANUARY 1998**

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# Quantitative Model Studies on the Effectiveness of Different Precursor Systems in the Formation of the Intense Food Odorants 2-Furfurylthiol and 2-Methyl-3-furanthiol

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The yields of the two intense food odorants 2-furfurylthiol (FFT) and 2-methyl-3-furanthiol (MFT) obtained by heating mixtures of possible precursors in model systems varying in temperature, pH value, or water content were determined by using stable isotope dilution assays. Although pentoses generated much higher amounts of FFT and MFT than hexoses when heated in the presence of cysteine, glucose and rhamnose also gave significant yields. Studies on several intermediates indicated the highest yields for MFT (1.4 mol %) when hydroxyacetaldehyde and mercapto-2-propanone were reacted for 6 min at 180 °C in the absence of water. Both intermediates also generated significant amounts of FFT (0.05 mol %). However, the system furan-2-aldehyde/$H_2S$ showed a 10 times higher efficiency in generating FFT. Thiamin and norfuraneol/cysteine were less effective precursors of MFT. The results imply that different formation pathways may run in parallel during food processing and may account for the different amounts of the two odorants present in the respective food.

**Keywords:** *2-Furfurylthiol; 2-methyl-3-furanthiol; 4-hydroxy-5-methyl-3(2H)-furanone; furan-2-aldehyde; flavor precursors; mercapto-2-propanone; 3-deoxyribosulose*

## INTRODUCTION

2-Furfurylthiol (FFT) and 2-methyl-3-furanthiol (MFT) exhibit intense roasted, coffee-like, and meatlike odor notes at the extremely low odor threshold of 0.0025 ng/L in air (Hofmann and Schieberle, 1995). FFT was reported for the first time as a food constituent in a study on roasted coffee (Reichstein and Staudinger, 1926) and, later, among the volatiles of cooked meat (Garbusov et al., 1976). MFT was identified for the first time among the food volatiles in an investigation on the constituents of heated canned tuna fish (Withycombe and Mussinan, 1988).

Using aroma extract dilution analysis (AEDA), both FFT and MFT have recently been established as key aroma contributors to several thermally processed foods, such as cooked beef (Gasser and Grosch, 1988) and roasted coffee (Blank et al., 1992). Furthermore, FFT was elucidated as an important odorant in freshly popped corn (Schieberle, 1991), roasted white sesame seeds (Schieberle, 1996), and, very recently, in heated commercial as well as in self-prepared baker's yeast extracts (Münch et al., 1997).

A great number of investigations have been performed in the past 30 years to clarify the precursors of FFT and MFT in foods. Model studies have elucidated pentoses and cysteine as precursors of either FFT [e.g. Ledl and Severin (1973), Mottram (1987), Whitfield et al. (1988), Farmer and Mottram (1990a), Zeiler-Hilgart (1994), and Mottram and Whitfield (1995)] or MFT [e.g. Mottram (1987), Whitfield et al. (1988), Martin (1988), Farmer and Mottram (1990a,b), Meynier and Mottram (1995), and Mottram and Whitfield (1994)]. In addition, FFT has been reported to be formed by heating glucose in the presence of either hydrogen sulfide and ammonia (Shibamoto and Russel, 1976, 1977) or cysteine (Yeo and Shibamoto, 1991).

Very recently, by application of the AEDA, FFT and MFT have also been established as key contributors to the overall odors of processed flavors manufactured by heating ribose (Hofmann and Schieberle, 1995) and glucose or rhamnose (Hofmann and Schieberle, 1997) in the presence of cysteine.

Shibamoto and Russell (1977) and Silwar and Tressl (1989) reported the formation of FFT from furan-2-aldehyde, a major dehydration product of pentoses, in the presence of sulfur sources, such as cysteine or hydrogen sulfide. Quantitative studies performed by using stable isotope dilution assays (Münch et al., 1997) recently confirmed that FFT is indeed formed in high concentrations during thermal treatment of furan-2-aldehyde in the presence of cysteine. Further data (Münch et al., 1997) suggested the importance of retro-Aldol reactions in FFT formation.

Whitfield et al. (1993) and Mottram and Whitfield (1994) reported that MFT is formed by reacting 4-hydroxy-5-methyl-3(2*H*)-furanone [norfuraneol (NF)], another major degradation product of pentoses, with cysteine or hydrogen sulfide, respectively. However, van den Ouweland and Peer (1975) were unable to identify MFT in such reaction systems. Quantitative studies (Zeiler-Hilgart, 1994) confirmed these results showing that the norfuraneol/cysteine system is indeed not very effective in generating MFT.

The thermal degradation of thiamin has been discussed as an alternative reaction pathway in MFT formation during food processing (van der Linde et al., 1979; Hartman et al., 1984; Reineccius and Liardon,

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S0021-8561(97)00598-0 CCC: $15.00 © 1998 American Chemical Society
Published on Web 01/19/1998

Case No.: IPR2023-00208
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1985; Gasser, 1990; Güntert et al., 1990; Werkhoff et al., 1990; Hincelin et al., 1992; Zeiler-Hilgart, 1994).

To reveal the effectiveness of the precursor systems discussed above in generating both odorants, exact quantitative data are a prerequisite. However, the instability of FFT, and especially that of MFT, may cause severe losses during enrichment procedures needed to quantify these trace odorants (Schieberle and Hofmann, 1997). Stable isotope dilution analysis (SIDA) is the best method to overcome these problems [cf. Schieberle (1995)]. The following quantitative model studies were, therefore, undertaken to gain more detailed insight into the efficiency of possible precursors and, also, in reaction mechanisms governing the formation of FFT as well as that of MFT during food processing.

## EXPERIMENTAL PROCEDURES

**Chemicals.** 2-Furfurylthiol, 2-methyl-3-furanthiol, 2-oxopropanal (30% solution in water), furan-2-aldehyde, D-ribose, D-xylose, D-glucose, L-rhamnose, D-glucose-6-phosphate, maltose, L-cysteine, thiamin, and sodium hydrosulfide hydrate (NaHS) were obtained from Aldrich (Steinheim, Germany), and hydroxyacetaldehyde (glycolaldehyde) was from Roth (Germany).

**Syntheses.** The following compounds were synthesized following closely the procedures given in parentheses: mercapto-2-propanone (Hofmann and Schieberle, 1995); [$^{13}C_4$]-mercapto-2-butanone (Hofmann and Schieberle, unpublished data); [$^2H_2$]furfurylthiol (Sen and Grosch, 1991); [$^2H_3$]-2-methyl-3-furanthiol (Sen and Grosch, 1991); [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (Sen et al., 1991).

*Preparation of 3-Deoxyribosulose.* The preparation was performed following the method described for 3-deoxyxylulose (Madson and Feather, 1981) with some modifications. A stirred solution of ribose (55 mmol) and *p*-toluidine (55 mmol) in ethanol/acetic acid (236 mL; 22:1 by volume) was refluxed for 30 min. After addition of benzoyl hydrazine (120 mmol), refluxing was continued for another 420 min. The reaction mixture was then cooled to room temperature and kept at −30 °C overnight. Separated 3-deoxyribosulose bis(benzylhydrazone) was isolated by filtration over a Büchner funnel and washed with ice-cooled ethanol (total volume = 100 mL; yield = 24 mmol, 43.6% of theoretical yield). The hydrazone (24 mmol) and benzaldehyde (16 mL) were then refluxed in a mixture of ethanol (300 mL), water (500 mL), and acetic acid (22 mL). After 2 h, the mixture was cooled to room temperature and the ethanol was evaporated in vacuo, while water (500 mL) was added within 30 min. The mixture was then cooled in an ice bath and the separated benzaldehyde−benzoylhydrazone was removed by filtration over a Büchner funnel. Mixed-bed $H^+/OH^-$-ion-exchange resin (Serdolyt MB3; 60 g; Serva, Heidelberg, Germany) was added to the solution, and after being stirred for 15 min in the dark, the suspension was filtered. The filtrate was concentrated in vacuo to ~100 mL, then washed with diethyl ether (6 × 50 mL), and finally concentrated in vacuo to ~5 mL. The residue was taken up in a mixture of ethanol (50 mL) and water (5 mL), mixed-bed $H^+/OH^-$-ion-exchange resin (Serdolyt MB3; 10 g) was added, and the suspension was stirred for a further 15 min in the dark. After filtration, the solution was concentrated in vacuo to ~5 mL. Freeze-drying yielded 3-deoxyribosulose as an amorphous powder (10 mmol; 42%).

*Preparation of NF.* Xylose (400 mmol), *n*-propylamine (200 mmol), and $KH_2PO_4$ (400 mmol) were dissolved in water (400 mL), and the pH was adjusted to 7.0 with aqueous NaOH (15%). After refluxing for 30 min, the mixture was cooled to room temperature, water (150 mL) was added, and the pH was adjusted to 4.0 with hydrochloric acid (1 mol/L). After filtration, the aqueous solution was extracted with ethyl acetate (8 × 200 mL), and the organic layer was dried over $Na_2SO_4$ and finally concentrated in vacuo to ~5 mL. The target compound was isolated from the mixture by column chromatography (30 × 4 cm) on silica gel (G60; 7% water). After flushing with ethyl acetate/hexane (3:1, by volume), an aliquot of the reaction mixture was applied onto the top of the column. Elution was performed with ethyl acetate/hexane (3:1, by volume), affording the target compound within 400 mL of the eluate. After recrystallization of the crude product from methanol (2 mL), NF (12 mmol; 3%) was obtained as a white solid: MS(EI), *m/z* (%) 43 (100), 114 (52), 55 (9), 58 (4), 71 (3); $^1H$ NMR (360 MHz in $CDCl_3$) (ppm; multiplicity; hydrogen at relevant carbon atom) δ 2.27 (s, 3H, $CH_3$), δ 4.52 (s, 2H, $CH_2$); $^{13}C$ NMR (360 MHz in [$^2H_4$]$CH_3OH$) (ppm; multiplicity; relevant carbon atom) δ 11.7 ($CH_3$), δ 72.6 ($CH_2$), δ 134.3 (C), δ 175.3 (COH), δ 196.6 (CO).

**Model Experiments.** In a first series of experiments, the reactants (cf. amounts detailed in the tables) were dissolved in phosphate buffer (0.5 mol/L) at pH 3.0, 5.0, or 7.0 and thermally treated for 20 min at 145 °C in a laboratory autoclave (200 mL; Type II; Roth, Germany). In a second series of experiments, the reactants were intimately mixed with silica gel (3.0 g containing 300 μL of the respective phosphate buffer; 2 mol/L) and reacted for 6 min at 180 °C.

**Quantitation of FFT and MFT by SIDA.** After cooling of the reaction mixtures to 20 °C, the internal standards [$^2H_2$]-2-furfurylthiol and [$^2H_3$]-2-methyl-3-furanthiol (10 μg each dissolved in 0.5 mL of pentane) were added to the aqueous reaction mixtures and, after equilibration for 10 min, the analyte and the labeled standard were isolated by extraction with diethyl ether (three times, total volume = 60 mL). After drying over $Na_2SO_4$, quantitation of FFT and MFT was performed by mass chromatography using the fragment ions and response factors as described recently (Guth et al., 1995).

**Quantitation of Furan-2-aldehyde.** After cooling, certain amounts of 5-methylfurfural were added to the reaction mixtures as the internal standard. After extraction of the analyte and the standard with diethyl ether (four times, total volume = 50 mL) followed by drying over $Na_2SO_4$, quantitation of furfural was performed by mass chromatography using the ion trap detector. An MS response factor of 0.95 was calculated from mixtures containing known amounts of furan-2-aldehyde and 5-methylfurfural by using the molecular ions obtained in the chemical ionization mode.

**Quantitation of Mercapto-2-propanone and NF.** After cooling to room temperature, certain amounts of the labeled homologues [$^{13}C_4$]mercapto-2-butanone and [$^{13}C_2$]-4-hydroxy-2,5-dimethyl-3(2*H*)-furanone were added to the reaction mixtures. After extraction with diethyl ether (four times, total volume = 50 mL) and drying over $Na_2SO_4$, quantitation was performed by mass chromatography using a gas chromatograph coupled to an ion trap detector. MS response factors for mercapto-2-propanone (1.08) and NF (0.98) were calculated from mixtures containing known amounts of the analyte and the labeled standard.

**High-Resolution Gas Chromatography/Mass Spectrometry (HRGC/MS).** HRGC analyses were performed by means of a gas chromatograph Type 5300 (Fisons, Mainz, Germany) and by using an FFAP capillary column (30 m × 0.32 mm; 0.5 μm; J&W Scientific, Fisons Instruments, Mainz, Germany). The samples (1 μL) were applied by on-column injection at 40 °C. After 2 min, the oven temperature was raised at 40 °C/min to 60 °C, held for 2 min isothermally, then raised at 6 °C/min to 230 °C, and held for 5 min. Quantitations were performed using an Finnigan 800 ion trap detector (Bremen, Germany), running in the chemical ionization mode with methanol as the reagent gas.

**NMR Spectroscopy.** $^1H$ and $^{13}C$ NMR spectra were recorded in [$^2H_4$]methanol (MSD isotopes, Montreal, Canada) by means of an AM 360 (Bruker, Karlsruhe, Germany). The $^1H$ signals were assigned according to tetramethylsilane (TMS) as the internal standard.

## RESULTS AND DISCUSSION

**Carbohydrates and Cysteine as Precursors of FFT and MFT.** To investigate their effectiveness in

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**Table 1. Amounts of FFT and MFT Generated from Cysteine and Various Carbohydrates[a]**

| carbohydrate | amount[b] (μg) FFT | MFT |
|---|---|---|
| ribose | 12.1 | 19.8 |
| xylose | 9.6 | 14.3 |
| fructose | 3.2 | 2.5 |
| glucose | 2.8 | 1.9 |
| glucose-6-phosphate | 0.9 | 0.6 |
| rhamnose | 0.8 | 0.8 |
| maltose | 0.6 | 0.3 |

[a] A solution (100 mL) of cysteine (3.3 mmol) and the corresponding carbohydrate (10.0 mmol) was reacted at pH 5.0 in a laboratory autoclave. [b] Data are mean values of triplicates and differed by not more than 10%.

**Table 2. Influence of pH on the Amounts of FFT and MFT Generated from Cysteine and Carbohydrates[a]**

| carbohydrate | FFT[b] (μg) at pH 3.0 | 5.0 | 7.0 | 5.0[c] | MFT[b] (μg) at pH 3.0 | 5.0 | 7.0 | 5.0[c] |
|---|---|---|---|---|---|---|---|---|
| ribose | 22.9 | 12.1 | 1.2 | 97.2 | 55.3 | 19.8 | 2.5 | 25.1 |
| glucose | 0.7 | 2.8 | 0.6 | 1.4 | 0.3 | 1.9 | 0.4 | 4.2 |
| rhamnose | 0.2 | 0.8 | 0.1 | 0.4 | 0.1 | 0.8 | 0.1 | 3.1 |

[a] A solution (100 mL) of cysteine (3.3 mmol) and the corresponding carbohydrate (10.0 mmol) was reacted in a laboratory autoclave. [b] See footnote *b* in Table 1. [c] A mixture of cysteine (3.3 mmol) and the corresponding carbohydrate (10.0 mmol) was dry-heated for 6 min at 180 °C.

generating FFT and MFT, aqueous solutions of several monosaccharides, as well as glucose-6-phosphate and the disaccharide maltose, were reacted in the presence of cysteine. The amounts of both odorants were determined by SIDA. The results established the pentoses ribose and xylose as the most effective precursors in the generation of both odorants (Table 1). Fructose and glucose were more efficient than glucose-6-phosphate, rhamnose, or maltose, which yielded only small amounts of FFT and MFT when heated in the presence of cysteine.

To gain a more detailed insight into factors governing the formation of FFT and MFT from pentoses and hexoses, the influence of the pH on odorant formation from ribose, glucose, and rhamnose was studied. The formation of both odorants from pentoses and hexoses was influenced by pH in a different way (Table 2). From ribose, the liberation of FFT and MFT increased significantly with lowering the pH from 7.0 to 3.0. Although comparatively lower amounts were formed, the generation of both odorants in the presence of glucose or rhamnose went through a maximum at pH 5.0.

To examine the influence of water and the reaction temperature, the three carbohydrates were heated under dry-heating conditions in the presence of cysteine. A small amount of concentrated buffer was added to maintain pH 5.0. Under these conditions, ribose generated much higher concentrations of FFT than under aqueous conditions, whereas MFT was only slightly increased. In contrast, FFT especially was lower when hexoses were reacted under dry-heating conditions.

In summary, the highest amounts of FFT were obtained by dry-heating the ribose/cysteine mixture (Table 2), whereas MFT was preferentially formed from this model mixture under aqueous conditions at pH 3.0. Substitution of ribose by hexoses generally gave different results. Besides lower yields, the formation of FFT from both hexose/cysteine systems was highest under aqueous conditions at pH 5.0, whereas the MFT was formed from these mixtures preferentially under dry-heating conditions. The data suggested that the formation of FFT and MFT from pentoses and hexoses follows different reaction pathways and prompted us to look into several possible intermediates of FFT and MFT formation in more detail.

**Table 3. Generation of FFT from 5-Carbon Precursors in the Presence of Hydrogen Sulfide[a]**

| precursor | FFT μg[b] | % |
|---|---|---|
| ribose | 9.2 | 0.008 |
| 3-deoxyribosulose | 78.6 | 0.08 |
| furan-2-aldehyde | 550.8 | 0.48 |

[a] An aqueous solution (50 mL) of hydrogen sulfide (1 mmol) and the precursor (1 mmol) was reacted at pH 5.0 in a laboratory autoclave. [b] See footnote *b* in Table 1.

**Table 4. Generation of MFT from Ribose or NF in the Presence of Hydrogen Sulfide[a]**

| precursor (1 mmol) | MFT μg[b] | % |
|---|---|---|
| ribose | 15.1 | 0.01 |
| NF | 211.2 | 0.19 |

[a] An aqueous solution (50 mL) of hydrogen sulfide (1 mmol) and the precursor was heated at pH 5.0 in a laboratory autoclave. [b] See footnote *b* in Table 1.

**Intermediates in FFT and MFT Formation from Pentoses.** Dehydration of pentoses may result in the formation of either the 1- or the 3-deoxypentosone. Their subsequent cyclization and elimination of water lead to the formation of either NF or furan-2-aldehyde as the major products. However, it is yet unknown whether both deoxyosones are formed in equal amounts.

The reaction of furan-2-aldehyde with hydrogen sulfide, which can be liberated from cysteine, was recently shown to generate the FFT very effectively (Münch et al., 1997). It might, therefore, be assumed that also the 3-deoxypentosone could act as a precursor of this flavor compound.

To compare their efficiencies as FFT precursors, furan-2-aldehyde and 3-deoxypentosone were each heated in the presence of hydrogen sulfide, and the amounts of FFT formed were compared with those formed from ribose. More FFT was liberated from the furan-2-aldehyde, compared to ribose and the 3-deoxyribosulose, by factors of 60 or 7, respectively (Table 3). A reaction mechanism explaining FFT formation via a reductive sulfhydrylation of furan-2-aldehyde with hydrogen sulfide has recently been proposed (Münch et al., 1997).

NF belongs to the major degradation product of pentoses and is assumed to be formed via the 1-deoxypentosone. When heated in the presence of cysteine, NF has been found to generate significant amounts of MFT (Whitfield et al., 1993). To compare its effectiveness with that of ribose, NF was reacted with hydrogen sulfide and the amount of MFT liberated was quantified. In agreement with literature data (Whitfield et al., 1993), the odorant was generated very efficiently from the furanone (Table 4). Compared to ribose, by a factor of ≈14 higher amounts of MFT were formed from NF. NF is generated in very high yields from ribose/cysteine (Table 5). This result therefore explains the preferential generation of MFT from the pentose via NF as the intermediate.

A hypothetical reaction sequence leading to MFT from NF and hydrogen sulfide as the intermediates is dis-

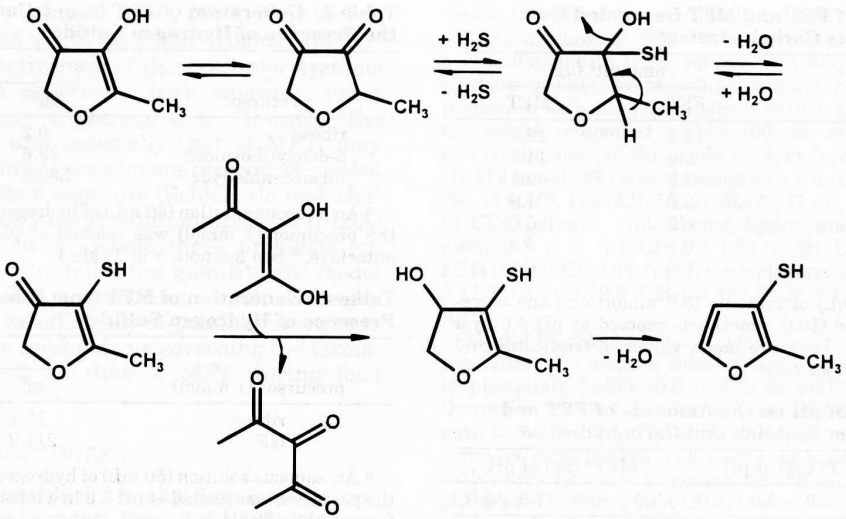


**Figure 1.** Hypothetical reaction sequence leading from NF and $H_2S$ to MFT.

**Table 5. Amounts (Micrograms)[a] of the Pairs Furan-2-aldehyde (FA)/FFT and NF/MFT Generated from Carbohydrates in the Presence of Cysteine[b]**

| carbohydrate | FA | FFT | NF | MFT |
|---|---|---|---|---|
| ribose | 67.5 | 12.1 | 54530.0 | 19.8 |
| glucose | 6.9 | 2.8 | 13.5 | 1.9 |
| rhamnose | 6.2 | 0.8 | 19.1 | 0.8 |

[a] See footnote *b* in Table 1. [b] An aqueous solution (100 mL) of cysteine (3.3 mmol) and the corresponding carbohydrate (10.0 mmol) was reacted at pH 5.0 in a laboratory autoclave.

played in Figure 1. Incorporation of a thiol group at carbon 3 yields 4-mercapto-5-methyl-3(2*H*)-furanone in a first reaction step, which in turn might be reduced by a reductone or by a further molecule of hydrogen sulfide. Elimination of water from the intermediate 3-hydroxy-4-mercapto-5-methyl-1,2-dihydrofuran would give rise to MFT.

**Intermediates in FFT and MFT Formation from Hexoses.** Because MFT and FFT were found to be generated also from hexoses (cf. Table 2), it might be speculated that NF and furan-2-aldehyde are also the key intermediates in the formation of both odorants. However, to form these precursors from hexoses, a cleavage of the carbohydrate skeleton must occur. This is possible by α-dicarbonyl cleavage and vinylogous retro-Aldol cleavage, leading to several cleavage products (C-5/C-1, C-4/C-2, or C-3/C-3). Such short-chain carbonyl compounds, e.g., hydroxyacetaldehyde and 2-oxopropanal, have been reported to be formed from hexoses (Hayashi and Namiki, 1986; Nedvidek et al., 1992). A hypothesis on the formation of NF and furan-2-aldehyde starting from 2-oxopropanal and hydroxyacetaldehyde is shown in Figure 2. Depending on the side of the nucleophilic attack, an Aldol reaction of the short-chain compounds might result in either furan-2-aldehyde or NF. Nucleophilic attack of the methyl group of 2-oxopropanal at the hydroxyacetaldehyde results in the formation of 3-deoxypentosone (**Ib**; Figure 2), which upon 2,5-cyclization and dehydration gives rise to furan-2-aldehyde (FA; **IIb**). However, the reaction of the methylene group of hydroxyacetaldehyde with the aldehyde function of the 2-oxopropanal might result in 1-deoxypentosone (**Ia**; Figure 2), which upon 2,5-cyclization and water elimination yields NF (**IIa**).

To check this hypothesis, hydroxyacetaldehyde and

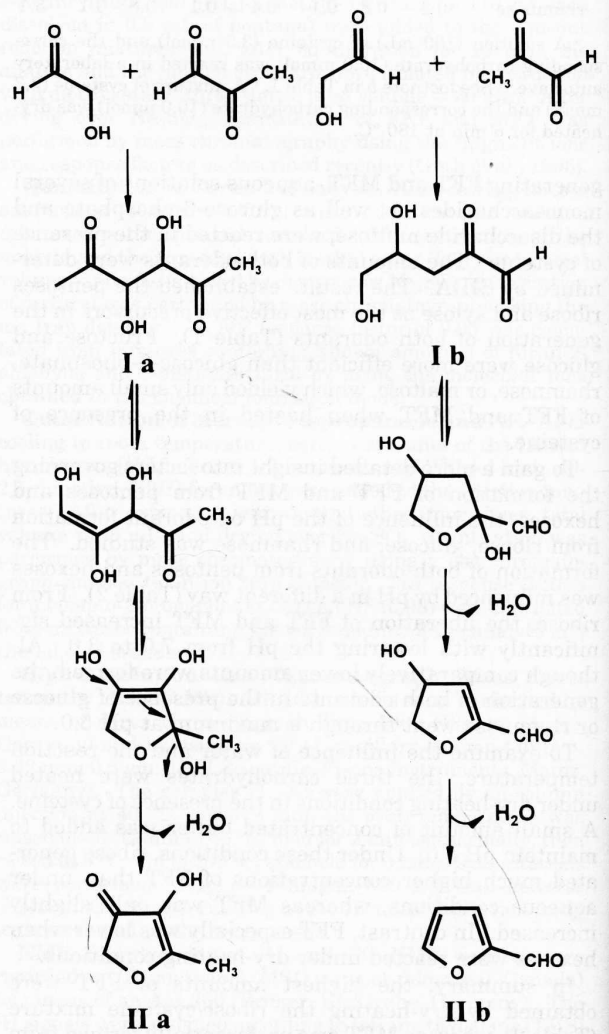


**Figure 2.** Formation of furan-2-aldehyde (FA) and NF by an Aldol reaction of 2-oxopropanal and hydroxyacetaldehyde.

2-oxopropanal were reacted in aqueous solution at different pH values and the amounts of FA and NF formed were quantified. Both compounds were formed

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**Table 6. Amounts of Furan-2-aldehyde (FA) and NF Generated from Hydroxyacetaldehyde and 2-Oxopropanal[a]**

| | FA | | NF | |
|---|---|---|---|---|
| pH | μg[b] | % | μg[b] | % |
| 3.0 | 101.2 | 0.11 | 153.1 | 0.13 |
| 5.0 | 364.5 | 0.40 | 885.1 | 0.78 |
| 7.0 | 1443.1 | 1.60 | 3610.5 | 3.18 |

[a] An aqueous solution (50 mL) of hydroxyacetaldehyde (1 mmol) and 2-oxopropanal (1 mmol) was reacted in a laboratory autoclave. [b] See footnote *b* in Table 1.

in significant yields, with neutral conditions (pH 7.0) favoring their formation (Table 6). It is interesting to note that in all reaction mixtures more NF than FA had been formed. Referring to the mechanism shown in Figure 2, these data indicate hydroxyacetaldehyde is the better nucleophile.

Assuming furan-2-aldehyde and NF to be the key intermediates in MFT and FFT formation, there should, consequently, be a correlation between the amounts of FA and FFT as well as between those of NF and MFT formed in processed carbohydrate/cysteine mixtures. As shown in Table 5, nearly 10 times more furan-2-aldehyde was generated in the ribose compared to the glucose system and simultaneously more FFT was generated. However, these data also corroborate the key role of furan-2-aldehyde in FFT generation from hexoses. In contrast, the amounts of NF were not well correlated with those of MFT. From ribose, by a factor of 3000 more NF was formed compared to MFT, whereas in the glucose system only by a factor of 10 more NF was present compared to MFT. This discrepancy implied that the MFT formation might not run exclusively via NF as the key intermediate.

We, therefore, thought of an alternative route for the formation of MFT starting from the possible precusors mercapto-2-propanone and hydroxyacetaldehyde. As proposed in Figure 3, depending on the side of the nucleophilic attack, the Aldol reaction of mercapto-2-propanone and hydroxyacetaldehyde might result in the formation of either MFT or FFT. Reaction of the methylene group of the mercapto-2-propanone gives the intermediate 4,5-dihydroxy-3-mercapto-2-pentanone (**Ia** in Figure 3), which upon cyclization and dehydration should give MFT (**Ib**). On the other hand, a nucleophilic attack of the methyl group of the mercapto-2-propanone on hydroxyacetaldehyde leads to the formation of 4,5-dihydroxy-1-mercapto-2-pentanone (**IIa**), which, after cyclization and elimination of water, might result in FFT (**IIb**). To elucidate this hypothesis, first, the formation of mercapto-2-propanone from 2-oxopropanal and $H_2S$ was studied. The data indicated that high amounts of mercapto-2-propanone were generated from $H_2S$ and the oxoaldehyde (Table 7). Because higher $H_2S$ concentrations increased the formation of the mercaptoketone (Table 7), it might be assumed that $H_2S$ is the limiting factor in this reaction.

In a next step, hydroxyacetaldehyde was reacted with mercapto-2-propanone in aqueous solution and the amounts of FFT and MFT were determined (Table 8). At pH 3.0, low concentrations of both odorants were formed. Increasing the pH to 5.0 significantly favored the formation of the MFT, whereas the increase in FFT concentration was low (Table 8). To study the influence of the reaction temperature and the water content on the formation of FFT and MFT, both intermediates were

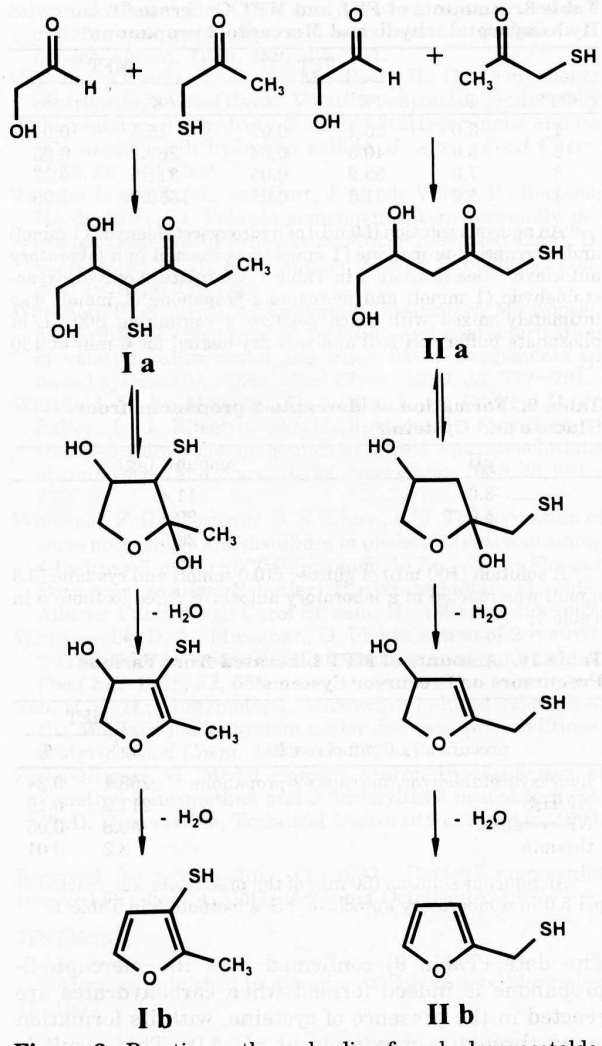


**Figure 3.** Reaction pathway leading from hydroxyacetaldehyde and mercapto-2-propanone to FFT and MFT.

**Table 7. Generation of Mercapto-2-propanone from 2-Oxopropanal and Hydrogen Sulfide[a]**

| reaction system | | mercapto-2-propanone | |
|---|---|---|---|
| 2-oxopropanal (mmol) | $H_2S$ (mmol) | μg[b] | % |
| 1 | 1 | 1650 | 1.8 |
| 1 | 2 | 3600 | 4.0 |

[a] An aqueous solution (50 mL) of hydrogen sulfide and 2-oxopropanal was reacted at pH 5.0 in a laboratory autoclave. [b] See footnote *b* in Table 1.

dry-heated and the odorant formation was monitored (experiment 4; Table 8). The results showed that compared to an aqueous system (cf. experiments 2 and 4; Table 8), the FFT formation increased slightly during dry-heating. However, the MFT generation was enhanced by a factor of nearly 6. The significant increase of MFT during dry-heating was well in line with the influence of the heating conditions on its generation from glucose or rhamnose (cf. Table 2).

The results indicate an important role of mercapto-2-propanone and hydroxyacetaldehyde in the formation of MFT from hexoses. To establish that this reaction is involved in MFT and FFT formation, the concentration of mercapto-2-propanone generated in thermally treated carbohydrate/cysteine mixtures was measured.

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**Table 8. Amounts of FFT and MFT Generated from Hydroxyacetaldehyde and Mercapto-2-propanone[a]**

| | | FFT | | MFT | |
|---|---|---|---|---|---|
| expt | pH | μg[b] | % | μg[b] | % |
| 1 | 3.0 | 26.1 | 0.02 | 15.5 | 0.01 |
| 2 | 5.0 | 40.5 | 0.04 | 268.1 | 0.23 |
| 3 | 7.0 | 58.2 | 0.05 | 311.5 | 0.27 |
| 4 | 5.0[c] | 51.3 | 0.05 | 1553.9 | 1.39 |

[a] An aqueous solution (50 mL) of hydroxyacetaldehyde (1 mmol) and mercapto-2-propanone (1 mmol) was reacted in a laboratory autoclave. [b] See footnote *b* in Table 1. [c] A mixture of hydroxyacetaldehyde (1 mmol) and mercapto-2-propanone (1 mmol) was intimately mixed with silica gel (3.0 g containing 300 μL of phosphate buffer, pH 5.0) and was dry-heated for 6 min at 180 °C.

**Table 9. Formation of Mercapto-2-propanone from Glucose and Cysteine[a]**

| pH | amount[b] (μg) |
|---|---|
| 3.0 | 11.4 |
| 5.0 | 59.6 |
| 7.0 | 26.5 |

[a] A solution (100 mL) of glucose (10.0 mmol) and cysteine (3.3 mmol) was reacted in a laboratory autoclave. [b] See footnote *b* in Table 1.

**Table 10. Amounts of MFT Liberated from Various Precursors or Precursor Systems[a]**

| | MFT | |
|---|---|---|
| precursor (1.0 mmol) each | μg[b] | % |
| hydroxyacetaldehyde/mercapto-2-propanone | 268.1 | 0.24 |
| NF/$H_2S$ | 211.2 | 0.19 |
| NF/cysteine | 50.8 | 0.05 |
| thiamin | 8.2 | 0.01 |

[a] An aqueous solution (50 mL) of the precursors was reacted at pH 5.0 in a laboratory autoclave. [b] See footnote *b* in Table 1.

The data (Table 9) confirmed that the mercapto-2-propanone is indeed formed when carbohydrates are reacted in the presence of cysteine, with its formation going through a maximum at pH 5.0. This result is consistent with the preferential generation of both odorants at this pH value, e.g., in the glucose/cysteine mixture (Table 2).

Besides carbohydrates, thiamin especially has been reported as an important precursor of MFT. To study the effectiveness of MFT formation from thiamin, MFT was quantified in a heated aqueous solution of thiamin and the yields were compared with those obtained in the various precursor systems described above. The data established the binary mixtures hydroxacetaldehyde/mercapto-2-propanone followed by NF/$H_2S$ as the most effective precursors systems (Table 10). Thiamin was less efficient, because by factors of 30 or 25 less MFT was generated compared to the two binary precursor mixtures.

Because, on the one hand, the efficiency of thiamin in MFT generation is very low and, on the other hand, thiamin is only a minor compound in foods, the liberation of MFT from thiamine during food processing should not contribute much to food flavors. Furthermore, since the carbohydrate fraction of foods contains usually more hexoses than pentoses, it seems likely that mercapto-2-propanone and hydroxyacetaldehyde are important intermediates involved in MFT generation during food processing.

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Received for review July 11, 1997. Revised manuscript received October 14, 1997. Accepted October 15, 1997.[⊗]

JF9705983

[⊗] Abstract published in *Advance ACS Abstracts,* November 15, 1997.

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