Transformation Pathways of Reductones in the Advanced Maillard Reaction

M. KONEČNÝ, K. CEJPEK, L. ČECHOVSKÁ and J. VELÍŠEK*

*Department of Food Chemistry and Analysis, Institute of Chemical Technology in Prague, 166 28 Prague, Czech Republic, *E-mail: karel.cejpek@vscht.cz*

Abstract: The transformation of methylene-active reducing Maillard intermediates 4-hydroxy-5-methyl-2*H*-furan-3-one (norfuraneol, 1) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) was studied in heated (at 70–95°C up to 2 h) model aqueous binary systems containing various reactive carbonyl Maillard intermediates. Among them, furan-2-carbaldehyde and its derivatives 5-hydroxymethylfuran-2-carbaldehyde and pyrrol-2-carbaldehyde react intensely with the above reductones resulting in significant formation of consecutive reducing products. The active products formation and changes in total electrochemical activity were evaluated by using HPLC system with amperometric detection. The active products are consisted of primary reductone-carbaldehyde adducts (2a, b) that dehydrate to major active stereoisomeric condensation products (3a, b). The latter are hydrolysed to still electrochemically active compounds (4a, b) with yet unknown structure. Norfuraneol is transformed by 67–94% after 2 h heating at 95°C and pH 7 depending on a carbaldehyde, while DDMP react much slowly. Up to 42% of the initial norfuraneol electrochemical activity remains retained in the consecutive products depending on time and carbaldehyde involved.

Keywords: Maillard reaction; antioxidants; reductones; norfuraneol; carbaldehydes

INTRODUCTION

During food processing, considerable changes in level and nature of antioxidant activity can frequently occur as a result of various reactions such as hydrolysis, oxidation and the Maillard reaction. In the early stage of the Maillard reaction highly reactive intermediates known as reductones together with structurally similar products possessing active methylene moiety are formed. In spite of great interest in reducing activity arising in the Maillard reaction, surprisingly little is known about the fate of these reactive reducing intermediates. In the presence of reactive Maillard intermediates and other food components, they may be largely transformed by non-oxidative processes. It is, therefore, helpful to study suitable model systems to obtain detailed information on the consecutive products, especially those that retain reducing activity.

Norfuraneol (1) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) are the principal reducing intermediates in pentose and hexose systems, respectively. They are thought to be formed *via* the 1-deoxy-2,3-pentodiulose and 1-deoxy-2,3-hexodiulose pathway, respectively (Nursten 2005). However, there are only few references on the transformations of these active intermediates in literature. Kim and Baltes (1996) reported numerous volatile products formed from DDMP by heating of aqueous solutions as well as under roasting conditions. 5-Hydroxymaltol is believed to be the principal oxidation product of DDMP. Norfuraneol, one of the main furanones formed in neutral Maillard reaction systems of pentoses, has been found as a key intermediate in the formation of several colored products. 2-[(2-Furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one has been identified as one of the first low-molecular Maillard colorants. It arises as one of the main colored reaction products in heated aqueous solutions of xylose or arabinose in the presence of an amino compound (Severin & Krönig 1972; Nursten & O'Reilly

1986; Hofmann 1998) and was synthesised by the controlled condensation of furan-2-carbaldehyde (2-FF) and norfuraneol. In comparison to mostly pentose-derived norfuraneol, less information is available regarding the reaction of hexose-derived methylene-active pyranone DDMP with 2-FF and its derivatives (Ledl & Severin 1982; Hofmann & Heuberger 1999). This study is the first report on the reducing properties of non-oxidative transformation products of common reductones within the Maillard reaction.

MATERIAL AND METHODS

Model reaction systems. Aqueous binary equimolar 0.1M mixtures of electrochemically active compounds, viz norfuraneol, DDMP, maltol, 5-hydroxymaltol, 4-hydroxy-5-hydroxymethyl-2-methyl-2*H*-furan-3-one, cyclotene and Sotolon, and reactive carbonyl intermediates – 2-FF, 5-hydroxymethylfuran-2-carbaldehyde (HMF), pyrrol-2-carbaldehyde (2-PC), benzaldehyde, glyoxal, methylglyoxal, biacetyl, glycoladehyde and glyceraldehyde – were heated at 70°C and 95°C in closed vials for 0–2 h. The reaction mixtures were cooled, filtered through nylon 0.45 µm filters (Alltech), and diluted with water by a factor of ten before HPLC analysis.

Syntheses. Syntheses of DDMP and 2-[(2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one (NOR–2-FF 3) were accomplished according to modified procedures described by Kim and Baltes (1996) and Ledl and Severin (1978), respectively.

HPLC method. Atlantis C_{18}, 150 × 3.9 mm× 3 µm with a pre-column, gradient elution (pH 6.5/MeCN/5mM NaCl, diode-array (PDA, 996) & electrochemical detectors (ELD, 2465, all Waters, E_a = +0.8 V) and mass spectrometer (Q-TOF-ESI, APCI, Micromass, 10mM $HCOONH_4$/MeCN as eluent) were used.

GC-MS (EI) method. Isolated products 3a, b were analysed on DB-1HT 15 m × 0.25 mm × 0.1 µm, Agilent): NOR–2-FF 3a (EI 192(100), 177(5), 121(94), 108(5), 80(7). NOR–HMF 3a (EI 222(91), 204(31), 191(28), 176(18), 151(26), 133(100), 121(64), 105(75). NOR–2-PC 3a (EI 191(100), 176(4), 120(86), 107(3), 92(22), 79(16). DDMP–2-FF 3a (EI. 222(100), 193(58), 179(4), 167(7), 151(9), 121(17).

NMR spectroscopy. ^{1}H- and ^{13}C-NMR spectra of the synthesised or isolated compounds were recorded on a Bruker Avance DRX 500 spectrometer in $CDCl_3$ and DMSO-d_6. Heteronuclear two-dimensional ^{1}H – ^{13}C correlations in HMQC and HMBC experiments were performed for the identity confirmation of 3a products.

RESULTS AND DISCUSSION

In complex Maillard systems, the amino acid-catalysed conversion of reducing carbohydrates results in a large variety of reactive aldehydes and other carbonyl compounds. Therefore, binary reaction models of a reductone and a carbonyl intermediate were used to minimise the product multiplicity to the key secondary reducing compounds. In this paper we report on the change in electrochemical activity during transformations of norfuraneol and DDMP in the presence of carbaldehydic Maillard intermediates at various conditions and on the temporary preservation of a part of original reducing power of norfuraneol and DDMP in consecutive products.

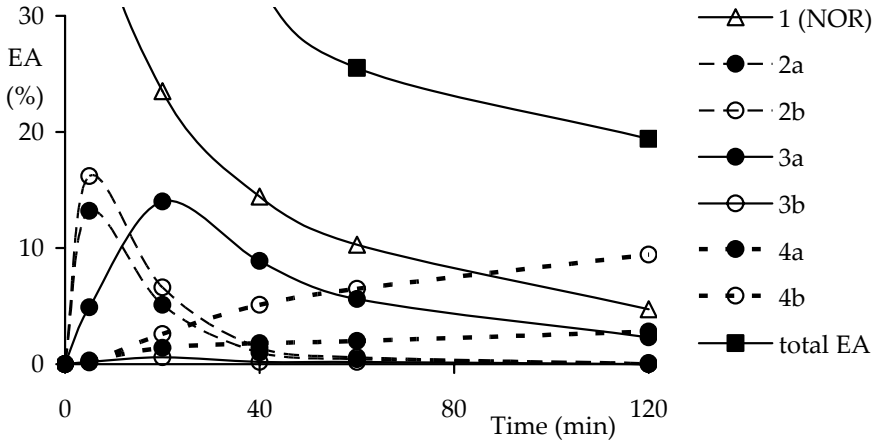

Figure 1. Changes in total electrochemical activity (EA) and contribution of the products to EA during reaction of norfuraneol with furan-2-carbaldehyde (0.1M, pH 7, 95°C)

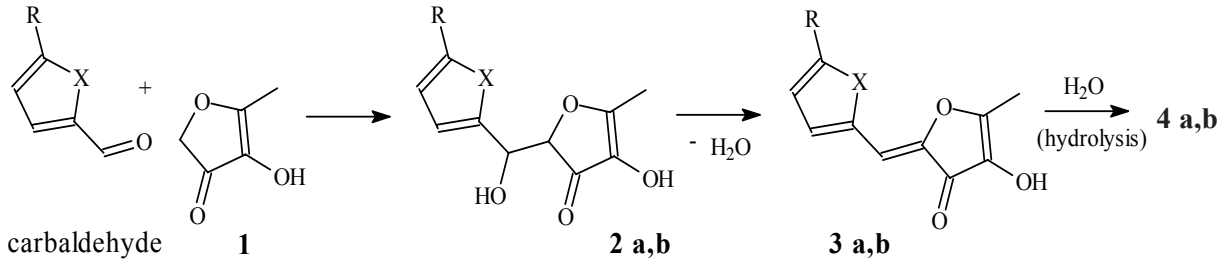

Figure 2. Proposed reaction scheme of the formation of addition (2a, b), condensation (3a, b) and hydrolytic (4a, b) electrochemically active products of norfuraneol (1) and carbaldehydes (X = N or O, R = H or CH_2OH)

Significant contribution of the consecutive EA products on reducing power of the mixture can be well illustrated by the reaction of norfuraneol and 2-FF in pH 7 phosphate buffer at 95°C (Figure 1). After 2 h heating, residual norfuraneol kept about 6% of initial EA, while products 3a and 4a, b comprised about 15% of initial norfuraneol activity. HMF reacted with norfuraneol with the rate comparable to 2-FF, but the carryover of activity was by 55% higher. The reactivity as well as the reducing activity transfer of 2-PC was the lowest among the carbaldehydes tested. DDMP was less reactive than norfuraneol when reacted with carbaldehydes and 3.1–3.6 times lower yields of secondary active products were acquired.

On the basis of identifications and time-dependent developments, a general reaction mechanism can be drawn as shown in Figure 2. The primary EA adducts (optical isomers 2a, b) dehydrate yielding condensation products (*trans*/*cis*-isomers 3a, b) that are hydrolysed to 4a, b. Several active condensation products were isolated and identified, such as stereoisomers of 2-[(2-furyl)methylidene]-4-hydroxy-5-methyl-2*H*-furan-3-one formed from norfuraneol and furan-2-carbaldehyde (NOR-2-FF 3), 4-hydroxy-2-[2-(5-hydroxymethylfuryl)methylidene]-5-methyl-2*H*-furan-3-one (NOR–HMF 3), and 4-hydroxy-5-methyl-2-[(2-pyrroyl)methylidene]-2*H*-furan-3-one (NOR–2-PC 3). Identification of NOR–2-FF 3a, b was accomplished by comparison with the synthesised product, while structure elucidation of the other condensation products of 1 and DDMP with carbaldehydes was based on molecular weight determinations, spectral data and analogies. Products 3a, b derived from norfuraneol are of yellow color; NOR–2-FF 3a was described earlier as a significant low-molecular colorant of Maillard mixtures (Hofmann 1998). Reducing analogues formed from DDMP (e.g., DDMP-HMF 3) are colorless. As follows from the preliminary DPPH radical scavenging assay results (not shown), electrochemical activity balance and standard potential measurements (e.g., $E_{1/2}$ = +0.32 V for norfuraneol *vs* +0.71 V for NOR–2-FF 3a), the consecutive products seem to be weaker antioxidants in comparison to norfuraneol and DDMP.

The amount of 2–4 obtained from norfuraneol is highly dependent on the reaction conditions. Norfuraneol is transformed very quickly in pH 10 with no norfuraneol and only 5–11% residual activity after 1 h heating at 95°C. In acidic media (pH 4), norfuraneol is converted much slowly; only 43% of norfuraneol reacted with 2-FF after 1 h at 95°C. Moderate transformation rates along with the best yields of the reducing products were achieved in neutral solutions (pH 7) following the same mechanism. However, due to different pH-dependent development of the ultimate precursors (e.g., 2-FF and norfuraneol) from sugars (ribose, glucose) in the presence of amino acids, optimum pH for the advanced reducing products (e.g., NOR-2-FF 3) in these primary Maillard systems is acidic (pH from 3 to 5).

Acknowledgements: Supported by the Ministry of Education, Youth and Sports of the Czech Republic (Projects COST 927 and MSM 6046137305).

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