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Oxidation of Flavonols in an Electrochemical Flow Cell Coupled Online with ESI-MS

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Introduction

The combination of an electrochemical flow cell (EC) coupled online with electrospray ionization mass spectrometry (ESI-MS) experienced an increasing interest due to the simulation of relevant metabolic oxidation reactions and the identification of generated short-lived products [1]. Therefore, EC/ESI-MS is a suitable tool to investigate the oxidative behavior of flavonols.

Flavonols, a class of flavonoids, are jointly responsible for antioxidant activities as free radical acceptors in flowers, fruits and vegetables [2]. They consist of two benzene rings (A and B) linked by a pyrane ring (C), which is characterized by a hydroxyl group at the C_3 position and by C_2-C_3 double bond in ring C. The oxidation of chrysin, flavonol, kaempferol, morin, quercetin and myricetin is performed to identify the possible oxidation products and accordingly to determine the oxidation mechanisms.

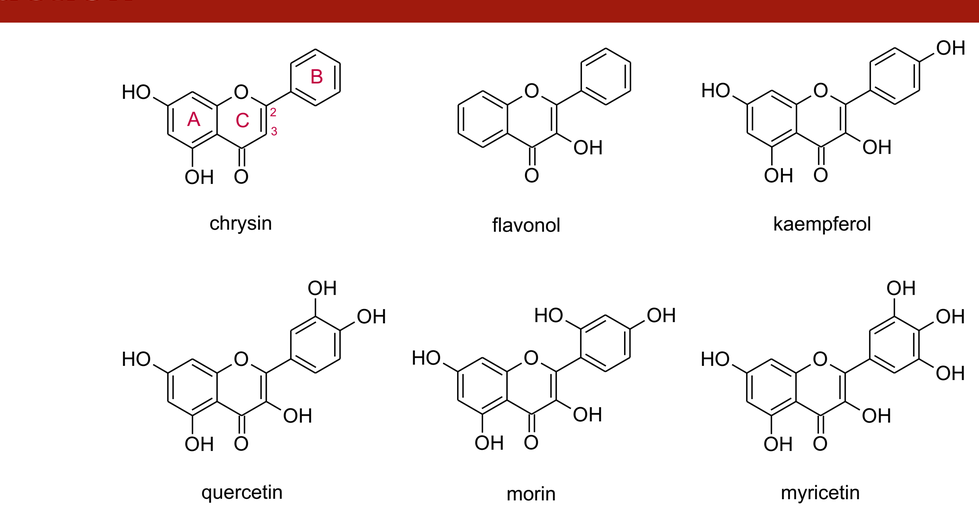


Fig. 1: Chemical structures of selected flavonoids.

Results

Experimental Setup

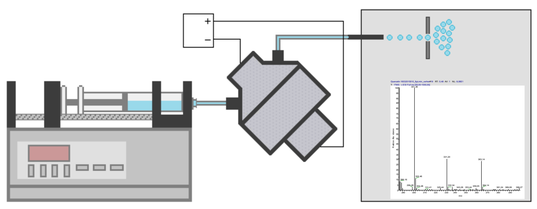
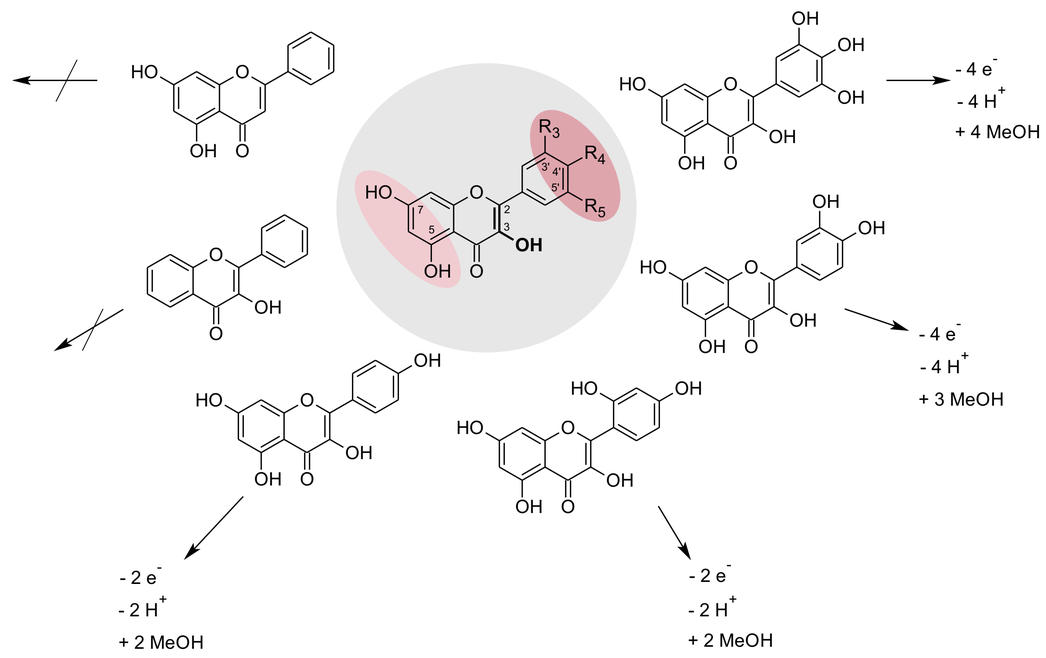


Fig. 2: Experimental setup consisting of syringe pump, flow cell with potentiostat and ESI-MS.

Flavonoid standards (20 µM, flavonol 100 µM) were oxidized in methanol with 0.1% formic acid in the flow cell. The working electrode was a glassy carbon electrode, HyREF was used as the reference and the inlet block was used as the auxiliary electrode (Antec). A 50 µm spacer defined the flow channel and resulted in a dead volume in the flow cell of 0.7 µL. The MS experiments were performed with electrospray ionization in the negative mode with an ion spray voltage of -2.2 kV (-2.0 kV for flavonol). The capillary from the flow cell to the mass spectrometer was 16.5 cm long and had an inner diameter of 0.01 inches, resulting in a response time of approximately 2-3 minutes, depending on the flow rate. The flow rate was varied between 8 and 15 µL/min to obtain the optimal conditions for ionization and detection in the mass spectrometer.

Comparison of the Electroactive Behavior



The electroactive behavior of the selected species during the oxidation in methanol with 0.1% formic acid are summerized in the above scheme. Certain structural features are required to initiate the oxidation of the selected flavonols: proximity to the C_2-C_3 double bond and the 3-OH group in ring C, along with additional OH groups in the backbone, are essential for high electroactive behavior. Therefore, chrysin and flavonol showed no oxidation products. Furthermore, the C_5- and C_7-OH groups have no influence on the electroactive behavior under these experimental conditions.

An OH group in ring B (kaempferol) or a second OH group in the *meta*-position in ring B (morin) result in few but distinctive oxidation products obtained through a two-electron, two-proton step and two methanol additions [3]. Two OH groups in the *ortho*-position (quercetin) give rise to a four-electron, four-proton step and three methanol additions, whereas the three OH groups in ring B (myricetin) further increase the electroactive behavior with a four-electron, four-proton step and four methanol additions.

Mass voltammograms of Flavonols

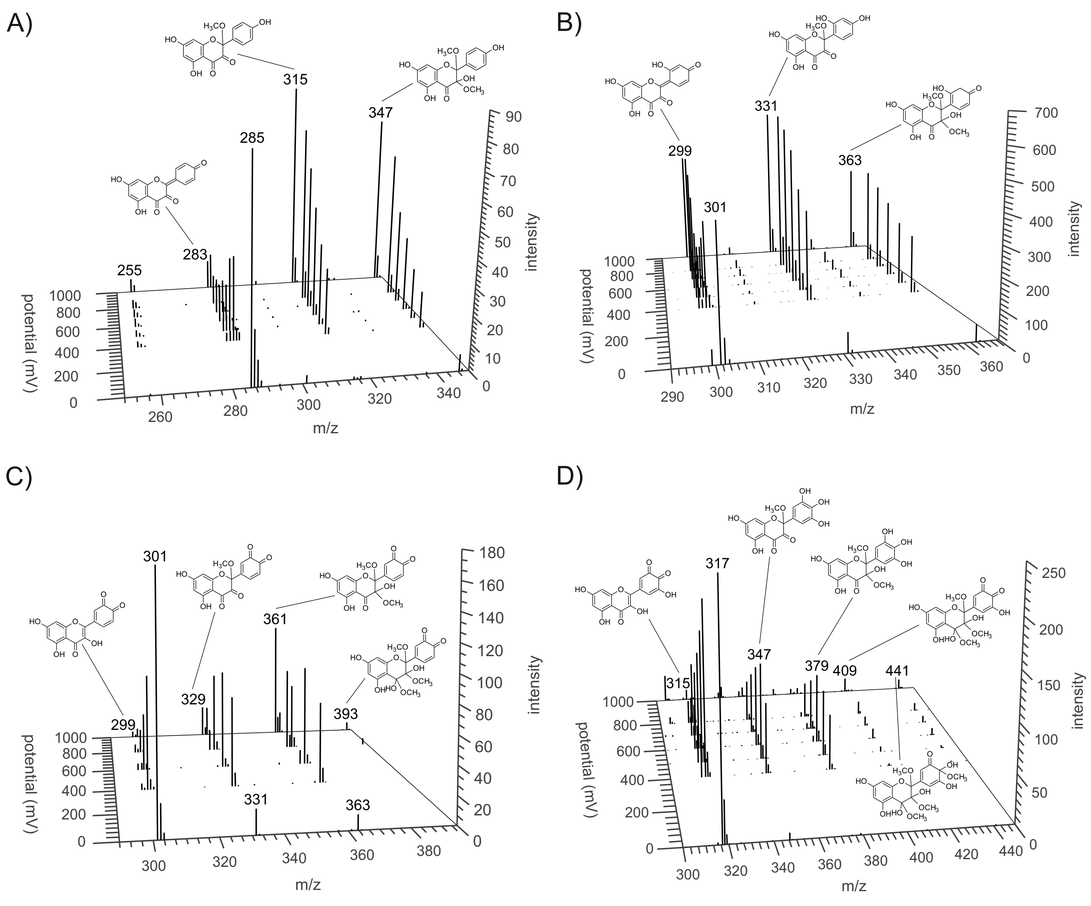


Fig. 3: Mass voltammograms of 20 µM species of A) kaempferol (MW = 286 g/mol), B) morin (MW = 302 g/mol), C) quercetin (MW = 302 g/mol) and D) myricetin (MW = 318 g/mol) in methanol with 0.1% formic acid in the negative ESI mode with their oxidation products.

Summary

Many intermediates and products for the oxidation of a structurally broad range of flavonols have been identified. The sites prone to oxidation were determined by varying the oxidation potential. Additionally, the order of the multiple methoxylations can be provided: the first methanol addition occurs at the C_2 position at ring C followed by the C_3 position for the second methoxylation and at the C_4 position for the third methoxylation. It was clearly shown that the number of methanol additions depends on the redox activity. Moreover, we identifed a second two-electron, two-proton oxidation of the formed oxidation products of quercetin and myricetin for the first time.

References: [1] A. Baumann, U. Karst, *Expert Opin. Drug Metab. Toxicol.* **2010** *6*, 715-731.
[2] P.-G. Pietta, *J. Nat. Prod.* **2000** *63*, 1035-1042.
[3] A.K. Timbola, C.D. de Souza, C. Giacomelli, A. Spinelli, *J. Braz. Chem. Soc.* **2006** *17*, 139-148.

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