Plasma-oxidative Degradation of Polyphenolics – Influence of Non-thermal Gas Discharges with Respect to Fresh Produce Processing

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Abstract: Non-thermal plasma treatment is a promising technology to enhance the shelf-life of fresh or minimaly processed food. An efficient inactivation of microorganisms comes along with a moderate heating of the treated surface. To elucidate the influence of highly reactive plasma-immanent species on the stability and chemical behaviour of phytochemicals, several polyphenolics were exposed to an atmospheric pressure plasma jet (APPJ). The selected flavonoids are ideal target compounds due to their antioxidant activity protecting cells against the damaging effects of reactive oxygen species such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. Reactions were carried out at various radio-frequency voltages, using Ar as a feeding gas. Degradation was followed by reversed-phase high-performance liquid chromatography.

Keywords: non-thermal plasma; Atmospheric Pressure Plasma Jet (APPJ); flavonoids; oxidation

INTRODUCTION

Food is subject to varying degrees of physical, chemical and biological deterioration, involving losses in nutritional value, safety, texture and flavour. To stop or greatly slow down spoilage while maintaining the characteristics of fresh and minimally processed foods, different food preservation techniques are known. Yet most of the conventional sterilisation processes suffer from significant shortcomings. This has fostered the development of mild preservation techniques which operate at room temperature and have a minor impact on the quality and fresh appearance of food products. Non-thermal atmospheric pressure plasma seems to be a promising alternative to thermal treatments since an efficient inactivation of microorganisms comes along with a moderate heating of the treated surface (Moisan *et al.* 2001). Despite the inherent advantages of cold plasmas for preservation purposes there are currently no studies known that investigate the interactions of energetically highly reactive plasma species (electrons, UV photons, radicals and reactive oxygen and nitrogen species (ROS, RNS)) with dietary bioactive compounds. This emphasises the need to ensure the application of plasma as a safe food preservation technology prior to possible health benefits to consumers.

From the many compounds that exist in plants flavonoids are most commonly known for their antioxidant activity protecting cells against the damaging effects of reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite (Roback & Gryglewski 1988; Hu *et al.* 1995; Van Acker *et al.* 1995; Haenen *et al.* 1997). They are thus ideal target compounds to study the interactions

of plasma-immanent reactive species. According to Makris and Rossiter (2001) an oxidative degradation of flavonoids upon interaction with hydroxyl free radicals takes place leading to the formation of low molecular weight phenolics. The degradation follows similar pathways as heat-induced, oxidative cleavage (Buchner *et al*. 2006). Thus, questions were raised concerning the plasmachemical interactions of flavonoids and how such interactions could result in flavonoid degradation. To elucidate the influence of plasma-immanent species on the stability, four flavonoids (Figure 1) differing in key structural features were exposed to a non-thermal atmospheric pressure plasma jet. Reactions were followed by means of reversed phase high-performance liquid chromatography (RP-HPLC).

MATERIALS AND METHODS

Chemicals. Quercetin (dihydrate, 99%) was obtained from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Myricetin, Kaempferol and Taxifolin (all reagent grade) were purchased from Extrasynthese SA (Genay, France). All solvents used were of HPLC grade quality (Carl Roth, Karlsruhe, Germany). Demineralised water (Milli-Q, Millipore, Germany) was used.

Atmospheric Pressure Plasma Jet (APPJ). Since the design of the APPJ has been discussed in more detail elsewhere (Brandenburg *et al.* 2007) only a brief description is given here. The plasma jet used for this study consists of a nozzle made of ceramics with a needle electrode mounted in the centre and a grounded ring electrode placed at the nozzle outlet. The plasma was generated with Ar as feeding gas by applying a 27.12 MHz electric field between the electrodes. The operating radio-frequency (Rf) power was 10–30 W at a gas flow of about 20 slm of argon. Since the bottom of the nozzle is open the plasma expands to the surrounding air, leading to a gradual mixing with the Ar gas, which enables the simultaneous and homogeneous treatment of spot contaminated quartz slides.

Treatment of flavonoids with plasma. Samples were prepared by putting the compounds dissolved in 50% methanol onto a quartz slide and allowing it to dry under room temperature and aseptic conditions, forming a spot of ~ 5 mm in diameter. The slide is then placed on a stage at a known distance (d = 8.5 mm) from the exit nozzle of the plasma jet. The APPJ was run at experimental conditions for 1–2 min to allow preheating and passivating of the electrodes. The dried sample spot was then placed in the plasma effluent for a measured length of time. The treatment time and the applied power were varied while three samples were treated per parameter set, keeping the pressure and gas flow rate constant. After exposure the samples were dissolved in 200 µl 50% MeOH and analysed by HPLC.

Isocratic reversed-phase high-performance liquid chromatography (RP-HPLC). The HPLC-System consisted of a HPLC pump (Model 64, Knauer, Berlin, Germany), an autosampler (Model 465, Kontron Instruments), and a variable UV/Vis wavelength detector (Knauer, Berlin, Germany), operating at 280 and 365 nm. The detector was

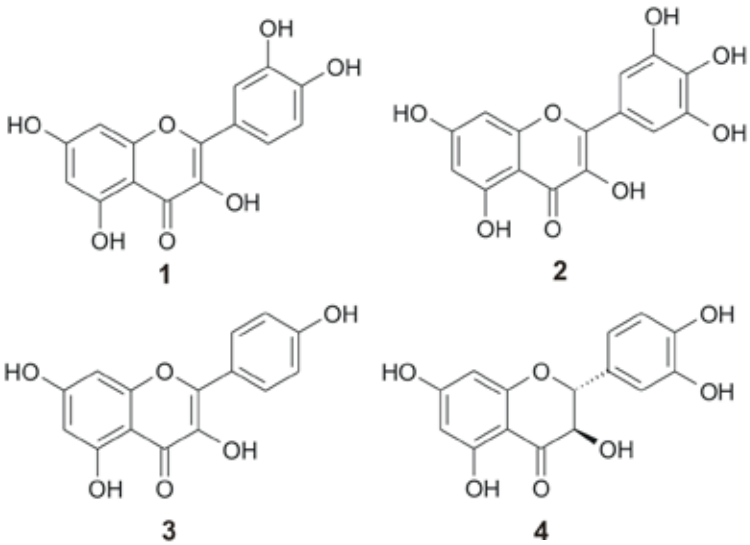

Figure 1. Chemical structures of the different flavonoid compounds applied (1 – Quercetin, 2 – Kaempferol, 3 – Myricetin, 4 – Taxifolin)

connected to a C-R4AX chromatopac data processor (Shimadzu Co., Kyoto, Japan).

The column was a 250 mm × 4.6 mm i.d., S-3 µm, YMC-Pack Pro C18 column (YMC Europe GmbH, Dinslaken, Germany) thermostatically controlled to maintain a temperature of 40°C. The mobile phase consisted of acetonitrile:water:acetic acid (30:65:5, v/v). Elution was performed at a flow rate of 0.4 ml/min.

RESULTS AND DISCUSSION

The catechol structure and the 2,3-double bond of the C-ring are characteristic structural elements responsible for antioxidative behaviour (Bors *et al.* 1990; Rice-Evans *et al*. 1995; Cook & Samman 1996; Rice-Evans & Miller 1996; Van Acker *et al.* 1996). The most acidic phenolics permit a high degree of π-electron delocalisation, which leads to a stabilisation of the anionic species formed after deprotonation by resonance phenomena. For flavonoids the 4'-position of the OH-group is the most acidic site. The stability of the anion is further enhanced when H-bonds between adjacent hydroxyls are possible (catechol structure) (Leopoldini *et al.* 2006). Structures lacking these structural features are known for their weaker antioxidant potential.

These phenomena can be confirmed by the observed different degradation rates of 1–4 upon exposure to an Ar plasma. All Ar plasma exposed compounds 1–4 show a strong degradation (Figure 2). Yet the degradation is higher for Quercetin 1 (reduction of 90%) and Kaempferol 2, the latter being almost completely degraded (to less than 2% amount) after 120 s exposure time. Myricetin 3 and Taxifolin 4, polyphenolic compounds that lack either a *o*-dihydroxy moiety in the B-ring of the flavonoid skeleton or the C = C double bond of the C-ring, show a weaker degradation (reduction to 23% and 26%, respectively). The degradation of the flavonoids is probably mediated by ROS leading to oxidation and cleavage of the flavonoid skeleton (Makris & Rossiter 2001; Buchner *et al.* 2006). The reaction rate significantly depends on the presence or absence of structural features. We assume that as a result volatile compounds not yet identified are formed by slow combustion due to reactive oxygen species or radicals emanating from the plasma.

When the power of the plasma is raised both the electron energy and its density increase, and the reactive particles become more energetic. Therefore the abstraction of H atoms and the subsequent scission of chains are supposed to be enhanced (Clouet & Shi 1992). Increasing the power while keeping all other operative parameters constant leads to an increased degradation (Figure 3). Even the weakest power (10 W) leads to a reduction of more than 60% after 120 s exposure to the Ar plasma.

The increase in the power is followed by an increase in the gas temperature. Thermal equilibrium is reached almost instantly, and the temperature remains constant with time. For 20 W the temperature of the plasma is 40°C, for 30 W the

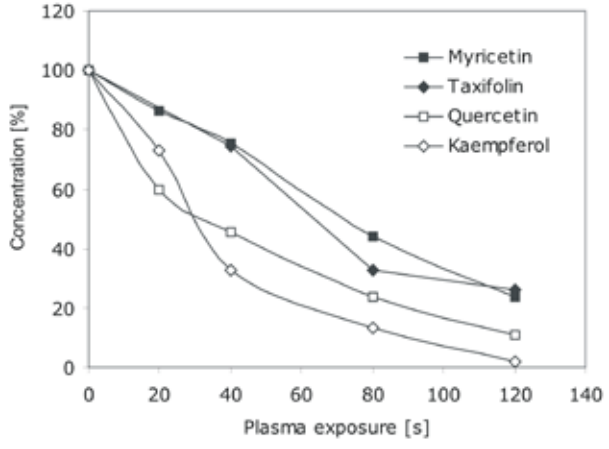

Figure 2. Degradation rate of the flavonoids 1–4 after exposure to APPJ

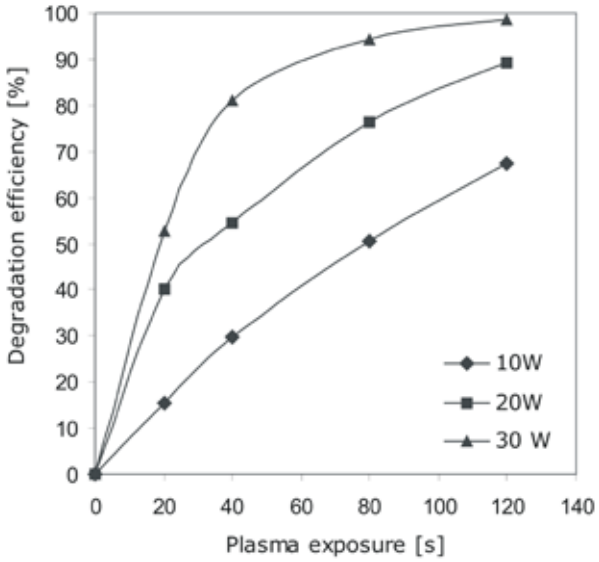

Figure 3. Depandance of the degradation rate of 1 on plasma driving voltage

temperature raises to 60°C. Experiments indicate that, whereas plasma exposure results in decrease of the flavonoids starting at temperatures as low as 30°C, degradation does even not begin for hot gas (without ionisation) at 120°C. We therefore conclude that ionisation and chemical processes in non-equilibrium plasmas are directly determined by the electron temperature and resulting immanent reactive species and are not sensitive to thermal processes and temperatures of the gas. Therefore a thermal-induced degradation can be neglected.

CONCLUSION

Rf-driven plasma jets in argon at atmospheric pressure have been shown to emit a significant amount of UV and VUV radiation (Foest *et al.* 2007). Photons below 275 nm (4.5 eV, 1 eV = 11 600 K or 96.485 kJ/mol) are energetic enough to break C-C or C-H bonds in a solid. Furthermore high energetic electrons (with mean electron temperatures typically in the range 1–2 eV) exist at the entire length of the jet. Thus considering the energy requirements for subsequent chemical reactions in the gas phase of a discharge volume the electronic and photonic energy in a non-thermal plasma is sufficient to break almost all type of bonds, excite or ionise atoms and molecules of the discharge gas. The presence of ground-state atomic oxygen (^{3}P), metastable molecular O_2 ($^1\Delta_g$ and $^1\Sigma_g^+$), O_3 or hydroxyl radicals can induce oxidation reactions (Jeong *et al*. 2000).

In order to elucidate the interactions of these highly reactive plasma-immanent species, we have investigated the stability of selected flavonoids with different antioxidative potential upon exposure to an atmospheric pressure Ar plasma jet.

Our results indicate that the flavonoids 1–4 degrade upon plasmo-chemical reactions probably due to existing ROS and radicals in the plasma effluent, whose presence, namely of ground-state O atoms (^{3}P) and OH radicals ($A^2\Sigma^+$), has directly been proven by optical emission spectroscopy (Brandenburg *et al.* 2007). This is in agreement to results showing that during roasting and cooking processes oxidative species lead to the formation of characteristic low molecular weight degradation products (Makris & Rossiter 2001; Buchner *et al.* 2006). The degradation rate directly depends on the operating power. The isolation and characterisation of volatile products emanating from plasma-induced photodesorption processes is part of further investigations.

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