Modification of polyphenols and cuticular surface lipids of Kale (B. oleracea convar. sabellica) with non-thermal oxygen plasma gaseous species

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ABSTRACT
The production and consumption of minimally processed food have grown rapidly over the past decades. Concomitant with popularity an increased number of microbial infections have been documented. Though efficiently inactivating microorganisms, conventional thermal food preservation significantly change the concentration, bioavailability and bioactivity of phytochemicals in food. In this context, non-thermal plasma (NTP) seems to be a promising alternative. Although much work has already been done in investigating the effects of non-thermal plasma on microorganisms, little is known about the influence of plasma treatment on food and food compounds.

To this end, we investigated the effects of a non-thermal oxygen plasma on the stability and functionality of phenolic compounds in kale (Brassica oleracea convar. sabellica). Reactions were followed by high performance liquid chromatography/diode-array detection (HPLC-DAD). Samples were characterized using scanning electron microscopy and attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FTIR).

Our results show that plasma treatment significantly influenced the flavonoid content in leaf tissue. 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. The degradation was not caused from photodesorption or thermodesorption processes at the adsorbate surface but clearly stemmed from the combined interaction of the various plasma reactive species in the plasma effluent. Epicuticular waxes on the abaxial side were visibly degraded.

The outcomes of this work represent a first step towards a molecular approach of plasma-food interactions and aim to open up novel insight into the reaction of flavonoids with reactive oxygen species at the solid-gas interface. This is especially important in view of future applications.

Keywords: Non-thermal plasma; flavonoids; kale; scanning electron microscopy; FTIR spectroscopy

INTRODUCTION
Food industry uses different preservation techniques to slow down or even stop food deterioration and to protect consumers from food-borne diseases. Conventional industrial and domestic thermal processes (i.e. boiling, frying, microwave cooking) are known to induce several detrimental effects on the food quality (Table 1). In addition to unacceptable flavor and texture changes, the usually high process temperatures are able to reduce the flavonoid content of vegetables [1]. Furthermore reduced antioxidant activities of specific bioactive compounds after heating an aqueous solution has been observed [2, 3]. Other preservation procedures suffer likewise from many shortcomings, such as formation of hazardous chemical residues as by-products of the preservation process or a low consumer acceptance. To retain the nutritional value and sensory quality of fresh or freshly-prepared food and beverages, mild preservation technologies are gaining
more and more importance. They are considered to have a minor impact on the quality and fresh appearance of food products as they usually operate at room temperature.

Table 1. Advantages and disadvantages of conventional thermal decontamination

<table>
<thead>
<tr>
<th>Thermal Decontamination</th>
<th>Beneficial</th>
<th>Detrimental</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inactivation of microorganisms</td>
<td>Inactivation of spores</td>
<td>High temperatures</td>
</tr>
<tr>
<td>Inactivation of spores</td>
<td>Inactivation of enzymes</td>
<td>Deterioration of bioactive compounds</td>
</tr>
<tr>
<td>Long shelflife of food products</td>
<td></td>
<td>Deterioration of flavoring substances</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decrease in the antioxidant activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prions are not degraded</td>
</tr>
</tbody>
</table>

Partially ionized gases, so-called non-thermal plasmas (NTP), might be promising alternatives to already existing technologies such as high-pressure processing or pulsed electric fields. An efficient inactivation of microorganisms has been observed for several pathogens, coming along with moderate heating of the treated surfaces [4]. Considering however the energy requirements for subsequent chemical reactions, plasma-immanent electrons and photons are able to break almost all type of bonds, excite or ionize atoms and molecules in the gas phase of a discharge volume or interact with at the plasma-solid interface (Table 2).

Table 2. Dissociation energies of organic compounds [5]

<table>
<thead>
<tr>
<th>Bond type</th>
<th>Bond energy (kJmol⁻¹)</th>
<th>Bond energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-H</td>
<td>411</td>
<td>4.25</td>
</tr>
<tr>
<td>C-C</td>
<td>346</td>
<td>3.56</td>
</tr>
<tr>
<td>C-N</td>
<td>276</td>
<td>2.86</td>
</tr>
<tr>
<td>C-O</td>
<td>358</td>
<td>3.70</td>
</tr>
<tr>
<td>C-S</td>
<td>272</td>
<td>2.80</td>
</tr>
<tr>
<td>C=C</td>
<td>602</td>
<td>6.23</td>
</tr>
<tr>
<td>C=O</td>
<td>724</td>
<td>7.50</td>
</tr>
<tr>
<td>C≡C</td>
<td>835</td>
<td>8.65</td>
</tr>
<tr>
<td>N-H</td>
<td>385</td>
<td>3.99</td>
</tr>
<tr>
<td>O-H</td>
<td>456</td>
<td>4.73</td>
</tr>
</tbody>
</table>

Despite the inherent advantages of cold plasmas in view of their decontamination potential, the influence of their reactive species on the stability and reactivity of food ingredients has not yet been clarified. It is however mandatory to elucidate and understand the basic interactions of radicals, reactive oxygen and nitrogen species (ROS, RNS) or energetic electrons and ions with bioactive compounds to avoid nutritional degradation or any other undesired effects in future applications.

MATERIALS & METHODS

Investigations in plasma-food interactions are done by a joint collaboration of three groups from different research centers. Within this network, research of the Food Chemistry group is aimed at investigating the chemical reactions of the various non-thermal plasma species with natural compounds. Main emphasis is put on the elucidation of the basic reaction mechanisms and potential structure-activity relationships with regard to oxidation reactions. Current investigations focus on the stability and reactivity of lipids at the plasma-solid interface and to polyphenolic compounds, known for their antioxidative, antimutagenic and antitumor efficacy. In addition the complex interplay of plant matrix compounds during oxidative photochemical processes is extensively studied.

For this purpose, we routinely use HPLC systems (analytical, semi-preparative, and preparative scale) coupled with various detection systems (UV, DAD, RID, fluorescence, ECD, ELSD) in addition to standard
RESULTS & DISCUSSION

Given that investigations on plasma-food interactions on a molecular level are still in their infancy, the main aim of our group is to ascertain if and how non-thermal plasma is changing the chemical composition and morphological structure of highly perishable vegetables. Plant systems, rich in polyphenolic and phenolic compounds are ideal systems to elucidate the interactions of plasma-immanent reactive species with 1,4-Benzopyrone derivates (flavonoids), known to protect cells against the damaging effects of ROS and RNS such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite [6, 7, 8]. Depending on the plasma feed gas composition and on various experimental variables, these highly reactive compounds are known intermediates in cold plasma effluents.

Recent experiments have shown, that a time- and structure-dependent degradation of specific flavonoids following a low-pressure oxygen plasma exposure can be observed [9]. This has been attributed to plasma-immanent reactive species such as O(3P), O₂ (¹Δg and ¹Σg⁺), or OH radicals leading to intermediate formation of oxygen functional groups at the adsorbate surfaces and finally to volatile organic compounds. However, since these compounds were adsorbed on solid surfaces, informations about the effects of low-pressure oxygen plasma on the stability and reactivity of flavonoids embedded in a food matrix are still rare. First measurements of lettuce plants have shown a clear relationship between plasma exposure and phytochemical composition [10]. As synergistic contributions of the numerous different concerted elementary reactions make the underpinning plasma chemistry rather complex, multiple reaction pathways are plausible and mechanistic details still have to be clarified.

In this talk, recent studies on plasma-induced changes in the plant phenolic profile of kale will be shown. Measurements were done by means of RP-HPLC/DAD and are compared to cooking experiments. Modifications in the adaxial leaf surface morphology are characterized using scanning electron microscopy (SEM). Changes in the surface chemical composition are monitored by Fourier-transformed infrared spectroscopy (FTIR) in attenuated total reflexion mode.

Our results indicate that the chemical composition in plants is changing upon plasma-chemical reactions, probably due to existing ROS and radicals in the plasma effluent. 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 [2, 11]. The food matrix significantly influences the chemical reactivity of embedded compounds as pure compounds have been shown to follow a differing reaction behaviour. Reaction rates directly depend on the operating power of the plasma source. A similar oxidation mechanism as for thermal treatment is suggested. The results of this talk are currently prepared to be published.

CONCLUSION

Non-thermal plasmas are usually regarded as a mild preservation technology. Their antimicrobial effects have been shown in several studies. The interactions of plasma with bioactive compounds embedded in a food matrix have not yet been elucidated. Our results suggest that non-thermal plasmas change the chemical composition in plants depending on the experimental conditions. The isolation and characterization of volatile products emanating from plasma-induced photodesorption is part of further investigations.

REFERENCES


