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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

The in-depth study of aroma compounds formed during tobacco combustion *via* Maillard reaction and/or caramelization
（シガレット燃焼過程でのメイラード反応およびカラメル化反応による加熱香気成分生成に関する研究）

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This dissertation demonstrated some of the investigation designed to lead to an increased understanding of the chemistry of the Maillard reaction and caramelization in real-life samples, based on recent analytical developments. The novel analytical techniques were developed and applied to precursor screening, intermediate monitoring and trace level detection of aroma compounds formed during tobacco combustion, a process of notorious complexity, to clarify the influence of tobacco leaf compounds on cigarette smoke aroma properties.

Firstly, analytical method for the aroma precursor compounds affecting the cigarette smoke aroma formed during tobacco combustion was discussed. In the first step, the aqueous tobacco leaf extract was fractionated using prep-LC. In the next step, the LC fractions were recombined in an omission approach. In this approach, a series of “pooled” extracts was obtained, and in each extract an LC fraction was omitted. In that way, certain tobacco leaf constituents are eliminated from the “pooled” extracts. All extracts were then concentrated and analysed by Py-GC–MS using a resistive heating pyrolysis system installed on a thermal desorption unit. The obtained pyrograms were statistically evaluated and compared with a whole extract (all LC-fractions present) and the key LC-fraction was identified that contribute most to the formation of typical cigarette smoke aroma. In addition, GC–MS analysis of derivatized LC-fraction was performed to identify the precursors of these aroma compounds in the key LC-fraction and the main constituents were identified as aspartic acid, proline, malic acid and sugars (fructose, glucose and sucrose). These are natural constituents in tobacco leaves and their degradations through Maillard-type reactions could contribute significantly to the aroma character of cigarette smoke.

For more in-depth study, then, analytical method was modified to analyze intermediate compounds. A recently developed resistive heating pyrolysis devices, namely a generic pyrolysis unit (PyroVial) was applied. Firstly, a mixture of glucose and proline was pyrolysed as model compounds. The volatile fractions of the pyrolysates were analyzed using headspace-GC–MS, semi-volatile using liquid injection GC–MS and non-volatiles using LC–MS. In addition, a tobacco leaf extract was analyzed by Py-LC–MS, whereby the micro-vial pyrolysis simulates the tobacco leaf

combustion process. Using MS deconvolution, molecular feature extraction and differential analysis it was possible to identify Amadori intermediates of the Maillard reaction in the tobacco leaf extract. The intermediate disappeared as was the case for 1-deoxy-1-prolino-β-D-fructose or the concentration decreased in the pyrolysate compared to the original extract such as for the 1-deoxy-1-[2-(3-pyridyl)-1-pyrrolidinyl]-β-D-fructose isomers indicating that Amadori intermediates are important precursors for aroma compound formation.

Finally, advanced comprehensive analytical systems applied to detect trace level of aroma compounds in cigarette smoke. Volatile sulfur compounds in food have received special attentions due to their extremely low odor threshold levels and high sensory impact, but these compounds are most often present at very low levels in complex matrices. Sulfur compounds which are derived from Maillard reaction of sugar and amino acids degradation products also play an important role in the aroma of cigarette smoke. In this study, large volume injection of cigarette smoke extract followed by selectable $^{1}D/^{2}D$ GC–Q-TOF-MS with parallel sulfur chemiluminescence detection (SCD) was applied for identification of trace sulfur compounds in cigarette smoke. In order to identify each individual sulfur compound, sequential heart-cuts of sulfur fractions from ^{1}D GC to ^{2}D GC were performed with the three MS detection modes (SCD/EI-TOF-MS, SCD/PCI-TOF-MS, and SCD/PCI-Q-TOF-MS). As a result, 41 sulfur compounds could be determined at ng mg TPM^{-1} levels. Of these sulfur compounds, 13 compounds have not previously been reported in cigarette smoke.

Through the efforts of this dissertation, innovative analytical work flows designed to lead to an increased understanding of the chemistry of the Maillard reaction and caramelization using real-life samples could be demonstrated. In tobacco science, but also in other fields such as food technology and biological application, sophisticated GC–MS and LC–MS techniques combined with computation analyses for aroma precursors screening, intermediates monitoring, trace level detection of aroma compounds generated by thermal processing and elucidating their formation pathways that has been developed in this dissertation are widely applicable, important and required for advanced research.