学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Application of untargeted lipidomics for the identification and characterization of novel lipids in plant and yeast samples
(植物および酵母サンプルにおける新規脂質の同定と特性評価のための非標的脂質オミクスの応用)

Abstract

Lipids are essential hydrophobic biomolecules which contribute to structural integrity, energy storage, and cellular signalling. Besides their cellular activities, bioactive lipids that are derived from food sources such as polyunsaturated fatty acids (PUFAs) showed significant health benefits, including anti-inflammatory, cardioprotective, and neuroprotective effects. Despite the significant importance of lipids derived from plant and microbial origins, research focusing on comprehensive lipidomic analysis and characterization of novel bioactive lipids in dietary plant and yeasts is still limited. This doctoral research aimed to cover these research gaps by comprehensive lipid profiling followed by the identification and characterization of new lipids of various plant samples, such as edible rose petals, herbal tea, grapes, oilseeds, and yeast, such as *Saccharomyces cerevisiae*, using an untargeted high-resolution liquid chromatography–mass spectrometry (LC/MS) approach. Through the untargeted LC/MS analysis, novel lipids such as acyl steryl glycoside (ASG), fatty acid esters of hydroxy fatty acids (FAHFAs), N-acyl-lysophosphatidylethanolamines (LPE-N), and acylhexosylceramides (AHexCer) were identified and characterized. The findings highlight the importance of dietary plant and yeast. The findings highlight the importance of untargeted lipidomics in food science, uncovering unique plant and fungal lipids with potential nutritional and biotechnological significances.

Chapter One introduces overall lipid information, the definition of lipids, classification, health benefits of bioactive lipids, lipidomic research in plant and yeast samples, prospects, research gaps, and objectives for this study. This also explains the principles of lipidomics, analytical methods, and the biological relevance of lipid classes, particularly highlighting the development of a targeted and untargeted LC/MS approach. The chapter addresses the limitations in current lipidomics methodologies, particularly the lack of knowledge on the comprehensive lipidome of food resources.

Chapter Two describes the lipid profiling of two edible rose petals of *Rosa hybrida* (Sahime and Apple roses), cultivated in Shimane Prefecture, Japan. By using a modified Bligh–Dyer method for lipid extraction and untargeted analysis using LC-Orbitrap-MS analysis, 166 lipid molecular species were identified from five major lipid categories, including fatty acyls (FAs), glycerophospholipids (GPs), glycerolipids (GLs), sphingolipids (SPs), and sterols (STs). Among the fatty acids, saturated fatty acids were abundant in both Sahime and apple roses compared to unsaturated fatty acids. Sahime rose petals contained significantly higher amounts of GPs and STs compared to Apple rose. Sphingolipids, such as phytoceramides, were relatively higher in Apple rose compared to Sahime. Importantly, eleven novel acyl steryl glycosides (ASG) were discovered for the first time in these rose petals. Correlation analysis revealed distinct lipidomic profiles between two rose species, demonstrating both compositional diversity and potential health-promoting lipid content in rose petals.

Chapter Three presents the comprehensive lipid compositional analysis of six herbal teas, including Fennel (*Foeniculum vulgare Mill.*), Ginger (*Zingiber officinale)*, juniper (*Juniperus communis*),

Lemon peel (*Citrus limon*), Orange peel (*Citrus aurantum*), and Rosehip (*Rosa canina*), using untargeted LC/MS analysis. A total of 204 lipid molecular species were identified and characterized by MS/MS analysis. Multivariate statistical analysis revealed distinct lipidomic profiles between herb teas. Cluster analysis showed that ginger was enriched in SPs, whereas fennel contained more GPs. Triacylglycerol levels did not differ markedly among the herbal teas. The nutritional parameter, PUFA/SFA ratio, was highest in orange peel, followed by lemon peel, suggesting potential health benefits of citrus teas. High-resolution mass spectrometry with MS^n analysis also enabled the identification of diverse lipids, including FAHFAs and LPE-N, across six herbal teas. Overall, the work delivers a comprehensive lipidomic profile of these teas and supports their potential use as functional ingredients in nutrition and food applications.

Chapter Four elucidates a comprehensive lipidomic investigation on five grape varieties, which include Kerner (*Vitis vinifera*), popularly known as white Herald, a white grape variety used in winemaking; Kiyomai, Kiyomi, and Yamasachi (*Vitis coignetiae*), Japanese hybrid grape varieties, and Zweigelt (*Vitis vinifera Zweigelt*), an Austrian hybrid grape variety used in red winemaking. The grapes were separated into fruits (containing pulp and skin) and seeds, and the comprehensive lipidomic analysis led to the identification of 245 lipid species across five major lipid categories. Multivariate analyses revealed distinct lipid composition between the five varieties. All the grape seeds showed higher lipid content compared to the fruits. Fatty acyls are abundant in Zweigelt seeds, including ω-3 and ω-6 fatty acids with a high PUFA/SFA ratio, suggesting their potential health benefits compared to other varieties. Importantly, this study identified new lipids, such as acylhexosylceramides (AHexCer), a subclass of sphingolipids, and characterised them for the first time in these grape varieties. AHexCer lipids were found to be abundant in Kerner seeds. Kyomi proved to be a potential source for natural ceramides, which are widely used in cosmetics. Additionally, several water-soluble metabolites, including antioxidants, were identified and characterized in the same samples. This study presents comprehensive lipid profiling of five grape fruit and seed varieties from Hokkaido, Japan, supporting their use in functional food development and quality winemaking.

Chapter Five focused on exploring the lipidomic analysis of five commonly used oilseeds: Chia (*Salvia hispanica*), Flax (*Linum usitatissimum*), Pumpkin (*Cucurbita pepo*), Sunflower (*Helianthus annuus*), and Hemp (*Cannabis sativa*). This untargeted analysis identified 259 lipid molecular species. Sunflower seeds showed higher ω-3 and ω-6 fatty acid levels, whereas pumpkin displayed the overall lipid abundance and diversity, particularly enriched with triacylglycerols and GPs. Saturated fatty acids predominated over unsaturated fatty acids in most varieties. Interestingly novel lipids such as FAHFAs were also identified and characterized in oil seeds for the first time with the aid of untargeted lipidomics, highlighting their potential implications for functional foods and dietary supplements.

Chapter Six examines the compositional lipid diversity across 48 yeast strains (*Saccharomyces cerevisiae* (SC)), which are important in fermentation and biotechnological utilization. The LC/MS analysis resulted in the identification of 135 lipid molecular species, representing five lipid classes and 15 subclasses. Correlation analysis of lipid species in 48 yeast strains was performed. Furthermore, several bioactive lipids, such as LPE-N, were identified and characterised for the first time in yeast varieties. This study provides new insights into yeast lipid metabolism for understanding strain-specific metabolic adaptations during wine fermentation and improving industrial fermentation quality.

Overall, this doctoral research set a comprehensive lipidomic framework, encompassing plant and yeast analysis by integrating untargeted LC/MS techniques with advanced bioinformatics approaches. This research emphasizes a deeper understanding of the diversity, functionality, and bioactivity of lipids in natural food matrices and microbial systems. However, the identification of new lipids, such as FAHFAs, AHexCers, ASGs, and LPE-Ns, offers new insights into the biochemical landscapes of lipid metabolism, underscoring their potential applications in functional foods and nutraceuticals. After all, this study aims to fill the gap between food science and analytical chemistry, fostering advancements in lipid research.