Exploring Lipid Diversity and Structure: From Hidden Lipids to Biomedical Insights

G. Ventura, C.D. Calvano - Università degli studi di Bari Aldo Moro - Italy - giovanni.ventura@uniba.it

Novel Lipid Classes

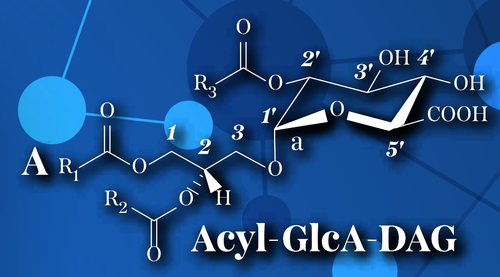
One of the most exciting frontiers in lipidomics is the ***identification and structural elucidation of unconventional lipid classes*** molecules that have historically escaped attention due to their atypical structures, low abundance, or analytical challenges. Advances in LC-MS/MS techniques, have enabled the discovery of entirely new lipid species with potential biological or nutritional relevance.

A striking example is **the discovery and in-depth characterization of acylated glucuronosyldiacylglycerols (acyl-GlcA-DAGs)**. These glycolipids consist of a **diacylglycerol backbone linked to a glucuronic acid headgroup, further acylated on the sugar moiety itself. Using RPLC-ESI-MS^n, more than 40 distinct acyl-GlcA-DAG species were structurally elucidated [1] (FIGURE 1a).**
Tandem mass spectra in both positive and negative ion modes provided complementary structural insights.
Positive ion mode enabled the identification of the acylation site, confirming esterification at the 2-hydroxyl position of the glucuronic acid, while in negative ion mode, characteristic fragmentations allowed for the assignment of sn-1 and sn-2 regiochemistry.
MS^n analysis were used to confirm structural hypotheses. Following this initial discovery, acyl-GlcA-DAGs have been subsequently identified in a variety of biological matrices, including brown algae and other food sources, suggesting a broader biological relevance and encouraging further investigation.

Another focus of the research was the structural characterization of unconventional phospholipids across diverse biological matrices. In plant-derived lipid extracts, attention was given to lyso-N-acylphosphatidylethanolamines (L-NAPE, **FIGURE 1c**), frequently misannotated as phosphatidylethanolamine (PE, **FIGURE 1b**) due to their isobaric nature. A method combining HILIC-LC and MS^3 fragmentation was developed to resolve these overlaps and enable unambiguous identification [3].

One example is phosphatidylhydroxyethylglycine (PHEG), an unusual aminophospholipid identified in brown algae, characterized by a unique headgroup whose distinct fragmentation behavior has been characterized [2] (**FIGURE 1d**).

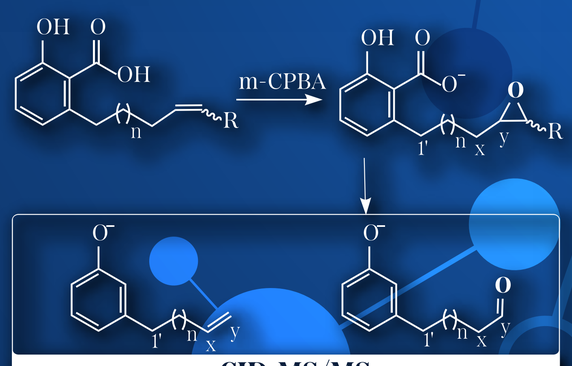
More recently, the investigation has extended to the effects of thermal processing on lipids. In edible insects and other roasted matrices, several Maillard-type lipid modifications were identified, including formyl-, acetyl-, and Amadori-modified PEs (**FIGURE 1d**) and their lyso-forms. Overall, six new lipid classes were described, demonstrating how heat-induced reactions can significantly alter the lipidome, and highlighting the importance of lipidomic safety evaluation in novel food systems.

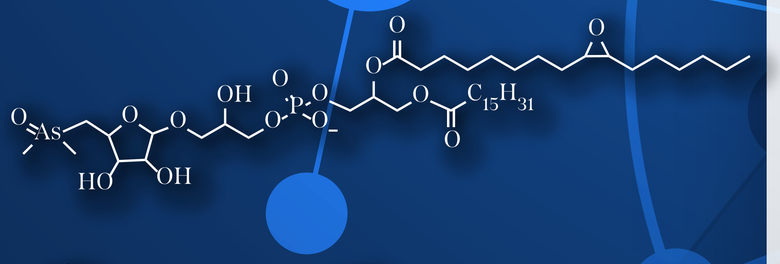


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Double bond position

One of the key challenges in lipidomics lies in determining **the position of double bonds** within fatty acyl chains. Recent studies have demonstrated the effectiveness of chemical derivatization strategies —most notably epoxidation using *m-chloroperoxybenzoic acid (m-CPBA)*— as a means *to locate C=C double bonds directly from complex biological extracts, without the need for prior purification.*

This strategy was pivotal in the structural elucidation of **anacardic acids** (AnAs, FIGURE 2a) [4], ***a class of phenolic lipids*** extracted from pistachio shell byproducts.
These molecules, characterized by ***a salicylic acid-derived headgroup*** and a long unsaturated alkyl chain, exist in multiple isomeric forms differing only by the location of C=C bonds.
After m-CPBA epoxidation, the AnAs were successfully separated and identified via LC-MS/MS.
The resulting fragmentation patterns allowed the distinction of regioisomers such as Δ8, Δ10, and Δ11 configurations in C15:1 chains.
This work demonstrated the power of derivatization in valorizing a***gricultural waste streams*** through high-resolution structural profiling.

The same approach was used for **arsenosugar phospholipids** (As-PLs, FIGURE 2b) [5 6].
These lipids, whose health implications are still debated due to their arsenic content, were shown to harbor common essential fatty acids such as linoleic (18:2 Δ9,12) and linolenic acid (18:3 Δ9,12,15). Upon derivatization, ***the double bonds were converted into epoxides, which fragmented yield diagnostic ions indicative of bond position.*** This allowed a direct assignment of the double bond locations and highlighted the nutritional potential of As-PLs when occurring in highly unsaturated and metabolically relevant forms .

Complementary to derivatization, non-derivatized approaches like ***HILIC-ESI-FTMS with All Ion Fragmentation (AIF)*** [7] scanning have proven to be powerful in profiling embedded fatty acyl chains. For example, a comprehensive lipidomic study using this method on human plasma allowed the identification of over 250 phospholipid species and 18 distinct fatty acids across multiple classes (PC, PE, PI, LPC).

Biomarker discovery & Software development

Lipidomics is increasingly being recognized as a powerful tool in biomedical research.

A representative case is the application of phospholipidomics to peripheral ***blood mononuclear cells (PBMCs)of children with autism spectrum disorder (ASD).***
This neurological condition lacks validated molecular biomarkers despite extensive genetic and neurological research.
In this study, PBMC lipid extracts from ASD children and their healthy siblings were analyzed using HILIC-ESI-FTMS and MS/MS.
The integration of two different data processing software platforms (XCMS and Alex123 and an in house developed tool for MS/MS attributions) provided complementary insights into both intact lipid species and fatty acyl chain composition [8 9].

The results revealed that *phospholipid profiles alone were not sufficient to distinguish ASD from controls.*
The study highlighted substantial inter-individual variability in lipid composition among ASD subjects, consistent with the known clinical and genetic heterogeneity of the disorder [8].

In another clinically context, the strategy was applied to lipid extracts from human dermal fibroblasts, aiming to identify lipid biomarkers of Parkinson's disease, r***evealing a downregulation of PI 38:3, containing the FA C20:3, in the most severe disease stage [10].***

Currently, my research is focusing on the analysis of nanovesicles derived from model systems of pancreatic cancer cells.
These vesicles are being investigated for their lipid composition and structural features, with the aim of understanding their potential roles in cell communication and disease progression, and exploring their relevance as biomarkers or therapeutic targets.

In parallel, I am working on the development of a MATLAB® platform aimed at the automated recognition and structural characterization of lipid species.

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UNIVERSITÀ DEGLI STUDI DI BARI ALDO MORO