Non-targeted NMR chemical profiling of vegetable oils: A two-path workflow for fast access to a detailed metabolome

Anaïs Cyatwa, Joseph Thiery, Lauriane Lenen, Alexis Kotland, Jane Hubert

Introduction

Vegetable oils are important in food, health, and cosmetic industries due to their nutritional properties, bioactive compounds and increasing use as extractive matrices. Oil-based matrices are mainly composed of saponifiable lipids (95–99%) and a small unsaponifiable fraction (~2%) rich in sterols, tocopherols, triterpenes, and other specialized metabolites which influence oil quality and biological effects. However, the chemical complexity of oils, especially the diversity of unsaponifiable constituents, requires dedicated analytical strategies to identify these minor metabolites highly diluted in the oily matrices. To address this challenge, we have developed tailored workflows based on Nuclear Magnetic Resonance (NMR) spectroscopy for the chemical profiling of a wide range of primary and secondary metabolites present in complex mixtures from both virgin oils and oil macerates.

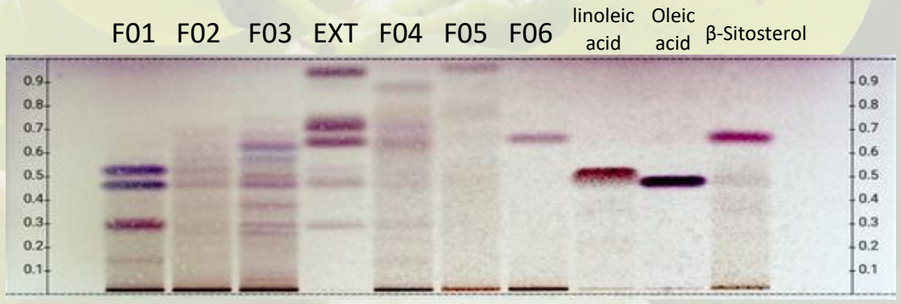
This study introduces two NMR-based workflows for the detailed chemical profiling of grape seed oil, with a particular focus on the identification of key bioactives. Both strategies can be used separately or in combination to investigate the metabolome of oily samples, such as vegetable oils and oily plant macerates.

Methods

Workflow 1

Liquid-Liquid Extraction

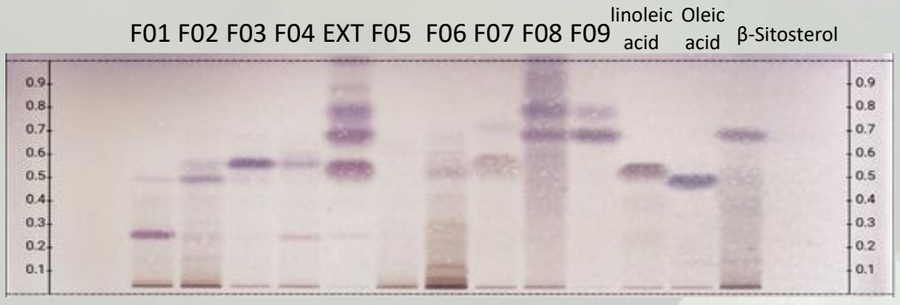
A grape seed oil sample was extracted by creating a biphasic system with a MeOH/H_2O hydroalcoholic phase. The resulting extract was then fractionated by centrifugal partition chromatography (CPC). Six final fractions were grouped based on their HPTLC profile similarities.



Workflow 2

Saponification

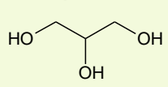
A grape seed oil sample was saponified using a hydroalcoholic KOH solution, followed by extraction. The extract was fractionated by CPC under the same conditions as in workflow 1. Nine final fractions were grouped based on their HPTLC profiles.



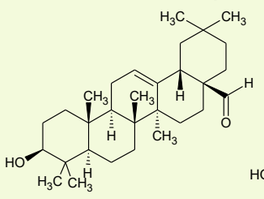
Results

Metabolites identified using liquid-liquid extraction and/or saponification

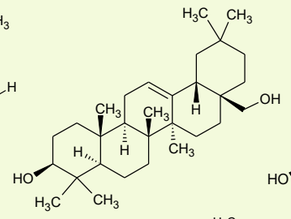
Glycerol



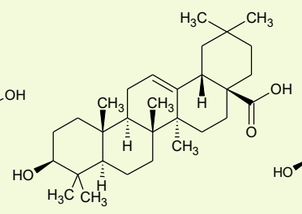
Oleanolic aldehyde



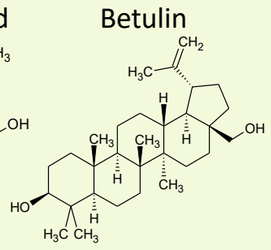
Oleanolic alcohol



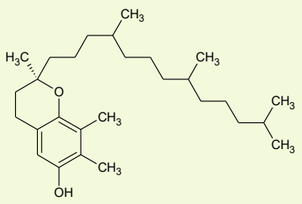
Oleanolic acid



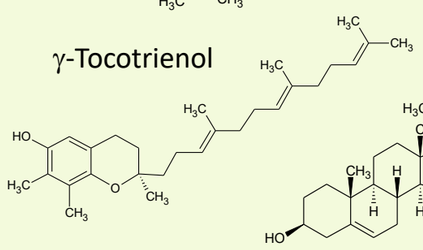
Betulin



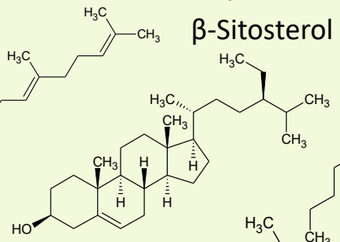
γ-Tocopherol



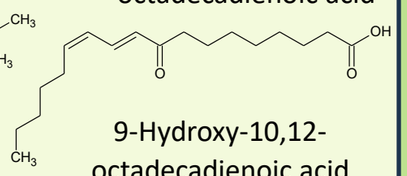
γ-Tocotrienol



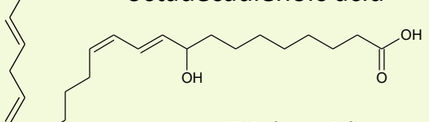
β-Sitosterol



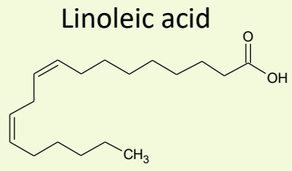
9-Oxo-10,12-octadecadienoic acid



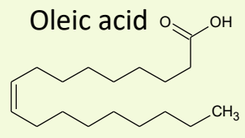
9-Hydroxy-10,12-octadecadienoic acid



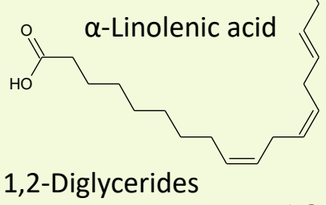
Linoleic acid



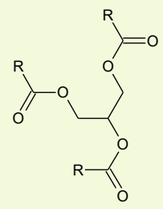
Oleic acid



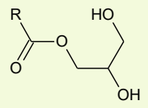
α-Linolenic acid



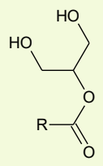
Triglycerides



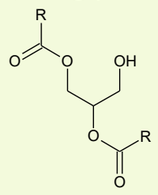
1-Monoglycerides



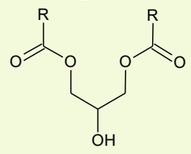
2-Monoglycerides



1,2-Diglycerides



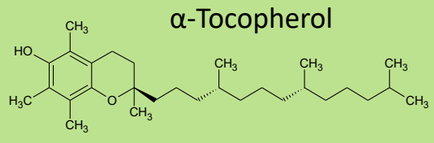
1,3-Diglycerides



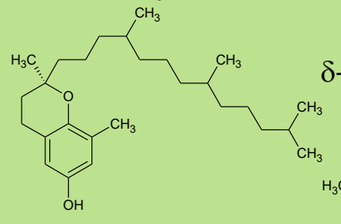
R=fatty acid

Additional metabolites identified using saponification

α-Tocopherol

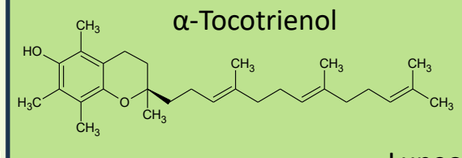


δ-Tocopherol

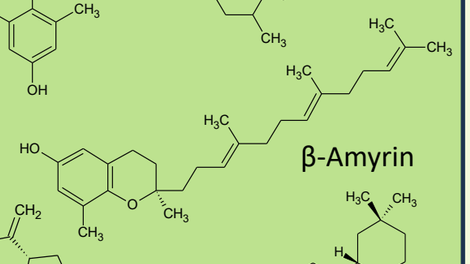


δ-Tocotrienol

α-Tocotrienol

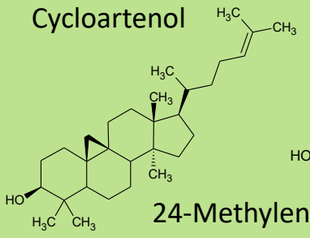


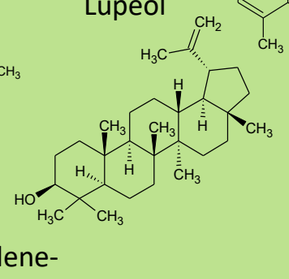
β-Amyrin

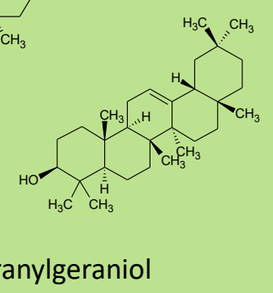


Lupeol

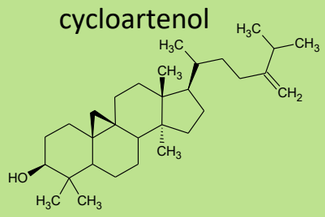
Cycloartenol



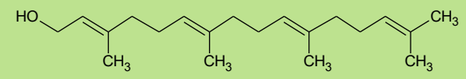




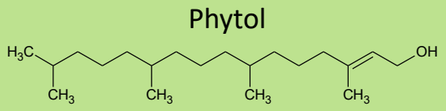
24-Methylene-cycloartenol



Geranylgeraniol



Phytol



Conclusion

- Both workflows successfully highlighted the complex metabolome of grape seed oil.
- Saponification provides deeper access to unsaponifiable and highly unpolar metabolites.
- NMR dereplication is robust, sensitive, and does not require reference standards for unambiguous metabolite identification.
- Applicable to a wide range of vegetable oils and oily extracts for untargeted chemical profiling, quality control, authentication, or sample comparison.

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