Metabolomic approach using LC-MS/MS analysis and molecular networking to follow up bioactive constituents of Callophyllum inophyllum nuts during drying process

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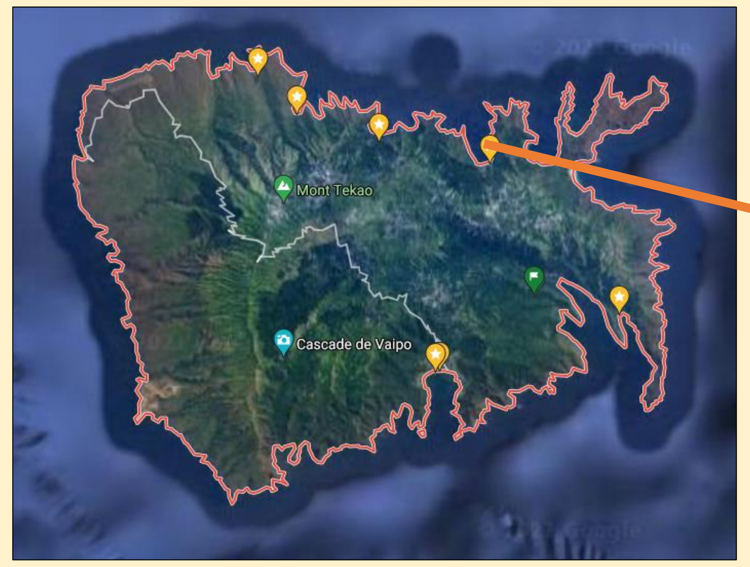
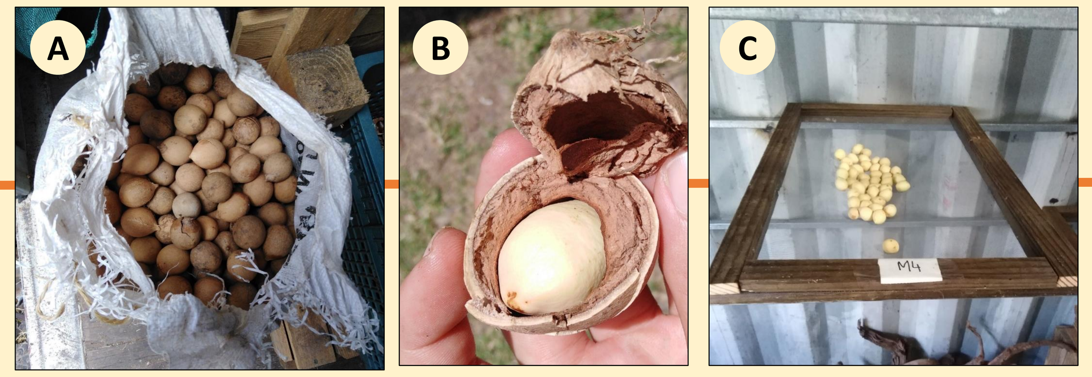
Strategy



Nuku Hiva collection sites

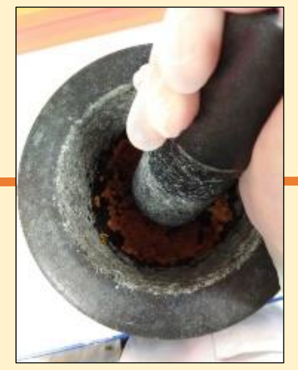
DRYING PROCESS

Hatiheu, S08°49.679' W140°04.897'



(A) Harvesting between July and September 2020 in the Marquesas Islands
(B) Opening the fruits
(C) Drying of the nuts from 0 to 10 weeks

PRESS EXTRACTION



Laboratory scale extraction: Grinding of 15 ± 2 g of dry nuts, maceration with EtOAc (ethyl acetate), sonication and drying

LC-DAD and UPLC-MS/MS analysis

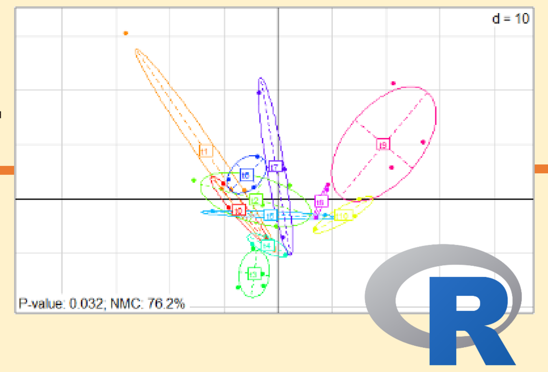
≈ 135 resinoid extracts analyzed in French Polynesia (LC-DAD, Luna C18 (250 x 4.6 mm, 3 µm)) and Marseille (UPLC-QqToF-MS/MS, Luna C18 (150 x 2.1 mm, 1.6 µm))

PRE-PROCESSING ANALYSIS

Workflow4metabolomics

Alignment on W4M
110 Samples
1176 ions

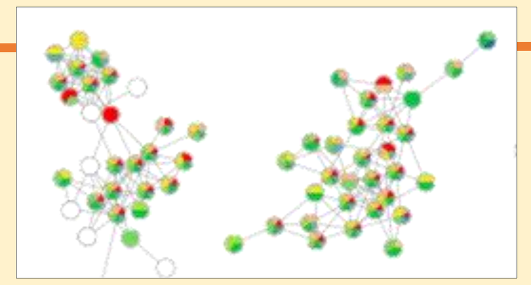
DISCRIMINANT ANALYSIS



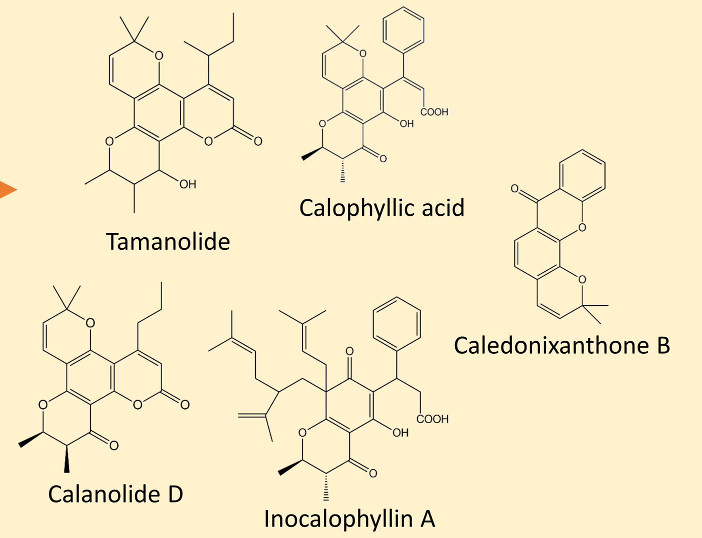
R packages: mixOmics (*plsda* function); ade4 (graphical representation); RVAideMemoire (discriminant compounds)

MOLECULAR NETWORKS

GNPS Cytoscape



ANNOTATION AND IDENTIFICATION



Context

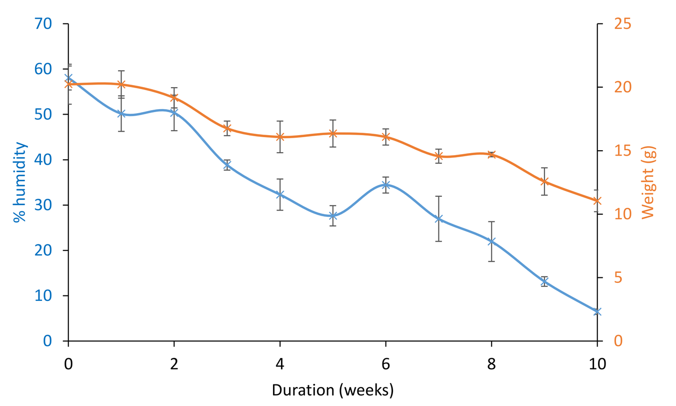
Tamanu oil, obtained from the nuts of *Calophyllum inophyllum* L. (Calophyllaceae), was traditionally used to cure various skin problems and ailments in French Polynesia [1]. Nuts and containing oil are also used for skin care [2]. They were reported to treat different kinds of skin affections and used as natural cosmetic ingredient [3]. Since the drying of nuts is an important stage for oil preparation, the objective of our study was to **develop an analytical method to evaluate oil quality** (chemical composition and oil yields) during nuts drying process following different drying parameters (abiotic factors or duration). In this project, we propose to use a metabolomic approach combining LC-MS/MS analysis and molecular networking to identify markers inducing **chemical composition variability during drying process of *tamanu* nuts** and previously unidentified constituents.

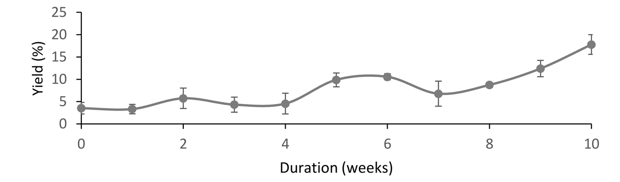
Objectives:

(1) Evaluate quality of oil by oil yields and chemical composition during drying process
(2) Assess the drying process efficiency by a metabolomics approach

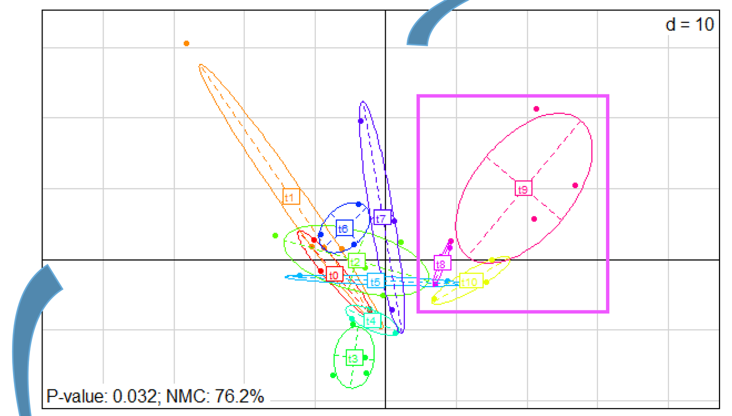
Results

Evolution of metabolites in *tamanu* nuts during the drying process

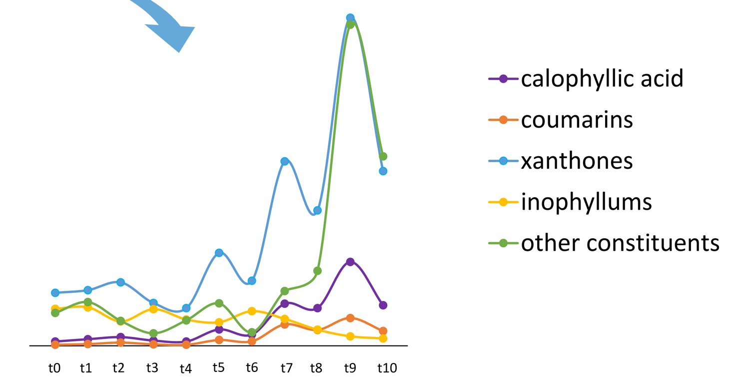




Evolution of the percentages of humidity, weight of the nuts and the oil yields of *tamanu* during the drying process from 0 to 10 weeks.



PLS-DA score plot (discriminant analysis) on the chemical composition of *tamanu* oils according to the drying process from 0 to 10 weeks.



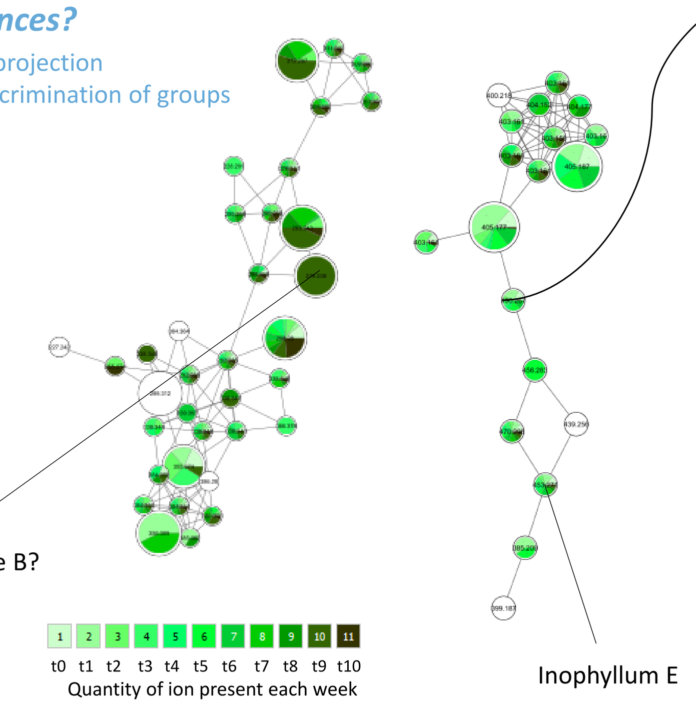
Evolution of the most represented chemical families in VIPs during drying process.

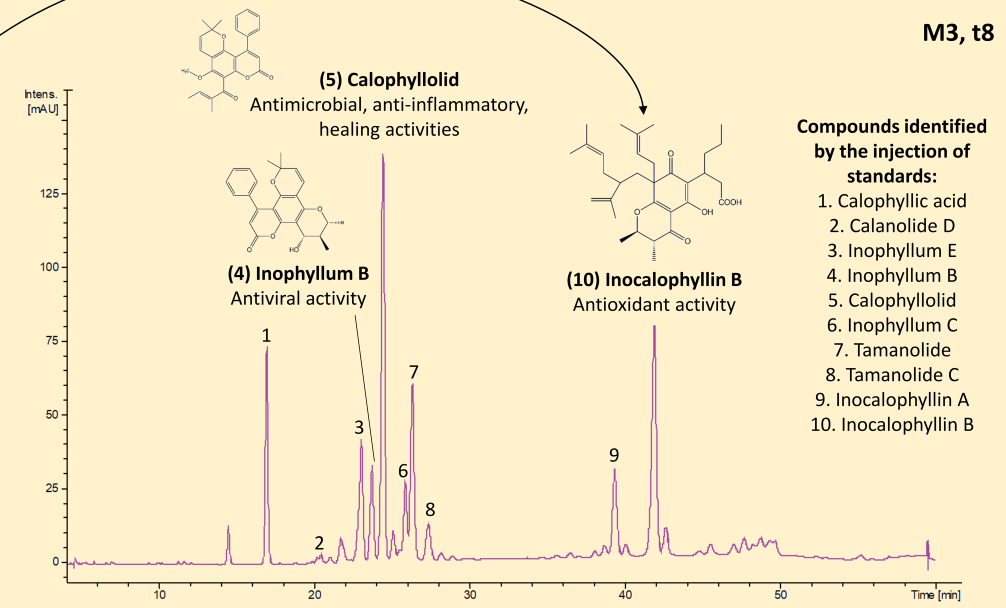
Which compounds may influence these differences?
VIP = Variable importance in the projection
= m/z which impact in the discrimination of groups

Main observations:

- Loss of nut mass of about 10% from t0 to t10 (from 20 to 11%)
- Decrease in moisture content from t0 to t10 (from 58% to about 6%)
- Increase of oil extraction yield (from 3.5% at t0 to 17.8% at t10)
- Different chemical compositions from t8 to t10 compared to the other durations (*P*-value < 0.05)
- ↑ node size = ↑ VIP score
- **Inocalophyllin B and inophyllum E, chemical markers of nuts at the beginning of drying**
- **Caledonixanthone B, marker at the end of drying**

Number of misclassifications (NMC): percentage of samples which are wrongly classified by the model
P-value < 0.05: significant difference between the two groups

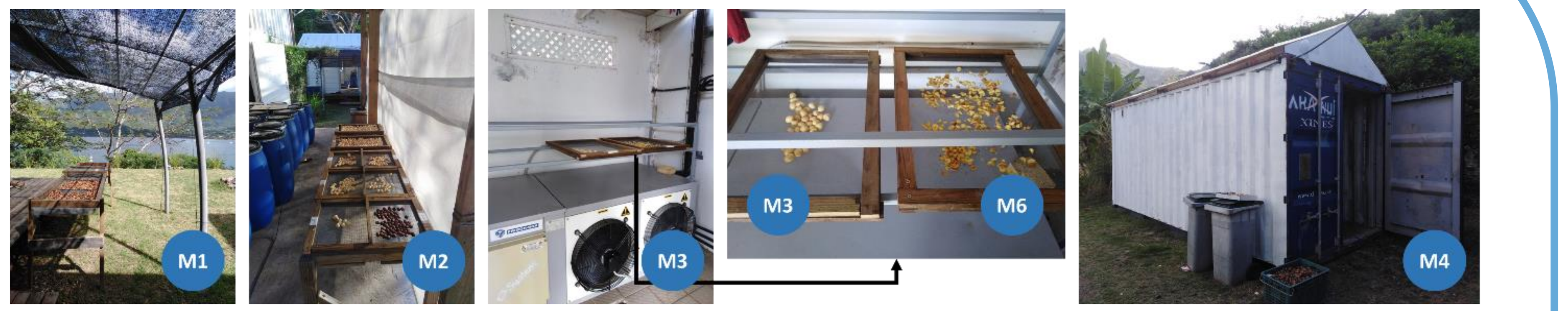




Compounds identified by the injection of standards:
1. Calophyllic acid
2. Calanolide D
3. Inophyllum E
4. Inophyllum B
5. Calophyllolid
6. Inophyllum C
7. Tamanolide
8. Tamanolide C
9. Inocalophyllin A
10. Inocalophyllin B

Chromatogram of *tamanu* oil from nuts dried in M3 condition (under hot air ventilation) for 8 weeks (LC-DAD, 280 nm).

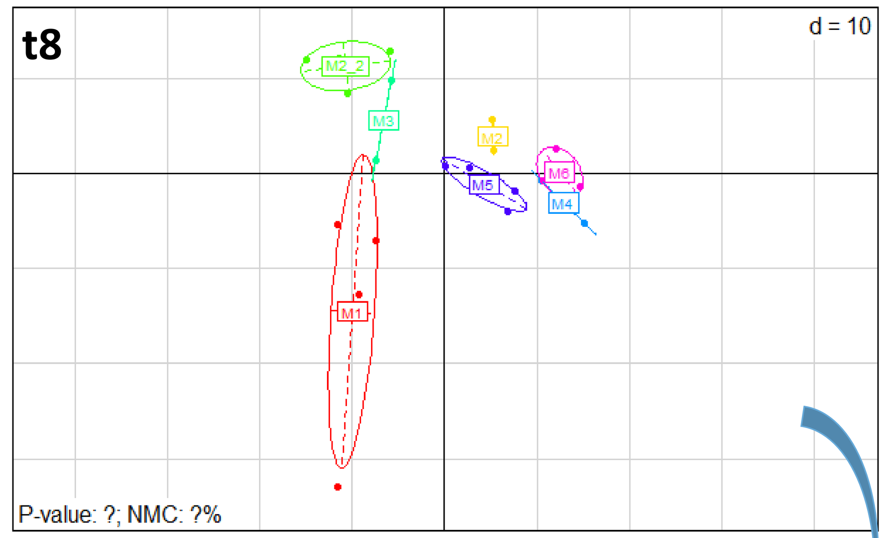
Chemical composition and drying methods



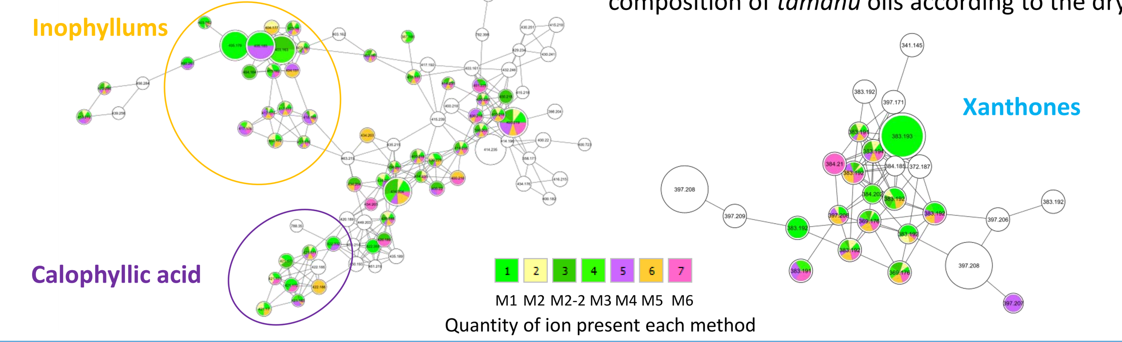
6 different drying methods tested in Taiohae, Nuku Hiva (September 2020):
M1: outdoor; M2: outdoor covered; M3: On ventilation (hot air); M4: in ventilated container; M5: in closed container (not ventilated); M6: the same as M3 but with cut nuts
M2-2: the same as M2 but harvest on january 2020 (dried seeds)

Main observations:

- No significant difference between methods?
- Similitude of chemical composition (3 clusters):
  - M1+M2-2+M3 (1)
  - M2+M5 (2)
  - M4+M6 (3)
- ↑ diversity of compounds in M1, M2-2 and M3
- Better yields for M3 and M5



PLS-DA score plot (discriminant analysis) on the chemical composition of *tamanu* oils according to the drying methods.



Conclusion and perspectives

Using a metabolomic approach combining LC-MS/MS analysis and molecular networking, the obtained data revealed differences in the metabolites chemical classes occurring in resulting oils (neoflavonoids, coumarins, xanthones and triterpenes) and provided the identification of precedently unreported constituents. **Inophyllums seemed to be more abundant in the beginning of drying process compared to calophyllic acid** after nine weeks. For the first time, our study provides new findings regarding the occurrence and evolution of the metabolites in *tamanu* nuts during the drying process. The developed method provided a powerful analytical tool aiming a better identification of bioactive components formed in *tamanu* nuts during drying process and will be helpful to control high quality oil for a natural active cosmetic ingredient. An efficient metabolomic approach was thus implemented to identify markers inducing variability in chemical composition during drying process of *tamanu* nuts and previously unidentified constituents. Thus, this set-up analytical method successfully applied in *C. inophyllum* could be used to the study of metabolites of a wide range of plant metabolites content.

Acknowledgements

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