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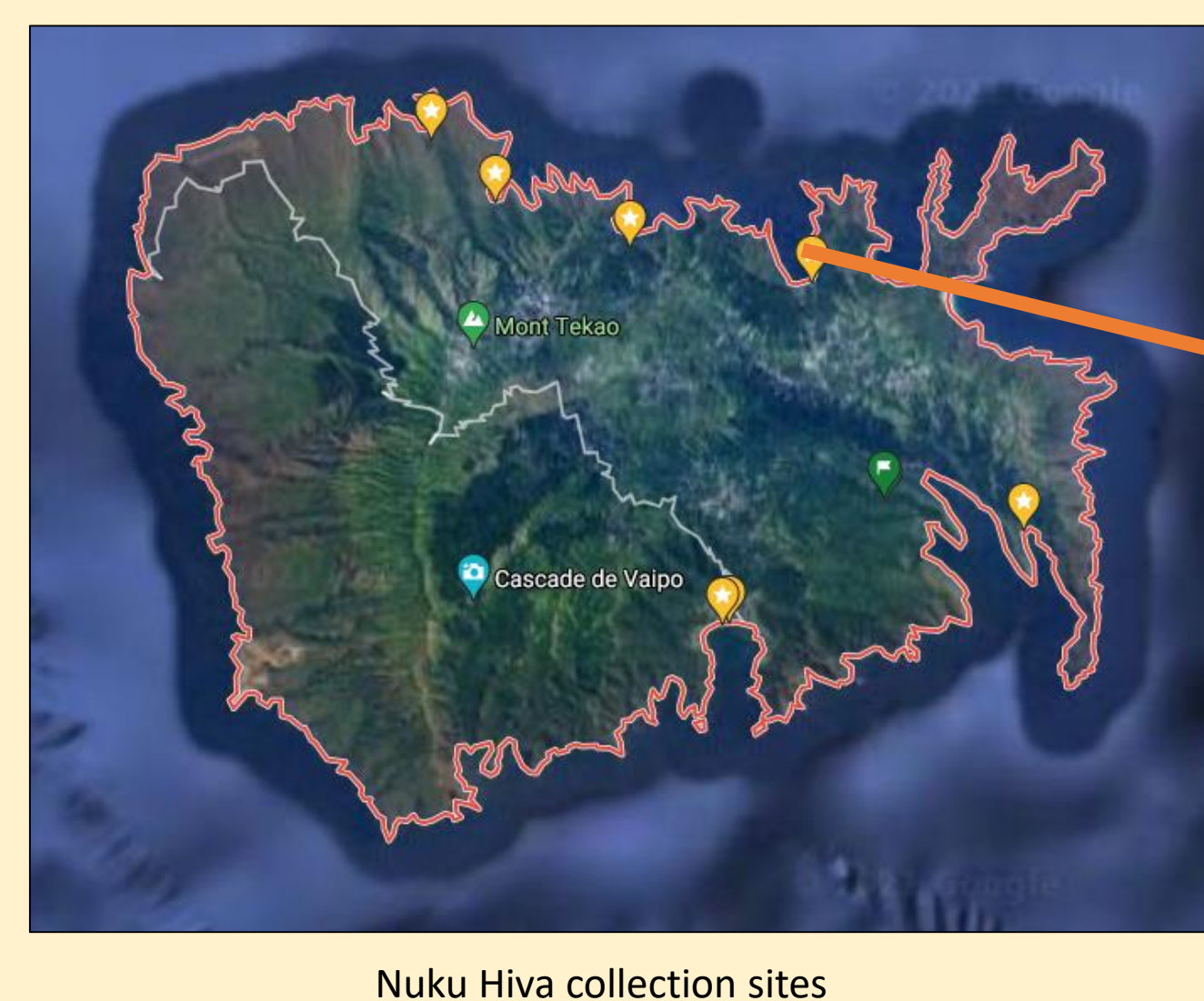
# Metabolomic approach using LC-MS/MS analysis and molecular networking to follow up bioactive constituents of *Calophyllum inophyllum* nuts during drying process

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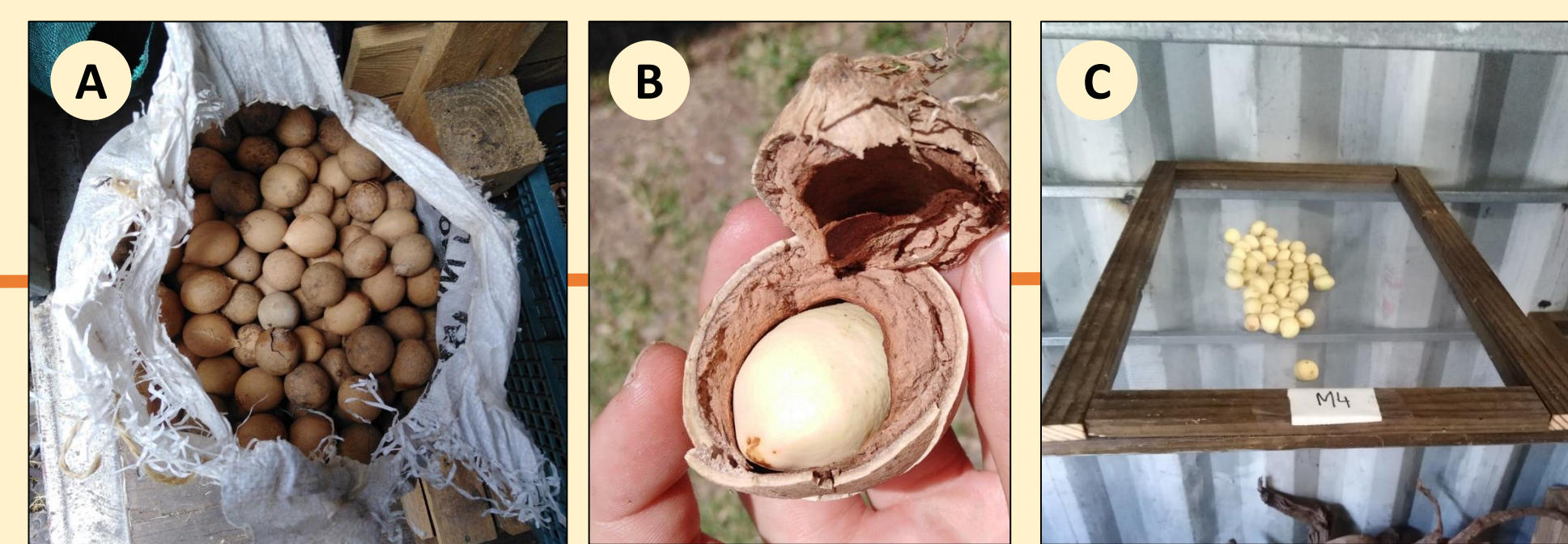
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## Strategy



### DRYING PROCESS



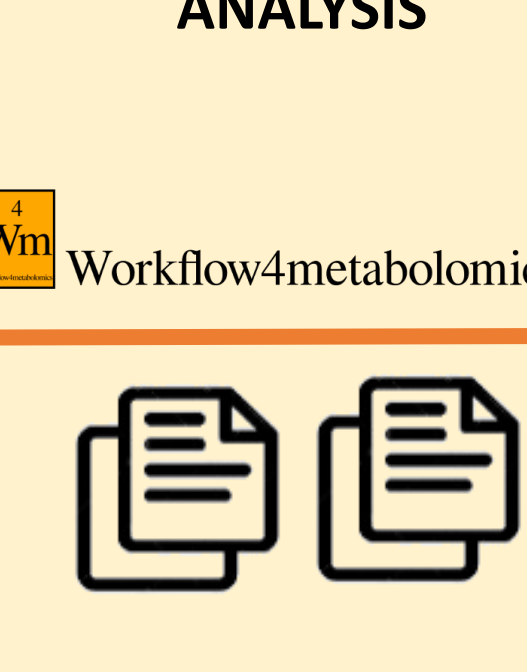
### PRESS EXTRACTION



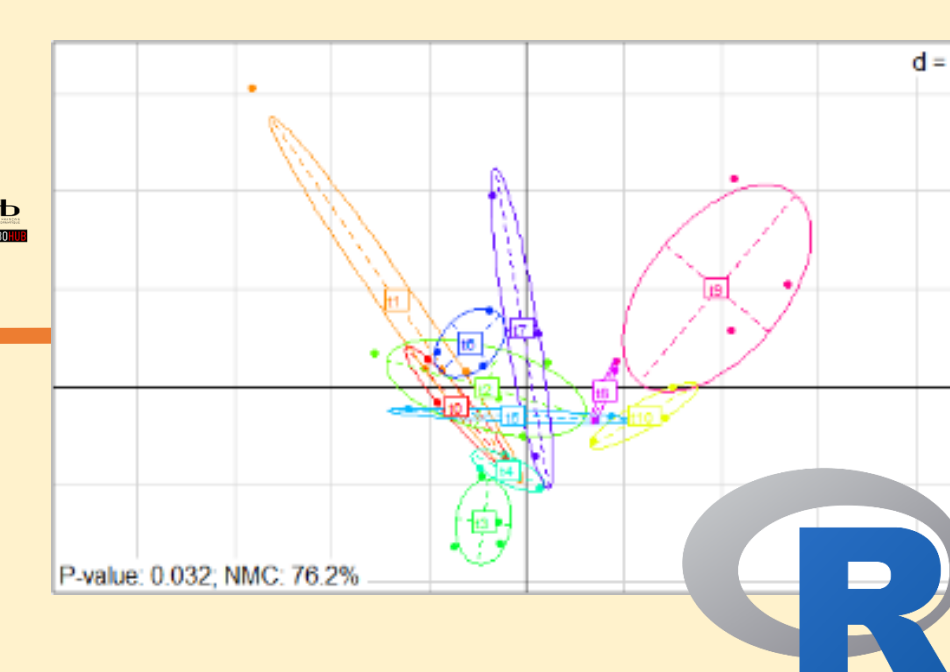
### LC-DAD and UPLC-MS/MS analysis



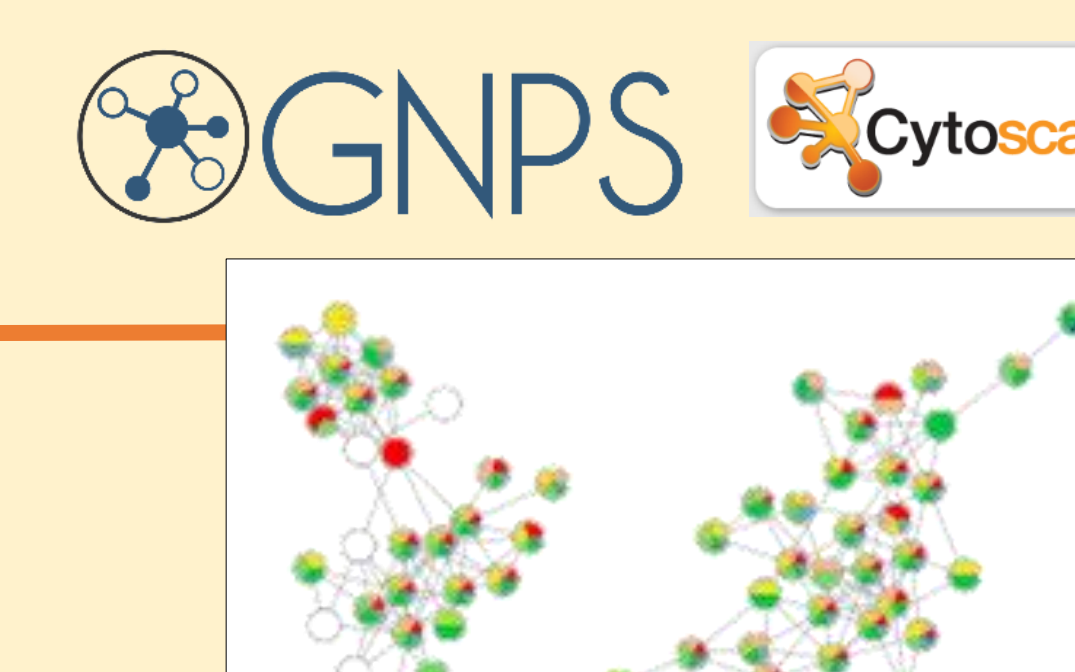
### PRE-PROCESSING ANALYSIS



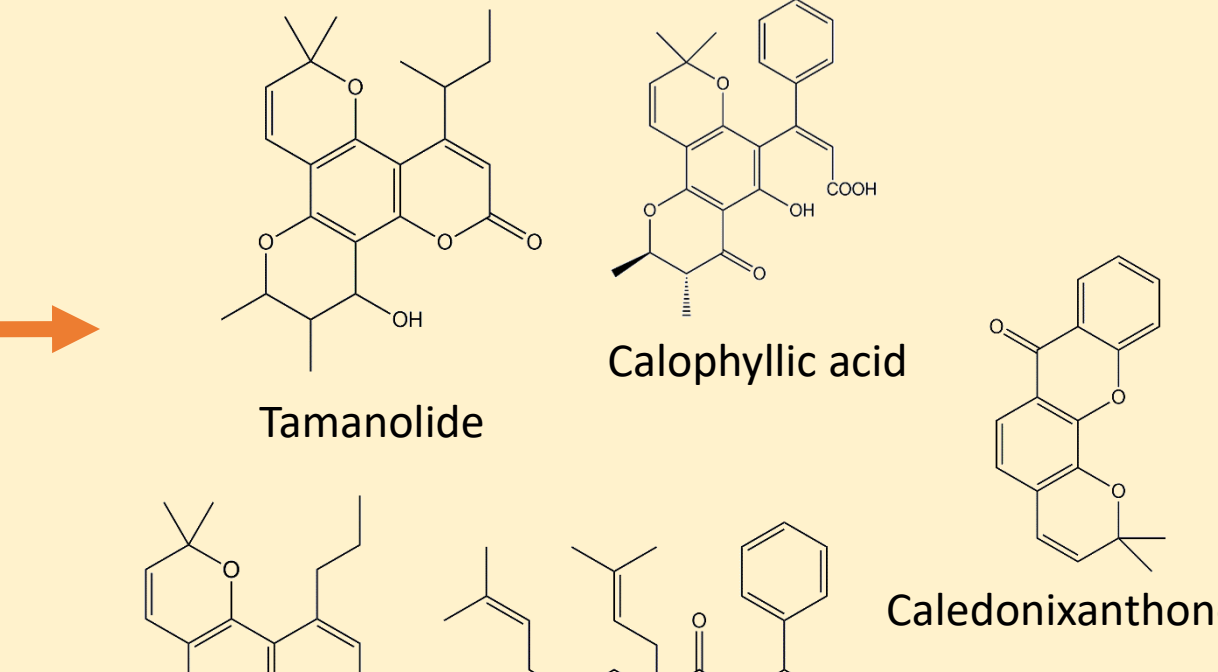
### DISCRIMINANT ANALYSIS



### MOLECULAR NETWORKS



### ANNOTATION AND IDENTIFICATION



## Context

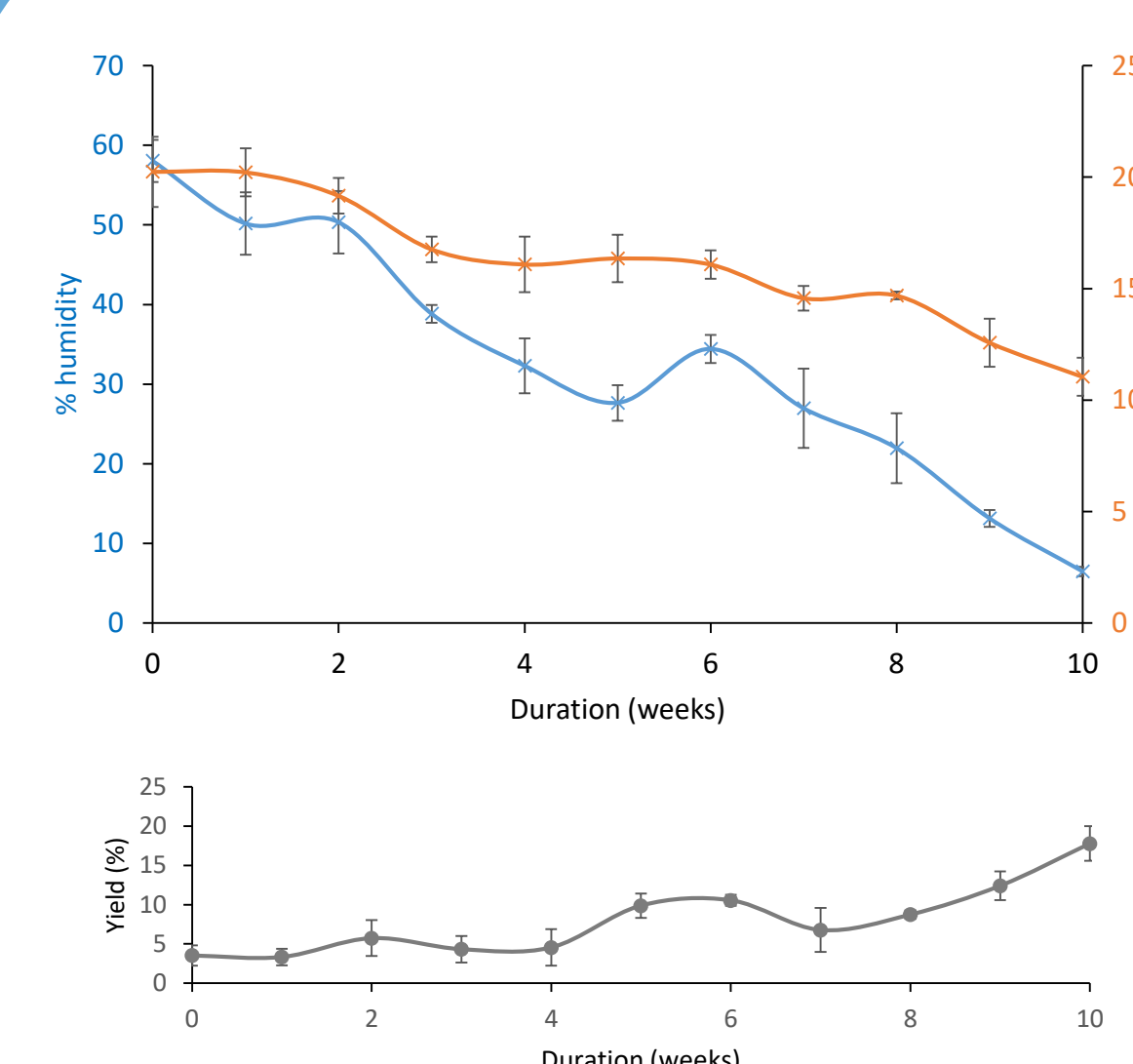
*Tamanu* oil, obtained from the nuts of *Calophyllum inophyllum* L. (Calophyllaceae), was traditionally used to cure various skin problems and ailments in French Polynesia [1]. Nuts and containing oil are also used for skin care [2]. They were reported to treat different kinds of skin affections and 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

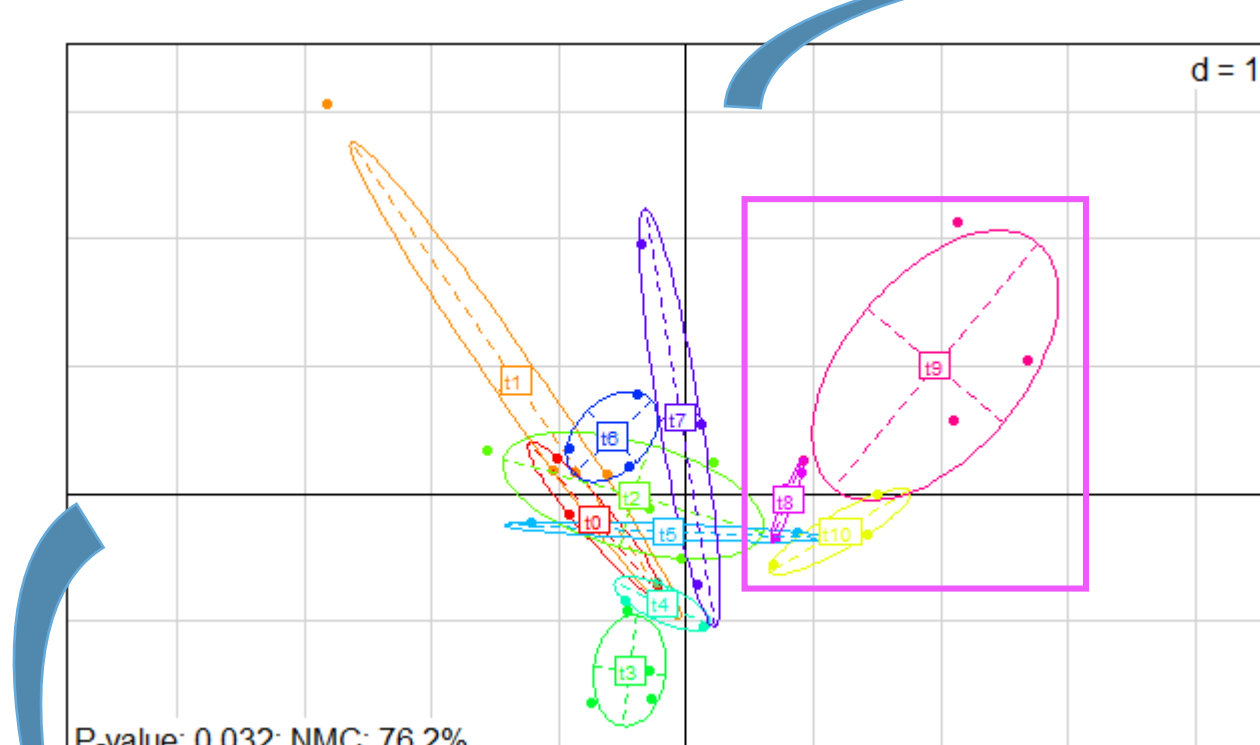


Evolution of the percentages of humidity, weight of the nuts and the oil yields of *tamanu* during the drying process from 0 to 10 weeks.

#### Main observations:

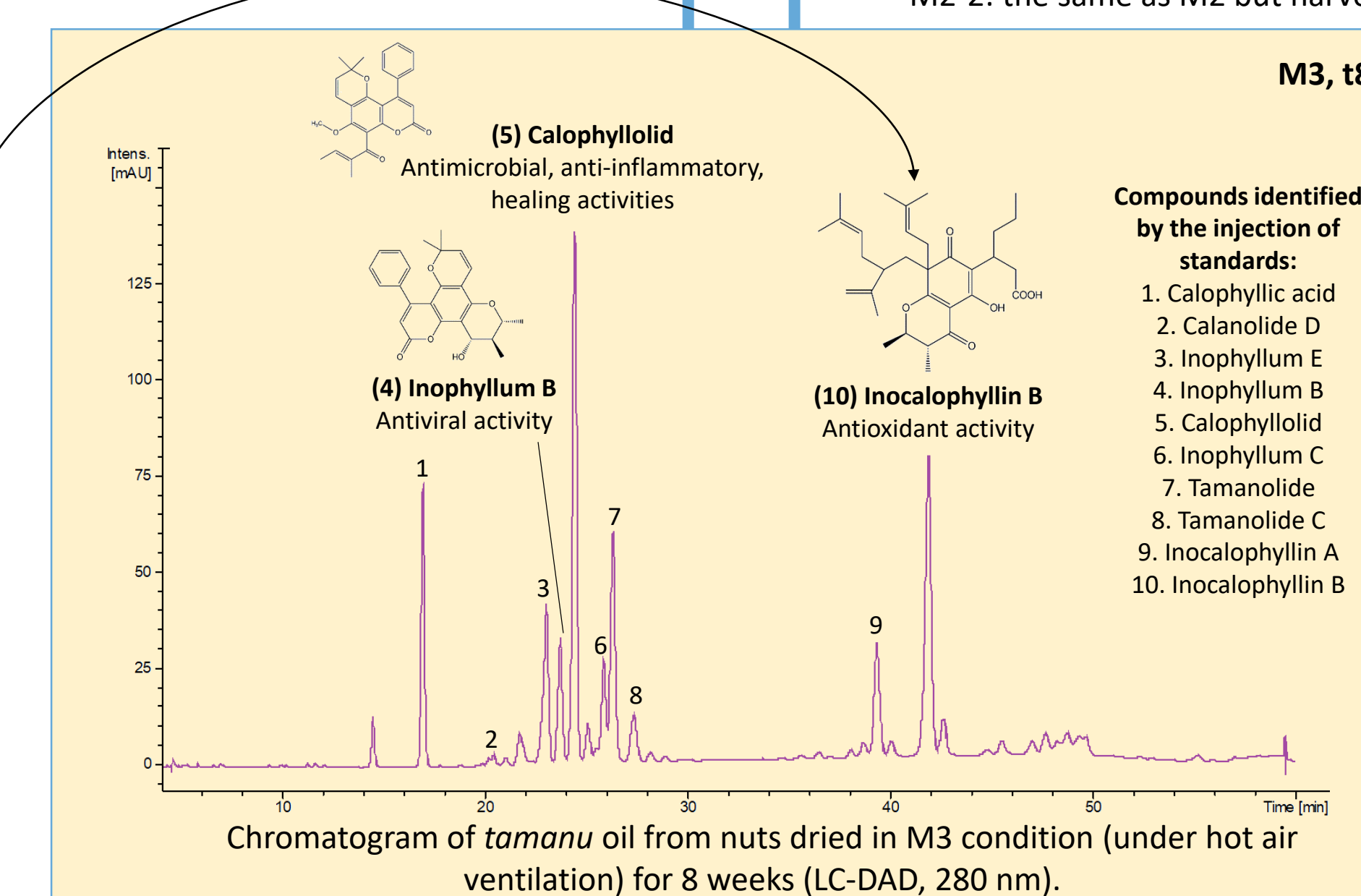
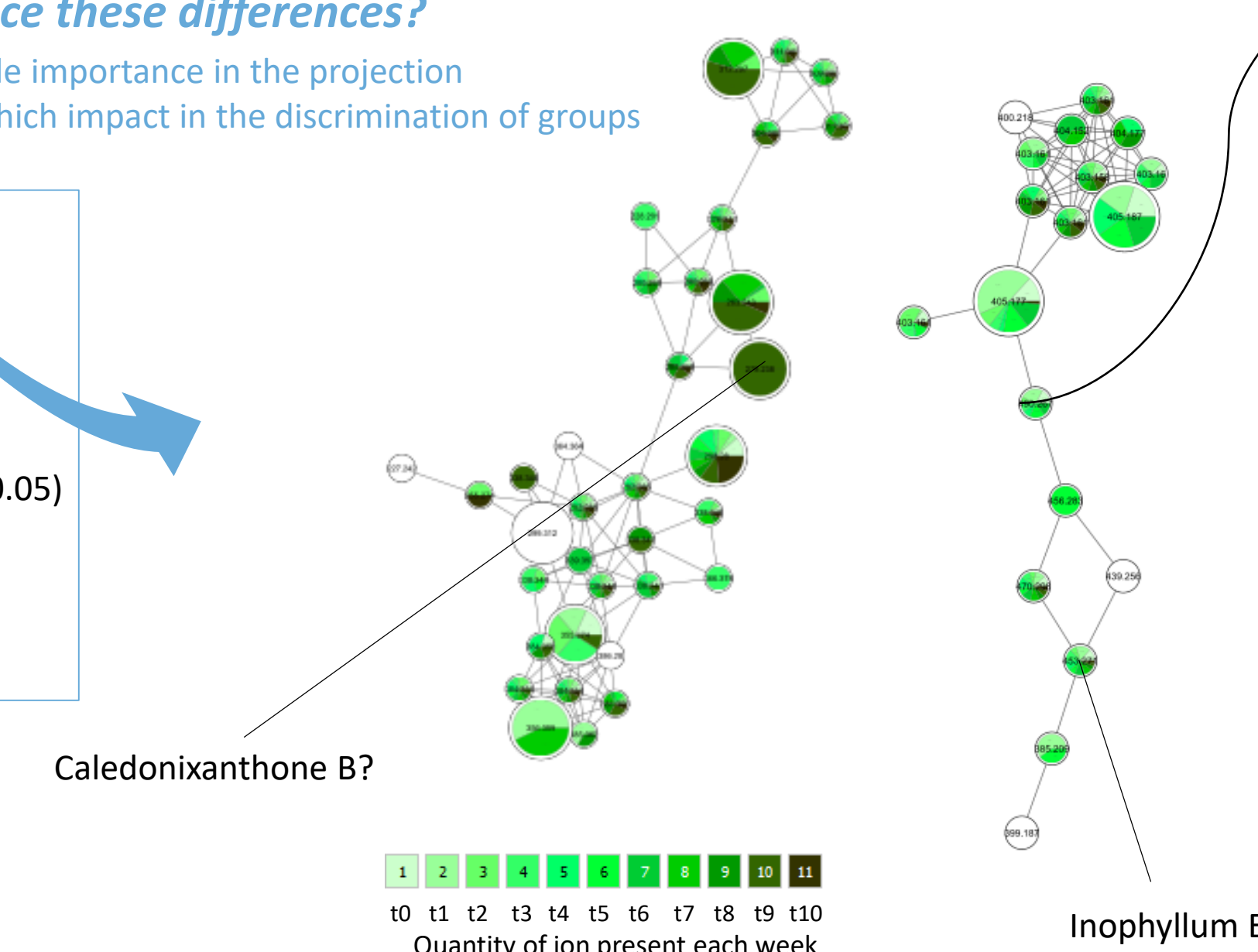
- Loss of nut mass of about 10% from t0 to t10 (from 20 to 11%)
- Decrease in moisture content from t0 to t10 (from 58% to about 6%)
- Increase of oil extraction yield (from 3.5% at t0 to 17.8% at t10)
- Different chemical compositions from t8 to t10 compared to the other durations (P-value < 0.05)
- ↑ 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



#### Which compounds may influence these differences?

VIP = Variable importance in the projection  
= m/z which impact in the discrimination of groups



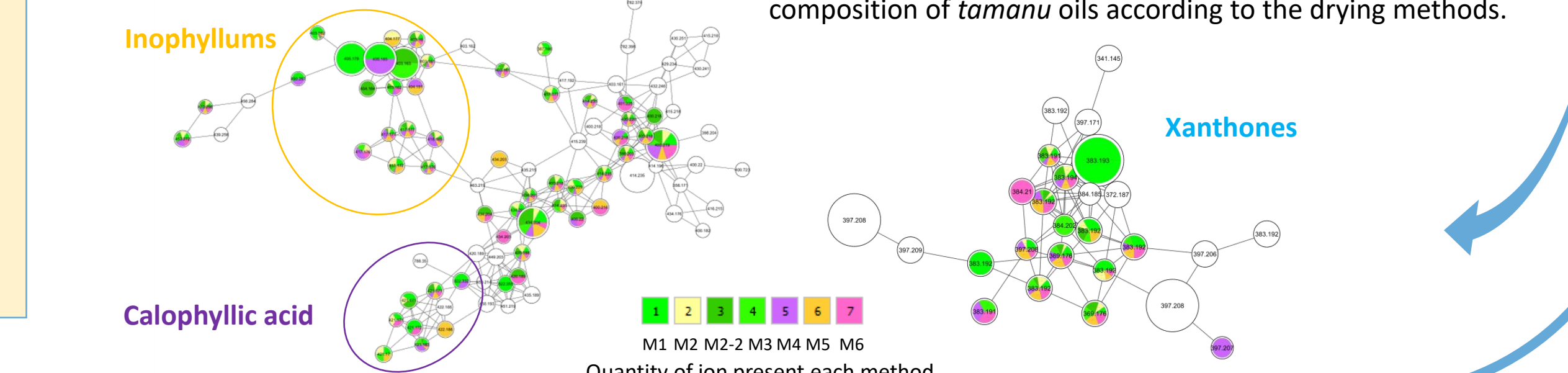
### Chemical composition and drying methods



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?
- Similarity 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



## 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, xanthenes 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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## References

- [1] Pétard P., Ed. Revue et augmentée HaerePo No Tahiti, 1986, 224-225.
- [2] Légoullier *et al.*, PlosOne, 2015, 10(9):e0138602, doi:10.1371/journal.pone.0138602.
- [3] Raharivelomanana *et al.*, OCL, 2018, 25(5), doi:10.1051/oc/2018048.