

## PhD PROPOSAL FOR THE DOCTORAL SCHOOL « Végétal, Animal, Aliment, Mer, Environnement »

### GENERAL INFORMATION

<p><b>Thesis title:</b></p> <p>Tannins and Oxidation in model-real matrix containing Protein, Interaction and Complexation monitoring.</p>
<p><b>Acronym of the project:</b> TOPIC-MIX</p>
<p><b>Disciplinary field 1:</b> analytical chemistry and reactivity</p> <p><b>Disciplinary field 2:</b> food science and chemistry</p>
<p><b>Three keywords:</b> proanthocyanidins, reactivity, proteins, metabolomics</p>
<p><b>Registration establishment:</b> ONIRIS</p>
<p><b>Research unit:</b> INRAE, UR1268 BIA</p>
<p><b>Name of the thesis director HDR (Accreditation to supervise research) required:</b> Guyot Sylvain</p> <p><b>Email address of the thesis director:</b> <a href="mailto:sylvain.guyot@inrae.fr">sylvain.guyot@inrae.fr</a></p> <p><b>Name of the thesis co-director (if applicable):</b> HDR (Accreditation to supervise research) required:</p> <p><b>Email address of the thesis co-director (if applicable):</b></p> <p><b>Name of the thesis co-supervisor 1 (if applicable):</b> Billet Kevin</p> <p><b>Email address of the thesis co-supervisor 1 (if applicable):</b> <a href="mailto:kevin.billet@inrae.fr">kevin.billet@inrae.fr</a></p> <p><b>Name of the thesis co-supervisor 2 (if applicable):</b></p> <p><b>Email address of the thesis co-supervisor 2 (if applicable):</b></p>
<p><b>Contact(s) (mailing address and E-mail):</b></p> <p>UR1268 BIA, 3 rue Yvette Cauchois, La Géraudière CS 71627 44 316 Nantes Cedex 3, France</p>
<p><input type="checkbox"/> <b>Doctoral school contest</b></p> <p><input checked="" type="checkbox"/> <b>Interview</b></p> <p><input type="checkbox"/> <b>Other (specify):</b></p>

COLLEGE	VEGETAL
DOCTORAL	ANIMAL, ALIMENT
PAYS DE LA LOIRE	MER, ENVIRONNEMENT

## SCIENTIFIC DESCRIPTION OF THE PhD PROJECT

### Socio-economic and scientific context: (10 lines)

Vegetal matrices have rising interest from the agri-food sector as promising alternatives for the food transition. To reach agroecological concerns, plant production in low input systems will rely on increase plant defense toward both a-/biotic stressors. Such situation will trigger increased polyphenols and pathogenesis related proteins content in plant products. The polyphenolic and especially tannins susceptibility to oxidation associated to the enriched proteic content will favored interaction establishment during transformation steps (flour, isolates...). Polyphenols oxidation lead to quinone formation that interact with proteins through covalent (irreversible) and non-covalent (reversible) bounding. Even if some polyphenol-protein interactions might benefit to technico-functional properties, it also can led to off-flavors. Despite their major effects on polyphenol-protein interactions, neoformed tannins structure remained poorly known.

### Assumptions and questions (8 lines)

What is the structure of newly formed tannins through oxidation process? Are the new structures dependent on the proanthocyanidin starting structure (extension unit nature, degree of polymerization...)? How do these structural changes resulting from oxidation of proanthocyanidin modify their ability to form complex/conjugates with proteins? What are the determinants of the interactions (non-/covalent) depending on the matrix composition and food processing steps ?

### The main steps of the thesis and scientific procedure (10-12 lines)

The first objective is the better understanding of the oxidation reaction on condensed tannins and thus the effect of the matrix composition (pH, temperature, pression...) on neoformed structures. The approach will be based on non-targeted LC-HRMS of vegetal matrix originated from agri-food transformation steps in the way to find oxidized tannin biomarkers. Proteins matrix differences (quality/quantity) will enabled to have a glimpse on the proteins impact on the proanthocyanidins reactivity towards oxidation. Then, a design of experiment will focus on proteins and non-/oxidized proanthocyanidins select according case of study (legume crops, astringency and salivary proteins, allergenicity, beverage fining...). This approach in model solution will provide a better understanding on mechanisms and determinants of polyphenols-proteins interactions and resulting structures through non-/covalent bounding. Finally, chemometrics analysis on model solutions will confirm biomarkers revealed through the LC-HRMS profiling of processed vegetal matrix.

### Methodological and technical approaches considered (4-6 lines)

- Non-targeted metabolomics matrix analysis: UHPLC-DAD-HRMS (Exploris 240, Orbitrap)
- Conduct and monitoring of polyphenols' oxidation in model solution
- Polyphenolic screening through both direct and depolymerization: UHPLC-DAD-MSn (LTQ-XL)
- Fluorescence spectroscopy, microcalorimetry, electrophoresis
- Chemometrics (PCA, (O)PLS-DA, multivariate tests)
- Proteomics

### Scientific and technical skills required by the candidate

Analytical chemistry and chemical reactivity knowledge and experiences as well as in vegetal biochemistry and properties in food. Desired experiences on separative and MS techniques on vegetal

matrix. Skills in high throughput data treatment and statistics. Ability to work in team and in project mode, writing and communication skills (English, willingness to perform/learn French).

## THESIS SUPERVISION

<b>Unit name:</b> INRAE, UR1268 BIA (Biopolymères Interactions et Assemblages)	<b>Team name:</b> PRP (Polyphénols, Réactivité & Procédés)
<b>Unit director name:</b> Bernard Cathala	<b>Team director name:</b> Sylvain Guyot
<b>Mailing address of the unit director:</b> INRAE, UR1268 BIA, 3 rue Yvette Cauchois, La Géraudière CS 71627 44316 Nantes Cedex 3 France email : <a href="mailto:bernard.cathala@inrae.fr">bernard.cathala@inrae.fr</a>	<b>Mailing address of the team director:</b> PRP Group – Polyphenols, Réactivité & Procédés UR1268 BIA – Biopolymers, Interactions & Assemblies INRAE, Bat. 305, Domaine de la Motte, 35653 Le Rheu Cedex email : <a href="mailto:sylvain.guyot@inrae.fr">sylvain.guyot@inrae.fr</a>
<b>Thesis director</b> Surname, first name: Guyot Sylvain Position: Research Director Obtained date of the HDR (Accreditation to supervise research): 2006 Employer: INRAE Doctoral school affiliation: VAAME Rate of thesis supervision in the present project (%): 50% Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):50% Number of current thesis supervisions/co-supervisions: 1	
<b>Thesis co-director</b> Surname, first name: Position: Obtained date of the HDR (Accreditation to supervise research):	

<p>Employer:</p> <p>Doctoral school affiliation:</p> <p>Rate of thesis supervision in the present project (%):</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):</p> <p>Number of current thesis supervisions/co-supervisions:</p>
<p><b>Thesis co-supervisor 1 (if applicable)</b></p> <p>Surname, first name: Billet Kevin</p> <p>Position: Research Associate</p> <p>Accreditation to supervise research <input type="checkbox"/> yes <input checked="" type="checkbox"/> no If yes, date diploma received:</p> <p>Employer: INRAE</p> <p>Doctoral school affiliation: VAAME</p> <p>Rate of thesis supervision in the present project (%): 50%</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%): 50%</p> <p>Number of current thesis supervisions/co-supervisions: 1</p>
<p><b>Thesis co-supervisor 2 (if applicable)</b></p> <p>Surname, first name:</p> <p>Position:</p> <p>Accreditation to supervise research <input type="checkbox"/> yes <input type="checkbox"/> no If yes, date diploma received:</p> <p>Employer:</p> <p>Doctoral school affiliation:</p> <p>Rate of thesis supervision in the present project (%):</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):</p> <p>Number of current thesis supervisions/co-supervisions:</p>
<p><b>Private partner (if CIFRE funding, private funding...)</b></p> <p>Surname, first name:</p> <p>Position:</p> <p>Employer:</p> <p>Rate of thesis supervision in the present project (%):</p> <p>Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):</p> <p>Number of current thesis supervisions/co-supervisions:</p>

**International partner (if Cotutelle thesis)**

Surname, first name:

Position:

Employer:

Rate of thesis supervision in the present project (%):

Total rate of thesis supervision in ongoing theses (supervisions and co-supervisions) (%):

Number of current thesis supervisions/co-supervisions:

**Professional status of previous PhD students supervised by both director and co-supervisors (from 5 years)**

*Please provide the following information for each PhD students supervised*

Surname, first name: CASTILLO-FRAIRE, Claudia-Mariana

Date of PhD beginning and PhD defence: 10/2016 – 05/2020

Thesis supervision: Sylvain GUYOT 50%, Pascal POUPARD 50%

Professional status and location: looking for a job, Bretagne

Contract profile (post-doc, fixed-term, permanent): 2021-2022 : Temporary Assistant Professor, AgroParis Tech

List of publications from the thesis work:

Castillo-Fraire, C., Poupard, P., Guilois-Dubois, S., Le Quere, J., Salas, E., Guyot, S., 2023. Detailed LC-UV-MS quantification of native and oxidized phenolic compounds in experimental and commercial apple juices revealed highly contrasting compositions. JOURNAL OF FOOD COMPOSITION AND ANALYSIS 122. <https://doi.org/10.1016/j.jfca.2023.105450>

Castillo-Fraire, C., Pottier, S., Bondon, A., Salas, E., Bernillon, S., Guyot, S., Poupard, P., 2022. NMR structural elucidation of dehydrodimers resulting from oxidation of 5-O-caffeoylquinic acid in an apple juice model solution. FOOD CHEMISTRY 372. <https://doi.org/10.1016/j.foodchem.2021.131117>

Castillo-Fraire, C., Branda, E., Poupard, P., Le Quere, J., Salas, E., de Freitas, V., Guyot, S., Soares, S., 2021. Interactions between polyphenol oxidation products and salivary proteins: Specific affinity of CQA dehydrodimers with cystatins and P-B peptide. FOOD CHEMISTRY 343. <https://doi.org/10.1016/j.foodchem.2020.128496>

Castillo-Fraire, C., Poupard, P., Guilois-Dubois, S., Salas, E., Guyot, S., 2019. Preparative fractionation of 5'-O-caffeoylquinic acid oxidation products using centrifugal partition chromatography and their investigation by mass spectrometry. JOURNAL OF CHROMATOGRAPHY A 1592, 19–30. <https://doi.org/10.1016/j.chroma.2019.01.071>

**Five main recent publications of the supervisors on thesis subject:**

Castillo-Fraire, C., Poupard, P., Guilois-Dubois, S., Le Quere, J., Salas, E., Guyot, S., 2023. Detailed LC-UV-MS quantification of native and oxidized phenolic compounds in experimental and

commercial apple juices revealed highly contrasting compositions. JOURNAL OF FOOD COMPOSITION AND ANALYSIS 122. <https://doi.org/10.1016/j.jfca.2023.105450>

Balaj, G., Tamanai-Shacoori, Z., Olivier-Jimenez, D., Sauvager, A., Faustin, M., Bousarghin, L., Gall, S., Guyot, S., Nebija, D., Tomasi, S., Abasq, M., 2022. An insight into an intriguing oxidative biotransformation pathway of 5-O-caffeoylquinic acid by a gut bacterium. FOOD & FUNCTION 13, 6195–6204. <https://doi.org/10.1039/d1fo04304h>

Rinaldo, D., Sotin, H., Petro, D., Le-Bail, G., Guyot, S., 2022. Browning susceptibility of new hybrids of yam (*Dioscorea alata*) as related to their total phenolic content and their phenolic profile determined using LC-UV-MS. LWT-FOOD SCIENCE AND TECHNOLOGY 162. <https://doi.org/10.1016/j.lwt.2022.113410>

Morzel, M., Canon, F., Guyot, S., 2022. Interactions between Salivary Proteins and Dietary Polyphenols: Potential Consequences on Gastrointestinal Digestive Events. JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY 70, 6317–6327. <https://doi.org/10.1021/acs.jafc.2c01183>

Billet, K., Salvador-Blanes, S., Dugé De Bernonville, T., Delanoue, G., Hirschberger, F., Oudin, A., ... & Lanoue, A. (2023). Terroir Influence on Polyphenol Metabolism from Grape Canes: A Spatial Metabolomic Study at Parcel Scale. Molecules, 28(11), 4555. <https://doi.org/10.3390/molecules28114555>

Castillo-Fraire, C., Pottier, S., Bondon, A., Salas, E., Bernillon, S., Guyot, S., Poupard, P., 2022. NMR structural elucidation of dehydrodimers resulting from oxidation of 5-O-caffeoylquinic acid in an apple juice model solution. FOOD CHEMISTRY 372. <https://doi.org/10.1016/j.foodchem.2021.131117>

Ferrier, M., Billet, K., Drouet, S., Tungmunnithum, D., Malinowska, M. A., Marchal, C., ... & Lanoue, A. (2022). Identifying Major Drivers of Antioxidant Activities in Complex Polyphenol Mixtures from Grape Canes. Molecules, 27(13), 4029. <https://doi.org/10.3390/molecules27134029>

Castillo-Fraire, C., Branda, E., Poupard, P., Le Quère, J., Salas, E., de Freitas, V., Guyot, S., Soares, S., 2021. Interactions between polyphenol oxidation products and salivary proteins: Specific affinity of CQA dehydrodimers with cystatins and P-B peptide. FOOD CHEMISTRY 343. <https://doi.org/10.1016/j.foodchem.2020.128496>

Guilois-Dubois, S., Guyot, S., Poupard, P., 2021. Preparative isolation of apple flavan-3-ols monomers and oligomers using pH-zone-refining centrifugal partition chromatography combined with reversed-phase liquid chromatography. JOURNAL OF CHROMATOGRAPHY A 1653. <https://doi.org/10.1016/j.chroma.2021.462382>

Liu, X., Le Bourvellec, C., Guyot, S., Renard, C., 2021. Reactivity of flavanols: Their fate in physical food processing and recent advances in their analysis by depolymerization. *COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY* 20, 4841–4880. <https://doi.org/10.1111/1541-4337.12797>

Billet, K., Unlubayir, M., Munsch, T., Malinowska, M. A., de Bernonville, T. D., Oudin, A., ... & Lanoue, A. (2021). Postharvest treatment of wood biomass from a large collection of European grape varieties: Impact on the selection of polyphenol-rich byproducts. *ACS Sustainable Chemistry & Engineering*, 9(9), 3509-3517. <https://doi.org/10.1021/acssuschemeng.0c07875>

Bose, S., Munsch, T., Lanoue, A., Garros, L., Tungmunnithum, D., Messaili, S., Billet, K., ... & Giglioli-Guivarc'h, N. (2020). UPLC-HRMS analysis revealed the differential accumulation of antioxidant and anti-aging lignans and neolignans in in vitro cultures of *Linum usitatissimum* L. *Frontiers in Plant Science*, 11, 508658. <https://doi.org/10.3389/fpls.2020.508658>

Anna Malinowska, M., Billet, K., Drouet, S., Munsch, T., Unlubayir, M., Tungmunnithum, D., ... & Lanoue, A. (2020). Grape cane extracts as multifunctional rejuvenating cosmetic ingredient: Evaluation of sirtuin activity, tyrosinase inhibition and bioavailability potential. *Molecules*, 25(9), 2203. <https://doi.org/10.3390/molecules25092203>

Millet, M., Poupard, P., Guilois-Dubois, S., Poiraud, A., Fanuel, M., Rogniaux, H., Guyot, S., 2020. Heat-unstable apple pathogenesis-related proteins alone or interacting with polyphenols contribute to haze formation in clear apple juice. *FOOD CHEMISTRY* 309. <https://doi.org/10.1016/j.foodchem.2019.125636>

Castillo-Fraire, C., Poupard, P., Guilois-Dubois, S., Salas, E., Guyot, S., 2019. Preparative fractionation of 5'-O-caffeoylquinic acid oxidation products using centrifugal partition chromatography and their investigation by mass spectrometry. *JOURNAL OF CHROMATOGRAPHY A* 1592, 19–30. <https://doi.org/10.1016/j.chroma.2019.01.071>

Millet, M., Poupard, P., Guilois-Dubois, S., Zanchi, D., Guyot, S., 2019. Self-aggregation of oxidized procyanidins contributes to the formation of heat-reversible haze in apple-based liqueur wine. *FOOD CHEMISTRY* 276, 797–805. <https://doi.org/10.1016/j.foodchem.2018.09.171>



## THESIS FUNDING

<b>Origin(s) of the thesis funding:</b> 50% INRAE + 50% Pays de la Loire region
<b>Gross monthly salary:</b> 2044€
<b>Thesis funding state:</b> Acquired
<b>Funding beginning date/duration of the thesis funding:</b> 01/09/2024 or 01/10/2024 (36 months)

**Date:** 22/03/2024

**Name, signature of unit director:**

Bernard Cathala



**Name, signature of team director:**

Sylvain Guyot



**Name, signature of thesis project director:**

Sylvain Guyot



**Documents to be send to:** [ed-vaame@doctorat-paysdelaloire.fr](mailto:ed-vaame@doctorat-paysdelaloire.fr)