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Dottorato in Scienze e Tecnologie Agrarie, Ambientali e Alimentari

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Valorisation of alternative protein sources by tailored biotechnological processes and non-thermal technologies to obtain new ingredients to be used in the formulation of innovative foods

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: I
Tutor: Rosalba Lanciotti; Co-tutor: Francesca Patrignani, Davide Gottardi

1. Curriculum

Solidea Amadei è nata a Faenza (RA) il 12/03/1998 ed è cittadina italiana. Da marzo 2023 è dottoranda in Scienze e Tecnologie Agrarie, Ambientali e Alimentari presso il Dipartimento di Scienze e Tecnologie Agro-Alimentari dell'Università di Bologna, sede di Cesena, nell'ambito della tematica Food Science and Biotechnology. A dicembre 2022 si è laureata al corso magistrale in Scienze e Tecnologie Alimentari con votazione 110/110 e lode, presso l'Alma Mater Studiorum, Università di Bologna, sede di Cesena, con una tesi sperimentale in Laboratorio di microbiologia applicata sviluppata nell'ambito del progetto europeo *NewTechAqua*, dal titolo "Valorizzazione di scarti ittici, mediante processi biotecnologici, per la produzione di ingredienti innovativi". Durante il periodo di studio magistrale ha svolto un semestre all'estero finanziato dal programma Erasmus+, presso l'Universidade Catolica Portuguesa (Porto, Portogallo) in cui ha frequentato corsi e sostenuto esami di Microbiologia industriale, Microrganismi e sicurezza alimentare e Progetto di ingegneria e innovazione alimentare. A luglio 2020 si è laureata al corso triennale in Tecnologie Alimentari con votazione 110/110 e lode, conseguita presso l'Alma Mater Studiorum, Università di Bologna, sede di Cesena, con una tesi compilativa in Ispezione ed igiene degli alimenti di origine animale, dal titolo "*Listeria monocytogenes* nei formaggi: elementi di valutazione del rischio per i consumatori vulnerabili".

2. Stato dell'arte

A causa del progressivo aumento demografico mondiale risulta necessario trovare nuove fonti di cibo e sviluppare nuove tecniche di valorizzazione delle risorse esistenti. Nel 2021 il consumo mondiale di proteine di origine animale si è attestato attorno a 478 milioni di tonnellate, con differenze che dipendono principalmente dall'area geografica, tradizioni e prezzi. Secondo la FAO, i quantitativi di fonti proteiche di origine animale prodotte nel mondo non aumenteranno nei prossimi dieci anni e si prevede che le quantità consumate pro-capite rimarranno pressoché costanti. Tuttavia, la domanda del mercato aumenterà, principalmente a causa dell'incremento della popolazione che necessita di queste risorse (FAO, 2021). Perciò, l'ottenimento e la valorizzazione di proteine ottenute da fonti alternative sta suscitando sempre più interesse, sia da parte dell'industria che della ricerca. Esempi di nuove fonti proteiche si possono ottenere dai vegetali come legumi, cereali e pseudocereali o scarti e sottoprodotti vegetali provenienti dall'industria alimentare e mangimistica (Molfetta et al., 2022). Questi ingredienti vengono opportunamente funzionalizzati mediante approcci biotecnologici basati su microrganismi per i quali l'Autorità Europea per la Sicurezza Alimentare (EFSA) ha riconosciuto la qualificata presunzione di sicurezza (QPS) e identificati come GRAS (Generally Recognized as Safe) dalla Food and Drug Administration (FDA), o processi non termici quali le alte pressioni di omogeneizzazione (HPH) e i campi elettrici pulsati che permettono di estrarre e valorizzare efficacemente proteine di alta qualità. Per esempio, batteri appartenenti al genere *Bacillus* sono stati utilizzati per valorizzare scarti vegetali per ottenere composti bioattivi; lieviti come *Saccharomyces cerevisiae* e batteri lattici sono stati utilizzati per fermentare gli scarti dell'industria di trasformazione di cereali, frutta, verdura e legumi, incrementando significativamente la quantità di peptidi funzionali nei prodotti estratti. Recentemente molti studi si stanno focalizzando anche sull'utilizzo di lieviti non convenzionali come *Debaryomyces* spp., *Kluyveromyces marxianus* e *Yarrowia lipolytica* per valorizzare scarti e sottoprodotti dell'industria agro-alimentare (Gottardi et al., 2021). Ad esempio, *Y. lipolytica* è stata impiegata per valorizzare un'altra fonte proteica alternativa come le farine di insetti (Molfetta et al., 2022). Infatti, a seguito della crescita di tale lievito sulla farina di grillo, si sono ottenuti idrolizzati proteici, con aumentata funzionalità e maggiore contenuto proteico/peptidico, utilizzati anche come ingredienti nella produzione di prodotti da forno (Patrignani et al., 2020; Rossi et al., 2022; Rossi et al., 2021). In generale, i processi biotecnologici permettono di ottenere composti a partire da scarti e sottoprodotti e da fonti proteiche alternative che dimostrano migliore attività antiossidante, antiipertensiva, antimicrobica, conservante e aromatica rispetto a quelli ottenuti con altre tecniche di valorizzazione. Pertanto, le proteine e i sottoprodotti opportunamente funzionalizzati possono essere riutilizzati nelle formulazioni di alimenti tradizionali e/o innovativi, in linea con gli obiettivi odierni di sostenibilità ed economia circolare. EFSA ha già riconosciuto la sicurezza di alcune proteine alternative derivanti da fonti vegetali e da scarti e sottoprodotti vegetali fermentati, identificate come novel food ai sensi del regolamento (UE) 2015/2283 e utilizzabili come ingredienti innovativi in prodotti alimentari.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di mettere a punto ingredienti, a partire da fonti proteiche alternative, proteine vegetali e/o scarti e sottoprodotti dell'industria alimentare opportunamente funzionalizzati mediante processi biotecnologici basati su microrganismi sicuri e riconosciuti da EFSA come QPS e trattamenti non termici, e di utilizzarli in formulazioni di alimenti innovativi. Il progetto di tesi di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt riportato in tabella 1:

A1) Ricerca bibliografica inerente all'argomento, funzionale per l'individuazione delle matrici di partenza e dei microrganismi potenzialmente utilizzabili, tra cui lieviti non convenzionali, Bacilli e batteri lattici.

A2) Caratterizzazione dei ceppi individuati ed ottimizzazione delle performance microbiche attraverso la valutazione delle caratteristiche tecnologiche, funzionali e di sicurezza dei microrganismi, al fine di scegliere i più interessanti per le loro attività enzimatiche e metaboliche.

A3) Caratterizzazione delle matrici di interesse dal punto di vista microbiologico, nutrizionale e di sicurezza.

A4) Ottenimento e caratterizzazione degli ingredienti a partire dalle matrici selezionate e l'utilizzo dei microrganismi più performanti: individuazione delle condizioni di processo più opportune per la messa a punto dei processi biotecnologici sulle matrici selezionate (tal quali o trattate non termicamente), caratterizzazione degli ingredienti innovativi da un punto di vista di sicurezza, valore nutrizionale e stabilità, loro inquadramento normativo secondo quanto stabilito da EFSA e messa a punto di protocolli *tailor-made* per la loro produzione su ampia scala.

A5) Sviluppo di prodotti tradizionali o innovativi utilizzando gli ingredienti più promettenti e caratterizzazione per valutare la loro sicurezza, *shelf-life* microbiologica, qualità, valore nutrizionale e funzionalità.

A6) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazione orale.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Ricerca bibliografica inerente all'argomento***																		
	1) Identificazione delle fonti proteiche alternative																		
	2) Identificazione di microrganismi sicuri e con QPS																		
A2)	***Caratterizzazione dei ceppi ed ottimizzazione delle performance microbiche***																		
	1) Valutazione delle caratteristiche tecnologiche, funzionali e di sicurezza dei ceppi microbici individuati, selezione di ceppi microbici ed ottimizzazione delle performance fermentative e tecnologiche																		
A3)	***Caratterizzazione delle matrici di interesse***																		
	1) Valutazione delle caratteristiche microbiologiche, nutrizionali e di sicurezza delle principali fonti proteiche alternative selezionate																		
A4)	***Ottenimento e caratterizzazione degli ingredienti***																		
	1)Allestimento di processi biotecnologici ed ottimizzazione delle condizioni di processo (tempo, T, livello di inoculo)																		
	2) Valutazione delle caratteristiche nutrizionali, di stabilità e sicurezza degli ingredienti ottenuti																		
	3) Inquadramento normativo																		
A5)	***Sviluppo di prodotti tradizionali o innovativi***																		
	1) Formulazione di alimenti tradizionali/innovativi includenti gli ingredienti precedentemente selezionati																		
	2) Caratterizzazione sugli aspetti di sicurezza, shelf-life microbiologica, qualità, valore nutrizionale e funzionalità																		
A6)	***Preparazione della tesi e di articoli***																		

4. Bibliografia

FAO (2021). Meat. OECD-FAO Agricultural Outlook 2021–2030, 163-177.

Gottardi D, Siroli L, Vannini L, Patrignani F, Lanciotti R (2021) Recovery and valorization of agri-food wastes and by-products using the non-conventional yeast Yarrowia lipolytica, Trends in Food Science & Technology, 115, 74-86.

Molfetta M, Morais EG, Barreira L, Bruno GL, Porcelli F, Dugat-Bony E, Minervini F (2022) Protein sources alternative to meat: State of the art and involvement of fermentation, Foods, 11(14), 2065.

Patrignani F, Parrotta L, Del Duca S, Vannini L, Camprini L, Dalla Rosa M, ... & Lanciotti R (2020) Potential of Yarrowia lipolytica and Debaryomyces hansenii strains to produce high quality food ingredients based on cricket powder, LWT, 119, 108866.

Rossi S, Parrotta L, Gottardi D, Glicerina VT, Duca SD, Dalla Rosa MD, ... & Lanciotti R (2022) Unravelling the potential of cricket-based hydrolysed sourdough on the quality of an innovative bakery product, Journal of Insects as Food and Feed, 1-16.

Rossi S, Parrotta L, Del Duca S, Dalla Rosa M, Patrignani F, Schluter O, Lanciotti R (2021) Effect of Yarrowia lipolytica RO25 cricket-based hydrolysates on sourdough quality parameters, LWT, 148, 111760.

Use of non-thermal treatments to improve the quality, safety, and shelf-life of products of animal origin

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1. Curriculum

Chiara Angelucci, born in 1998, is a PhD student (XXXVIII cycle) at the University of Bologna,
In July 2020, she obtained a bachelor's degree in "Food technology" with a degree of 110/110 cum laude at the *Alma Mater Studiorum* - University of Bologna - Cesena Campus with an experimental thesis in animal production. Her thesis had the aim of evaluating the technological and functional properties of poultry meat treated with Pulsed Electric Fields.
In July 2022, she obtained a master's degree in "Food and science Technology" with a degree of 110/110 cum laude, at the *Alma Mater Studiorum* - University of Bologna - Cesena Campus. Her experimental thesis, in collaboration with Amadori group, was aimed at comparing the qualitative characteristics and technological properties of meat from chickens belonging to different genotype.
In November 2022, she has got a PhD scholarship in "Agricultural, Environmental and Food Science and Technology" at the Department of Agricultural and Food Sciences (DISTAL) of the University of Bologna, in the research topic of Food Science and Biotechnology. Her current research is focused on the use non-thermal treatments to improve the quality, safety, and shelf-life of animal food products.

2. State-of-the-art

The market requires more and more natural products, less treated to safeguard the perceived safety, nutritional quality, and organoleptic aspects of products of animal origin. In fact, the heat treatments applied to food can cause chemico-physical, nutritional, and sensory changes that reduce the quality of the product. For this reason, the goal of the processing technologies is to be mild for foods especially with respect to their nutritional value while diminishing any pathogenic and spoilage risk or any quality deterioration (*Valdramidis & Koutsoumanis*, 2016). To obtain a safe food matrix, the most important challenge is to avoid the presence of pathogenic microorganisms. Among them, Salmonella spp., *Listeria monocytogenes* and *Escherichia coli* are the most involved pathogens in foodborne outbreaks, while *Pseudomonas aeruginosa*, *Staphylococcus sp*., *Brochothrix thermosphacta*, *Psychrobacter sp*., *Micrococcus* sp., lactic acid bacteria (LAB) and several strains of *Enterobacteriaceae* are the most reported microorganisms in the spoilage process (*Rosario et al*., 2021). Among the non-thermal technologies for the inactivation of microorganisms and the prolongation of the shelf-life of foods the high hydrostatic pressures (HHP) are studied. During HHP process, the matrix is subjected to pressures from 100 to 1,000 MPa in an aqueous medium at room temperature. The treatment can improve the microbiological quality of the products, promoting microbial inactivation by minimizing nutritional and sensory changes of the matrix as commercial applications generate a non-significant increase in the temperature of the treated food (*Aymerich et al*., 2008).
In addition to the application of non-thermal technologies for the treatment of products of animal origin, other strategies can be adopted, such as the application of natural antimicrobials (essential oils or their components, phenolic extracts, etc.), the use of bioprotective cultures and metabolites produced by them (bacteriocins). Consumers are aware of the negative health effects of food additives and, for this reason, processed meat products without adding chemicals are becoming increasingly important (*Balciunas et al*., 2013). In example, nitrates and nitrites can be reduced or replaced using natural substances present in spices, herbs, or essential oils, or deriving from microbial (bacteriocins) or animal (lysozyme) sources (*Oliveira et al*., 2018). However, the microorganisms used as bio-protection agents in foods have to show a high antagonistic activity against pathogenic and/or degrading microorganisms, must be safe for human health and must not have negative repercussions on the sensory and nutritional quality of the food. product (*Oliveira et al*., 2018).
In this regard, my PhD research project is aimed at evaluating the application of the most interesting of these strategies in order to improve the organoleptic and nutritional quality and to prolong the shelf-life of products of animal origin (fresh sausage, dairy products) and assuring food safety avoiding the proliferation of pathogenic or toxin-producing microorganisms.

3. Objectives and expected results

The present research project aims to evaluate the use of non-thermal treatments to improve the safety, quality, and shelf-life of products of animal origin, in particular fresh sausages, and dairy products.
The PhD thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:
A1) State of the art of non-thermal treatments on characteristics of foods of animal origin
A2) HPP application on fresh sausages to evaluate its impact on meat color, lipid oxidation and microbial growth.
A3) HPP application on dairy products to evaluate their safety and shelf-life prolongation.
A4) Use of plant extracts and/or essential oils for the safety and quality enhancement of product of animal origin. Plant extracts and/or essential oils will be characterized as regard their composition. Their antimicrobial effects will be studied *in vitro* against common foodborne pathogens. The most interesting matrices will be tested in animal food models.
A5) Use of bioprotective coltures to increase the safety and quality of product of animal origin. Some bioprotective lactic acid bacteria strains will be tested for their antimicrobial activity *in vitro* and in animal food models. In addition, the cell free supernatants and/or purified antimicrobial peptides will be studied in the same matrices.
A6) Writing and publication of the doctoral thesis, posters, scientific articles, and oral presentation.

Table 1. Gantt diagram for this Ph.D. thesis project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***State of the art***																		
A2)	***HPP application on fresh sausages***																		
	1) Evaluation of color by colorimeter and pigment analysis																		
	2) Evaluation of T-bars																		
	3) Microbiological analysis and evaluation of shelf-life																		
A3)	***HPP application on dairy products***																		
	1) Evaluation of product safety																		
	2) Prolongation of product shelf-life																		
A4)	***Study of plant extracts and/or essential oils***																		
	1) Study of the composition of plant extracts and/or essential oils																		
	2) Antimicrobial effects of plant extracts and/or essential oils																		
	3) Food model trials																		
A5)	***Study of bioprotective coltures***																		
	1) Cultures antimicrobial activity *in vitro*																		
	2) Cell free supernatants and/or purified antimicrobial peptides antimicrobial activity																		
	3) Food model trials																		
A6)	***Preparation of manuscripts, presentations, and thesis***																		

4. References

Aymerich T, Picouet PA, Monfort JM (2008) Decontamination technologies for meat products, Meat Science, 78(1-2), 114-129.

Balciunas EM, Martinez FAC, Todorov SD, de Melo Franco BDG, Converti, A., & de Souza Oliveira, R. P. (2013) Novel biotechnological applications of bacteriocins: a review, Food Control, 32(1), 134-142.

Oliveira M, Ferreira V, Magalhães R, Teixeira P (2018) Biocontrol strategies for Mediterranean-style fermented sausages, Food Research International, 103, 438-449.

Rosario DK, Rodrigues BL, Bernardes PC, Conte-Junior CA (2021) Principles and applications of non-thermal technologies and alternative chemical compounds in meat and fish, Critical Reviews in Food Science and Nutrition, 61(7), 1163-1183.

Valdramidis VP, Koutsoumanis KP (2016) Challenges and perspectives of advanced technologies in processing, distribution, and storage for improving food safety, Current Opinion in Food Science, 12, 63-69.

Microbial biopolymers for innovative packaging to increase food shelf-life and safety

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1. Curriculum

Marianna Ciccone è nata ad Atripalda (AV) il 29.06.1997 ed è una cittadina italiana. Dal novembre 2022 è dottoranda (XXXVIII ciclo) in *Agricultural, Environmental and Food Science and Technology* presso il Dipartimento di Scienze e Tecnologie Agro-alimentari (DISTAL) dell'Università di Bologna. La sua attività di ricerca riguarda lo studio di *Biopolimeri di origine microbica per la produzione di packaging innovativi per incrementare la shelf-life e la sicurezza degli alimenti*. Nel dicembre 2021 è risultata vincitrice di un assegno di ricerca intitolato *Approcci biotecnologici per l'ottenimento di ingredienti ad alto valore aggiunto da scarti e sottoprodotti ittici*, presso il Dipartimento di Scienze e Tecnologie Agro-alimentari (DISTAL) dell'Università di Bologna. Nell'ottobre 2021, Marianna ha conseguito la laurea magistrale in Scienze e Tecnologie alimentari con votazione di 110/110 presso l'Università di Bologna, con una tesi di laurea sperimentale in Qualità e formulazione degli alimenti, dal titolo *Microincapsulazione mediante spraizzazione di ficocianina estratta da Spirulina.* Nel settembre 2019, ha conseguito la laurea triennale in Tecnologie alimentari con votazione di 107/110, presso l'Università degli Studi di Napoli Federico II, con una tesi in Igiene, dal titolo *Rischi per la salute correlati alla presenza di glicosidi cianogenici in semi di albicocca*. Durante la sua carriera accademica, ha avuto l'opportunità di partecipare ad una Summer School presso l'Universidad ISA (Santiago de los Caballeros, Repubblica Domenicana) nell'ambito del progetto *LatinLAB*. Inoltre, ha partecipato come partner al meeting di progetto *NewTechAqua* presso l'università Nofima (Ås, Norvegia) e ha presentato poster relativi alla sua attività di ricerca presso convegni internazionali come *Aquaculture Europe 2022* (Rimini, Italia) e *EFFoST International Conference 2022* (Dublino, Irlanda).

Pubblicazioni con IF:

Gottardi D.; Ciccone M.; Siroli L.; Lanciotti R.; Patrignani F., Use of *Yarrowia lipolytica* to Obtain Fish Waste Functional Hydrolysates Rich in Flavouring Compounds, *Fermentation*, 2022, 8, pp. 708 – 724.

Poster in atti di convegno:

Ciccone M.; Gottardi D.; Braschi G.; Siroli L.; Lanciotti R.; Patrignani F., Production of functional and aroma compounds from fish waste and by-products using selected microorganisms, in: *Aquaculture Europe*, 2022.

Ciccone M.; Gottardi D.; Braschi G.; Siroli L.; Lanciotti R.; Patrignani F., High-added value compounds obtained from fish waste using microorganisms endowed with proteolytic and lipolytic activity, in: *36th EFFoST International Conference*, 2022.

2. Stato dell'arte

Negli ultimi decenni, vi è stato un crescente interesse, da parte della ricerca e del mondo industriale, nell'individuazione di valide alternative all'utilizzo di film sintetici derivanti dal petrolio. Infatti, sebbene polimeri di natura petrolchimica come il polietilene, il polipropilene, il polistirene e la poliammide siano prodotti a basso costo e abbiano buone proprietà meccaniche e ottime proprietà barriera, presentano un elevato impatto sull'ambiente, poiché non biodegradabili e derivanti da fonti non rinnovabili. Lo sviluppo di polimeri biodegradabili per imballaggi alimentari ecocompatibili potrebbe rappresentare un approccio sostenibile per minimizzare il problema dell'accumulo di plastiche nell'ambiente (Siracusa et al., 2008). Secondo la letteratura, i polimeri naturali utilizzati per sviluppare materiali biodegradabili sono polisaccaridi, proteine e lipidi. Inoltre, i biopolimeri stanno acquisendo grande importanza nel settore del packaging alimentare poiché possono fungere da carrier di altre molecole con proprietà antiossidanti. Grazie anche alle loro proprietà antimicrobiche è possibile ottenere packaging attivi per incrementare la shelf-life e la sicurezza di diversi prodotti alimentari (Cerutti et al., 2016). Tra le sostanze potenzialmente utilizzabili come biopolimeri, ci sono alcune fonti non ancora ben esplorate, ma con proprietà molto interessanti come, ad esempio, le biomasse dei lieviti, le cui pareti cellulari sono composte da glucani, mannoproteine e chitina. Ad esempio, i β-Glucani che compongono il 55-65% della parete cellulare dei lieviti sono polisaccaridi che, se combinati con le proteine della parete cellulare, hanno dimostrato essere buoni candidati per la formazione di film multicomponenti. Inoltre, il vantaggio di utilizzare la parete cellulare dei lieviti per la formazione di film utilizzabili come packaging è quella di evitare passaggi di purificazione (Choque et al., 2021). Attualmente, esistono diverse metodologie per rompere la parete cellulare del lievito e separare i componenti da quelli intracellulari come la sonicazione, ma anche i trattamenti ad alta pressione di omogeneizzazione che possono rappresentare una alternativa non termica sostenibile. Oltre alle pareti cellulari dei lieviti quali ceppi di *Saccharomyces cerevisiae* e *Yarrowia lipolytica*, anche alcuni biopolimeri come i pullulani prodotti da *Aureobasidium*

pullulans o le cellulose prodotte da diversi batteri acetici potrebbero rappresentare una valida alternativa per l'ottenimento di biopolimeri di natura microbica da utilizzare nel settore del packaging (Kraśniewska, Pobiega & Gniewos, 2019). Un ulteriore aspetto di sostenibilità potrebbe essere quello di produrre biomasse di lieviti e/o loro metaboliti per la formazione di film biodegradabili a partire da scarti e sottoprodotti dell'industria agro-alimentare, utilizzandoli come substrati di coltura per la crescita microbica. Infatti, il siero di origine lattiero-casearia, alcuni sottoprodotti dell'industria enologica o scarti vegetali e ittici potrebbero rappresentare, poiché ancora caratterizzati da un alto contenuto in sostanza organica, un buon substrato di crescita per i microrganismi potenzialmente utilizzabili.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di selezionare ceppi microbici in grado di produrre biopolimeri (estratti da pareti cellulari o come metaboliti) per la produzione di film, ad azione antimicrobica, al fine di incrementare la shelf-life e sicurezza di matrici alimentari. Al fine di raggiungere gli obiettivi attesi, il progetto di tesi di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt riportato in tabella 1:

A1) Ricerca bibliografica per l'individuazione dei microorganismi più idonei ed utilizzabili per il recupero dei costituenti della parete cellulare o loro metaboliti.

A2) Screening dei ceppi selezionati ed ottimizzazione delle performance microbiche su scarti e sottoprodotti dell'industria agro-alimentare.

A3) Selezione dei metodi e delle tecnologie più idonee alla rottura delle pareti cellulari e al recupero della frazione di interesse.

A4) Caratterizzazione dei biopolimeri ottenuti in relazione alle performance antimicrobiche e tecnologiche e formulazione dei film.

A5) Valutazione di shelf-life e sicurezza di matrici alimentari in relazione alle condizioni di packaging selezionate.

A6) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazione orale.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Ricerca bibliografica***																		
	1) Individuazione dei microrganismi più idonei																		
A2)	***Screening dei ceppi selezionati e ottimizzazione delle performance microbiche***																		
	1) Selezione e caratterizzazione tecnologica dei ceppi di lievito selezionati																		
	2) Ottimizzazione delle condizioni di crescita dei microrganismi su scarti e sottoprodotti dell'industria agro-alimentare																		
A3)	***Messa a punto di processi biotecnologici per il recupero dei biopolimeri di interesse***																		
	1) Applicazione di enzimi microbici per ottenimento dei composti cellulari																		
	2) Applicazione delle alte pressioni di omogeneizzazione, sonicazione, PEF																		
A4)	***Caratterizzazione antimicrobica e tecnologica dei biopolimeri ottenuti e formulazione dei film***																		
	1) Caratterizzazione antimicrobica e antiossidante dei biopolimeri																		
	2) Formulazione dei film e caratterizzazione																		
A5)	***Valutazione di shelf-life e sicurezza di matrici alimentari in relazione alle condizioni di packaging selezionate***																		
A6)	***Partecipazione a congressi, preparazione di articoli scientifici e tesi***																		

4. Bibliografia

Cerrutti P, Roldán P, García RM, Galvagno MA, Vázquez A, Foresti ML (2016). Production of bacterial nanocellulose from wine industry residues: Importance of fermentation time on pellicle characteristics, Journal of Applied Polymer Science, 133(14).

Choque E, Rezzani G, Salvay AG, Mathieu F, Peltzer MA (2021). Impact of fungal extracts on the physical and antioxidant properties of bioactive films based on enzymatically hydrolyzed yeast cell wall, Journal of Polymers and the Environment, 29(6), 1954-1962

Kraśniewska K, Pobiega K, Gniewosz M. (2019). Pullulan–biopolymer with potential for use as food packaging, International Journal of Food Engineering, 15(9).

Siracusa V, Rocculi P, Romani S, Dalla Rosa M (2008). Biodegradable polymers for food packaging: a review, Trends in Food Science & Technology, 19(12), 634-643.

Application of innovative technologies for the functionalisation of alternative proteins and the associated functional and rheological characterisation

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1. Curriculum

Work:
Scientific internship at Wroclaw University of Economics, Poland from 1st Mar. to 4th Apr. 2023
Academic Tutor for the Quality and Food Design course, UNIBO, from Nov. 2022
Research assistant, in the non-thermal technology and food processing group, UNIBO, from Feb. 2022 to Oct. 2022
Quality intern, internship in a fish processing company Ecopesce s.r.l. from Dic. 2020 to May 2021

Studies:
MSc Food Science and Technology, UNIBO, Italy 2020-2021, thesis: "Plasma Activated Water and Computer Vision System Application to Control and Evaluate Melanosis in Crustaceans"
Erasmus exchange in Lithuania at the Kauno Kolegija from Gen. 2018 to Jun. 2018
BSc Food Science, UNIBO, Italy, 2016-2019, thesis: "Effect of pulsed electric fields on the characteristic of fresh and freeze-dried strawberries"
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2. State-of-the-art

Following recent trends in the food sector, an increasing market share of plant-based products can be observed, ranging from dairy substitutes to alternative meats (GFI Europe, 2023), trend that is expected to increase further, also thanks to a shift in consumer preferences towards more environmentally friendly and cruelty-free products. Finally, especially in the richest countries, the development of protein foods from vegetable matrices has experienced tremendous growth (Fasolin et al., 2019; Aschemann-Witzel et al., 2021). As a result of this situation, more and more companies are launching such products, creating a need for specific ingredients. As it is well known, many plant-based products are characterised by a long list of ingredients used to overcome technological problems and meet specific requirements in terms of sensory properties and stability, to mimic the animal counterpart (Akharume FU et al., 2021). Unfortunately, proteins derived from plant sources differ not only in terms of nutritional value but also in terms of technological properties. Solubility and gelling properties are generally lower compared to those of animal origin (especially at pH close to neutrality), making their use in formulations more complex (Akharume FU et al., 2021).

One response to these needs can be the modification of plant proteins to obtain products with specific properties. Protein functionalisation has been used in the food sector for several years (Panyam et al., 1996; Messens et al., 1997). Recently, however, the interest of scientific research has shifted from the classical chemical-enzymatic modifications (e.g. glycosylation, acetylation, hydrolysis and cross-linking) to physical modifications obtained by applying non-thermal technologies such as cold plasma (CP), pulsed electric field (PEF), ultrasound (US), high Pressure processing (HPP) and extrusion, which make the whole functionalisation process more sustainable and efficient (Mirmoghtadaie et al., 2016; Sun-Waterhouse et al., 2017; O'sullivan et al., 2022).

Although the four technologies mentioned above are all considered non-thermal, they are based on different functional mechanisms. Cold plasma is able to favour the rearrangement of the protein structure thanks to the main action of the RONS formed (Basak et al., 2022), while in high pressure processing, the denaturing effect on the proteins is achieved by the compression that causes the collapse of the structures with empty spaces (such as the β-sheets) (Wang et al., 2022). One of the most studied technologies is the application of ultrasound, where the short and localised pressure and temperature shocks (thanks to the cavitation phenomenon) can act both at the macroscopic level on the size of the particles and at the microscopic level, denaturing the proteins and exposing the most lipophilic areas (O'sullivan et al., 2022). Finally, the least used technology for this purpose is pulsed electric field, as its efficacy and mechanism of action on proteins is still controversial (Han et al., 2018).

3. Objectives and expected results

The aim of the project is to evaluate the possibility of using innovative non-thermal treatments to modify the functional properties of legume flours and subsequently use these optimised ingredients in product formulation to meet specific needs. The project can be divided into the following tasks, which are also time-framed in Table 1.

A1) Literature review of previous studies on the application of non-thermal technologies for flour modification and protein denaturation and research on the specific needs in the formulation of new plant-based products.
A2) Evaluation of the properties of different legume flours with the aim of identifying one or two specific legumes to work on.
A3) Application of different treatments to the selected flours, with the aim of studying the effects on functional properties. The effect of PEF, US, HPP and CP will be evaluated and optimisation of parameters for the best treatment will also be studied.
A4) The modified flour will be evaluated in terms of its functional and rheological properties, with the aim of identifying some key aspects in which the flour has been modified and trying to find a use in a final product.
A5) Based on the ingredients obtained, a final bakery, snack or dairy replacement product will be developed to understand if the functionalisation process can improve the performance of the final product.
A6) This phase will assess whether the functionalisation process can be considered sustainable from both an economic and environmental perspective, taking into account other, more conventional functionalisation systems.
A7) This time is used to write the final dissertation, write articles and attend conferences.

Table 1. Gantt chart with the expected duration of different research activities

		Time [month]																
	RESEARCH ACTIVITY	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
A1	Literature review and research	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2	Flour characterization and identification of target characteristics	■	■	■	■														
A3	Treatments application and optimization for flour functionalization (PEF, US, HPP, CP)			■	■	■	■	■	■	■									
A4	Quality evaluation and identification of possible usages for the product						■	■	■	■	■	■	■						
A5	Application of the created flours for new final products										■	■	■	■	■				
A6	LCA and LCC of developed processes												■	■	■	■	■		
A7	Write dissertation, posters, scientific papers	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Bibliography

GFI Europe (2023) Europe plant-based food retail market insights, report.
Fasolin LH, Pereira RN, Pinheiro AC, Martins JT, Andrade CCP, Ramos OL, Vicente AA (2019) Emergent food proteins–Towards sustainability, health and innovation, Food Res Int 125, 108586.
Aschemann-Witzel J, Gantriis RF, Fraga P, Perez-Cueto FJ (2021) Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future, Crit. Rev. Food Sci. 61(18), 3119-3128.
Akharume FU, Aluko RE, Adedeji AA (2021) Modification of plant proteins for improved functionality: A review, Compr. Rev. Food Sci. Food Saf. 20(1), 198-224.
Panyam D, Kilara A (1996) Enhancing the functionality of food proteins by enzymatic modification, Trends Food Sci. Technol. 7(4), 120-125.
Messens W, Van Camp J, Huyghebaert A. (1997) The use of high pressure to modify the functionality of food proteins, Trends Food Sci. Technol. 8(4), 107-112.
Mirmoghtadaie L, Aliabadi SS, Hosseini SM. (2016) Recent approaches in physical modification of protein functionality, Food Chem. 199, 619-627.
Sun-Waterhouse D, Zhao M, Waterhouse GI (2014) Protein modification during ingredient preparation and food processing: approaches to improve food processability and nutrition, Food Bioproc. Tech. 7(7), 1853-1893.
O'sullivan JJ, Park M, Beevers J, Greenwood RW, Norton IT (2017) Applications of ultrasound for the functional modification of proteins and nanoemulsion formation: A review, Food Hydrocoll. 71, 299-310.
Basak S, Annapure US (2022) Recent trends in the application of cold plasma for the modification of plant proteins-A review, Fut. Foods 100119.
Wang W, Yang P, Rao L, Zhao L, Wu X, Wang Y, Liao X (2022) Effect of high hydrostatic pressure processing on the structure, functionality, and nutritional properties of food proteins: A review, Comp. Rev. Food Sci. Food Saf.
Han Z, Cai MJ, Cheng JH, Sun DW (2018) Effects of electric fields and electromagnetic wave on food protein structure and functionality: A review. Trends food Sci. Technol. 75, 1-9.

Rapid analytical assessment of aroma and visual quality on food products of animal origin

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1. Curriculum

Mara Antonia Gagliano was born in Chieri (Turin) in 1996. In 2019 she graduated in Food Technology at the University of Turin with a final report entitled "Termochromic labels in food products". In 2021 she achieved Master's degree in Food Science and Technologies (Alma Mater Studiorum – Università di Bologna) discussing a thesis entitled "Effect of low frequency ultrasound treatment on tenderness of chicken breast affected by Wooden Breast abnormality". In October of the same year, she got a research fellowship at the Department of Agricultural and Food Sciences (Alma Mater Studiorum - Università di Bologna). Research activities carried out during that year concerned the study of poultry meat affected by muscle abnormalities (i.e., white-striping, wooden breast and spaghetti meat) with particular reference to the evaluation of their technological properties and composition. Since November 2022, she is a PhD student in Agricultural, Environmental and Food Science and Technology (XXXVIII cycle) at the same Department conducting a research project entitled "Rapid analytical assessment of aroma and visual quality on food products of animal origin".

2. State-of-the-art

Generally, descriptive sensory assessment for food quality evaluation has been conducted by panels with trained experts (Alander et al., 2013). Although this approach is the most commonly used, it shows various disadvantages, such as being time consuming, and expensive (Chiofalo et al., 2017). In this regard, in recent years, human sense perception is frequently combined with "artificial senses" based instruments, which have applied in the food industry for e.g. quality control, freshness and maturity monitoring, shelf-life study, authenticity evaluation and microbial analysis. Such equipment shows many advantages, such as the rapidness, objectiveness, preciseness, efficiency, using low cost and non-destructive technique, and more sustainable with respect of environment (Ali et al., 2020). Novel artificial sensing devices, such as e-noses and e-tongues based on hybrid or electronic sensors, are being investigated. In addition to sensors-based e-nose, other analytical techniques are used in the identification and quantification of volatile organic compounds (VOCs) such as gas solid-phase micro extraction with chromatography and mass spectrometry (SPME-GC-MS), or flame ionization detector (SPME-GC-FID), also coupled with multivariate statistical analysis (Calvini et al.,2022). The electronic nose usually is made up by nonselective sensors that interact with volatile molecules; upon interaction, a signal is produced which constitutes a sort of fingerprint of the smells. The signal is used to identify the odour through comparison with a reference library of previously obtained measurements of known samples (Calvini et al.,2022). The electronic nose can be used to assess quality of beef fillets (Mohareb et al., 2016), measure flavour quality changes of oxidized chicken fat (Song et al., 2013), verify the authenticity of Parmigiano-Reggiano (Chiofalo et al., 2017) and identification of botanical origin and to determine quality honey (Huang et al., 2015). Another rapid analytical and non-destructive instrument is computer vision system (CVS) (electronic eye), which consist in an illumination device, a camera, a computer with a high-resolution monitor. CVS applications are mainly used for those food products for which the appearance is the among the main key quality attributes evaluated by the consumers (Chiofalo et al.,2017). In fact, CVSs are important to classify food products into specific grades, detect visual defects and estimate properties such as colour, shape, size, surface defects and contamination; examples are estimation of fat content in poultry products (Chmiel et al., 2011), or predicting colour grade in beef meat (Sun et al., 2011) and characterization of different types of honey with different botanical origin (Shafiee et al., 2014). Thus, it may be interesting the application of instrumental analysis to support results that are obtained by sensory analysis, in particular to investigate how animal farming systems and origin influence the quality and composition of meat and dairy products (El-Deek et al, 2016; Morand Fehr et al., 2007). In this framework, this PhD research project is focused on investigating the effect of animal farming systems and origin on the quality of several food products of animal origin. This will be realized by developing and applying rapid analytical techniques to assess the aroma and the visual quality and to combine and discuss the results with those obtained by sensory tests, both descriptive and affective, as well as by microbiological analyses.

3. Objectives and expected results

The aim of this PhD project is to assess the qualitative characteristics of different food products of animal origin (namely poultry, beef, dairy products and honey), with a focus on the evaluation of the aromatic profile and visual aspects, through the development and subsequent application of rapid, non-destructive and user-friendly analytical approaches. The

selected analytical techniques do not involve the use of reagents or solvents and for this reason are considered environmentally and operator health friendly, thus representing a sustainability advantage over other more conventional analytical approaches. The implementation of the project will require the following activities, which are reported in the Gantt diagram below (Table 1).

A1) Bibliographic research: research in the literature on image analysis, aromatic profile of food products of animal origin, as well as sensory analysis.

A2) Aroma and image analysis of food products from animal origin: use of HS-Flash GC and electronic eye to characterise the aromatic profile and visual aspects of food products, as dairy products, meat and honey.

A3) Sensory evaluation of food products of animal origin: quantitative descriptive analysis (QDA®) of cheese and milk, consumer test on cheese and beef meat.

A4) Statistical analysis: univariate and multivariate analysis. Joint elaboration of results from sensory analysis and instrumental analysis, taking consideration also results obtained by microbiological analysis.

A5) Dissemination, writing scientific papers and final thesis.

Table 1. Gantt diagram showing PhD activities.

Activities	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***HS-Flash GC and image analysis of animal food products***																		
	1) Study analytical methodologies																		
	2) Methods application of food products (cheese, meat, honey)																		
A3)	***Sensory evaluation of food products of animal origin***																		
	1) Quantitative descriptive analysis (QDA®) (cheese and milk)																		
	2) Consumer test (cheese and meat)																		
A4)	***Statistical analysis***																		
	1) Correlation between sensory, chemical and microbial results																		
	2) Univariate and multivariate approaches																		
A5)	***Dissemination, writing scientific papers and final thesis***																		

4. Bibliography

Alander JT, Bochko V, Martinkauppi B, Saranwong S, Mantere T (2013) A review of optical non destructive visual and near-infrared methods for food quality and safety, Int. J. Spectrosc, art ID 341402.

Ali MM, Hashim N, Abd Aziz S, Lasekan O (2020) Principles and recent advances in electronic nose for quality inspection of agricultural and food products, Trends Food Sci. Technol. 99: 1-10.

Calvini R, Pigani, L (2022) Toward the development of combined artificial sensing systems for food quality evaluation: A review on the application of data fusion of electronic noses, electronic tongues and electronic eyes, Sensors 22(2): 577.

Chmiel M, Slowinski M, Dasiewicz K, Florowski T (2016) Use of computer vision system (CVS) for detection of PSE pork meat obtained from m. semimembranosus, LWT - Food Sci. Technol. 65: 532-536.

Di Rosa AR, Leone F, Cheli F, Chiofalo V (2017) Fusion of electronic nose, electronic tongue and computer vision for animal source food authentication and quality assessment–A review, J. Food Eng. 210: 62-75.

El-Deek A, El-Sabrout K (2019) Behaviour and meat quality of chicken under different housing systems, Worlds Poult Sci J 75(1): 105-114.

Huang X, Xu H, Wu L, Dai H, Yao L, Han F (2016) A data fusion detection method for fish freshness based on computer vision and near-infrared spectroscopy, Anal. Methods 8 (14): 2929-2935.

Mohareb F, Papadopoulou O, Panagou E, Nychas GJ, Bessant C (2016) Ensemble-based support vector machine classifiers as an efficient tool for quality assessment of beef fillets from electronic nose data, Anal. Methods 8: 3711-3721.

Morand-Fehr P, Fedele V, Decandia M, Le Frileux Y (2007) Influence of farming and feeding systems on composition and quality of goat and sheep milk, Small Rumin. Res. 68(1-2): 20-34.

Shafiee S, Minaei S, Moghaddam-Charkari N, Barzegar M (2014) Honey characterization using computer vision system and artificial neural networks, Food Chem. 159: 143-150.

Song S, Yuan L, Zhang X, Hayat K, Chen H, Liu F, Xiao Z, Niu Y (2013) Rapid measuring and modelling flavour quality changes of oxidised chicken fat by electronic nose profiles through the partial least squares regression analysis, Food Chem. 141 (4): 4278-4288.

Sun X, Chen K, Berg EP, Magolski, JD (2011) Predicting fresh beef colour gradesing machine vision imagine and support vector machine (SVM) analysis, J. Animal Veterinary Adv. 10 (12): 1504-1511.

Study on Dealcoholized Wine and Exploitation of Winery Byproducts: A Multidisciplinary Perspective from Processing and Sensory Science

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1. Curriculum

Date of birth: 07/05/1998, Sikar, India
Bachelor of Technology in Food Technology and Management at the National Institute of Food Technology Entrepreneurship and Management (NIFTEM), India (2016-2020). *Thesis title:* Ultrasonication of mayonnaise formulated with xanthan and guar gums: Rheological modeling, effects on optical properties and emulsion stability.
Master's degree in Italian Food and Wine at the University of Padua (UNIPD), Italy (2020-2022). *Thesis title:* The effect of bentonite fining on the volatile and non-volatile profile of Italian white wines.
PhD. Food Science and Biotechnology, Department of Agricultural and Food Sciences, University of Bologna, Italy, Nov. 2022-present.
Seven articles were published in national/international journals, and three are under review.

2. Stato dell'arte

EU wine regulations define wine as "the product obtained exclusively from the total or partial alcoholic fermentation of fresh grapes, whether or not crushed, or of grape must with an actual alcoholic strength of not less than 8.5 % volume" (EU Regulation No 1308, 2013, p. 809). In a previous study, authors (Pickering, 2010; Saliba et al., 2013) proposed different wine categories based on the alcohol content as alcoholic (> 10.5% v/v), lower-alcohol (5.5% to 10.5% v/v), reduced-alcohol (1.2% to 5.5% or 6.5% v/v), low-alcohol (0.5% to 1.2% v/v), and alcohol-free (0.5% v/v) wine to consider potential social and health benefits for consumers. However, there were no official regulations at that time, and these categories were loosely based on labelling and legislative requirements. In 2021, a new EU regulation 2021/2117 introduced the category of "dealcoholized wine; if the actual alcoholic strength of the product is no more than 0.5 percent by volume" and "partially dealcoholized; if the actual alcoholic strength of the product is above 0.5 percent by volume and is below the minimum actual alcoholic strength of the wine category" (Regulation (EU) 2021/2117, 2021, p. 270). According to the most recent industry data, the non-alcoholic wine market is worth more than US$ 1.6 billion in 2021. It is predicted to grow at a compound annual growth rate (CAGR) of 10.4% to reach a valuation of US$ 4.5 billion by 2031, compared to a CAGR of 8.8% for 2016 to 2020 (Fact.MR, 2022).
Interestingly, reducing the alcohol content of red wines may reduce the risks associated with the consumption of alcoholic wines without compromising their cardio-protective effects (Chiva-Blanch et al. 2012). Moreover, polyphenolic compounds can have a positive biological effect on cardiovascular health due to their anti-inflammatory properties (Jiao et. al., 2019, Rojas Borquez et al., 2016). As a result, alcohol-free wine could be an excellent source of antioxidants to protect people suffering from oxidative stress, such as cancer, diabetes, alzheimer, etc., who should not consume alcohol. Furthermore, techniques for reducing alcohol content during post-fermentation include membrane techniques such as reverse osmosis (RO), nanofiltration (NF), pervaporation (PV), vacuum distillation (VD), osmotic distillation (OD), spinning cone column (SCC), and multi-stage membrane systems (Mangindaan et al., 2018; Sam et al., 2021; Schmitt & Christmann, 2022). Ongoing limitations in sensory quality, promotional issues, and a low level of awareness of the improvements in quality based on innovations in production methods were suggested as potential barriers to the market success of dealcoholized, low- and reduced-alcohol wine. In this view, there is a need to increase consumer knowledge related to alcohol reduction processes and increase consumer awareness about high-quality, low-alcohol wines with appealing sensory properties.
Furthermore, the production of wine and dealcoholized wine produced significant amounts of waste byproducts such as grapes, pomace, seeds, stems, and ethanol. Wine lees and pomace are considered byproducts according to the European Council Regulation (EC) N° 479/2008 on the common organization of the market in wine (EC, 2008). These byproducts are often discarded, leading to environmental pollution and economic losses. Ethanol fraction after

dealcoholization composed of anthocyanins, polymeric proanthocyanidins and other pigmented complexes. Also, byproducts contain several ions, such as sodium, potassium, phosphorus and even heavy metals. Even though they are not considered toxic to humans per se, they do display phytotoxic effects, and they may significantly impact the environment mainly because of the organic content (Salazar et al. 2018). Byproducts can be used as a source of phenolic compounds, which have antioxidant, anti-aging activity and antimicrobial properties. As a result, more applications must be investigated to address the problem of excessive waste in an environmentally friendly manner, as well as to improve the quality and health aspects of a variety of food products through the incorporation of valuable functional ingredients.

3. Obiettivi e risultati attesi

The overall objective of this PhD project is to investigate the effect of the dealcoholization process on the volatile and non-volatile profiles of wine and preserve product quality by combining processing, chemistry and sensory science and investigate innovative approaches for the valorization of its byproducts.
The doctoral thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:
A1) Literature review on Meta-Analysis of the volatile and non-volatile profile of dealcoholized wines.
A2) Optimization and browning kinetics of model white wine as a function of ethanol, phenolics, metal (Iron and Copper) and SO_2 concentrations.
A3) Study the volatile and non-volatile profiles of commercial wines before and after the dealcoholization process.
A4) Study the effects of packaging and storage temperature on the shelf-life of the dealcoholized wine.
A5) Characterization and optimization of extracted compounds (such as Polyphenols and Polysaccharides) from winery sludges, grape skins, lees and seeds.
A6) Management strategies towards using by-products in food product applications.
A7) Writing and publication of doctoral theses, posters, scientific papers and oral presentations

Table 1. Gantt Chart for the research activities in the scope of doctoral study

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Literature review***																		
A2)	***Pre-trials and optimization of experimental methods***																		
	1) Optimization and browning kinetics of model white wine																		
	2) Preparation of the publication																		
A3)	***Dealcoholization of commercial wines***																		
	1) Study the volatile and non-volatile profiles																		
	2) Statistical analysis and preparation of the publication																		
A4)	***Study the effects of packaging and storage temperature***																		
A5)	***Characterization and optimization of extracted compounds***																		
A6)	***Management strategies towards using by-products***																		
A7)	***Preparation of the publications and thesis***																		

4. Bibliografia

Fact.MR. (2022, December 21). Non-alcoholic wine market trends & industry forecast - 2033. https://www.factmr.com/report/4532/non-alcoholic-wine-market

Mangindaan, D., Khoiruddin, K., & Wenten, I. G. (2018). Beverage dealcoholization processes: Past, present, and future. Trends in Food Science & Technology, 71, 36–45. https://doi.org/10.1016/J.TIFS.2017.10.018

Pickering, G. J. (2010). Low- and reduced-alcohol wine: A review. Journal of Wine Research, 11(2), 129–144. https://doi.org/10.1080/09571260020001575

Regulation (EU) 2021/2117, p. 270. (2021). establishing a common organisation of the markets in agricultural products, (EU) No 1151/2012 on quality schemes for agricultural products and foodstuffs, (EU) No 251/2014 on the definition, description, presentation, labelling and the protection of geographical indications of aromatised wine products and (EU) No 228/2013 laying down specific measures for agriculture in the outermost regions of the Union. Official Journal of the European Union, 262–314. http://data.europa.eu/eli/reg/2021/2117/oj

Regulation (EU) No 1308/2013 of the European Parliament and of the Council of 17 December 2013 establishing a common organisation of the markets in agricultural products and repealing Council Regulations (EEC) No 922/72, (EEC) No 234/79, (EC) No 1037/2001 and (EC) No 1234/2007. (n.d.). Retrieved January 23, 2023, from http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32013R1308&from=en/

Saliba, A. J., Ovington, L. A., & Moran, C. C. (2013). Consumer demand for low-alcohol wine in an Australian sample. International Journal of Wine Research, 5(1), 1–8. https://doi.org/10.2147/IJWR.S41448

Sam, F. E., Ma, T. Z., Salifu, R., Wang, J., Jiang, Y. M., Zhang, B., & Han, S. Y. (2021). Techniques for dealcoholization of wines: Their impact on wine phenolic composition, volatile composition, and sensory characteristics. Foods, 10(10), 2498. https://doi.org/10.3390/FOODS10102498

Messa a punto di un coating attivo bio-based per cartone ondulato ad azione impermeabilizzante e antimicrobica

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Tutor: Rosalba Lanciotti; Co-tutor: Lorenzo Siroli e Claudio Dall'Agata

1. Curriculum

Joel Armando Njieukam è un dottorando del XXXVIII ciclo in Scienze e Biotecnologie degli Alimenti. Laureato magistrale in Scienze e Tecnologie Alimentari, presso l'Università degli Studi di Bologna, con tesi di Laurea in Microbiologia delle fermentazioni (Voto di laurea 110/110), svolge la sua attuale attività di ricerca presso il DISTAL-sede di Cesena. Tale attività di ricerca riguarda principalmente: lo studio di processi biotecnologici innovativi per lo sviluppo di materiali d'imballaggio di origine microbica e più specificamente di film bio-based, il miglioramento della qualità e il prolungamento della shelf-life di prodotti alimentari di origine vegetale, l'impiego di microrganismi per la produzione di molecole di interesse per l'industria alimentare (antimicrobici, cellulosa batterica, additivi alimentari) nonché l'identificazione, la caratterizzazione e la selezione di colture microbiche per la produzione di cellulosa o di monomeri utili per la sintesi di biopolimeri.
Da Novembre 2021 a Ottobre 2022, Joel Armando Njieukam è stato assegnista di ricerca presso il DISTAL-sede di Cesena con un progetto dal titolo "Qualità microbiologica di alimenti e ingredienti funzionali da frutta biologica ottenuti mediante tecnologie non termiche", finanziato sul progetto europeo "MILDSUSFRUIT" sotto la supervisione della Prof.ssa Francesca Patrignani.

2. Stato dell'arte

Tra i temi oggigiorno più dibattuti, ci sono sicuramente quelli della riduzione dello spreco alimentare e dell'impatto ambientale delle materie plastiche di origine fossile usate nel settore del packaging. Tali temi rappresentano dei punti chiave che le Nazioni Unite hanno inserito nell'Agenda per lo sviluppo sostenibile 2030. A livello globale, infatti, più del 40% delle materie plastiche prodotte sono utilizzate per il confezionamento alimentare (Rochman, et al., 2013). Per ridurre la dipendenza dell'industria alimentare dalle risorse non rinnovabili quali i combustibili fossili, le ricerche scientifiche si sono concentrate su soluzioni alternative tra cui, la realizzazione di packaging bio-based e quindi, con polimeri biodegradabili e rinnovabili. Tra i materiali di imballaggio a base di biopolimeri, stanno ricevendo enorme attenzione a causa del loro basso costo di produzione, biodegradabilità, ampia disponibilità e molteplici applicazioni quelli a base di polisaccaridi (Xu, Chen, Rosswurm, Yao, & Janaswamy, 2016), a base di lipidi composti da cere, acilgliceroli e acidi grassi o quelli a base di proteine come albume d'uovo, caseina, proteine della soia, collagene, glutine, proteine del siero di latte, gelatina di pesce, proteine miofibrillari e cheratina sono già utilizzati nell'industria alimentare (Meerasri & Sothornvit, 2020). Inoltre, numerosi studi hanno evidenziato la possibilità di utilizzare composti derivanti da biomasse microbiche o dal metabolismo microbico come la cellulosa batterica, ottenuta attraverso ceppi bi batteri acetici come *Komagataeibacter* spp. e *Novacetimonas* spp., per produrre imballaggi alimentari con proprietà tecnologiche pressoché simili a quelle degli imballaggi tradizionali e con il grande vantaggio aggiuntivo, di essere biodegradabili (Alshehrei, 2017; Lambert & Wanger, 2017). Inoltre, gli imballaggi bio-based destinati a venire a contatto con gli alimenti possono, in alcuni casi, essere funzionalizzati attraverso l'incorporazione di componenti ad attività antimicrobica o antiossidante che possono essere rilasciati nel prodotto alimentare confezionato o nel suo ambiente consentendo di incrementare la shelf-life del prodotto e riducendo di conseguenza, spreco e scarto alimentare. Si ottengono, in questo modo, imballaggi attivi in grado di garantire sicurezza, economicità e biodegradabilità (Almasi, Oskouie & Saleh, 2021).

3. Obiettivi e risultati attesi

Il presente progetto si inserisce nel vasto contesto delle ricerche sul packaging attivo bio-based focalizzandosi sullo sviluppo di un film di rivestimento innovativo per cartone ondulato destinato al confezionamento di prodotti ortofrutticoli. Nello specifico, il progetto si propone di mettere a punto nel triennio del dottorato un imballaggio in cartone ondulato rivestito di film bio-based, ottenuto a partire da cellulosa batterica prodotta da ceppi di batteri acetici selezionati, e attivato grazie all'addizione di antimicrobici naturali rilasciati nel tempo, in grado di ridurre la proliferazione microbica e di incrementare la shelf-life di prodotti ortofrutticoli confezionati.

Le aspettative in termini di risultati sono: selezione di ceppi microbici, tra quelli appartenenti alla collezione del DISTAL, in grado di produrre cellulosa batterica e antimicrobici naturali; ottimizzazione delle performance dei ceppi selezionati per quanto concerne la qualità e la quantità dei biopolimeri e degli antimicrobici prodotti; selezione di antimicrobici naturali, anche attraverso la loro estrazione da scarti e sottoprodotti dell'industria alimentare, e loro miscele più idonei per l'attivazione del film bio-based; messa a punto di un film attivo per cartone ondulato da ortofrutta ottenuto a partire dai biopolimeri ottenuti nelle fasi precedenti; definizione di protocolli per la produzione di biopolimeri integrati con composti bioattivi di origine microbica o estratti da scarti e sottoprodotti; caratterizzazione del film attivo bio-based in termini di valutazione dell'attività antimicrobica dei film selezionati nei confronti dei principali microrganismi patogeni e degradativi associati a prodotti ortofrutticoli; caratterizzazione del rivestimento bio-based in termini di biodegradabilità, resistenza all'umidità, proprietà di superficie, proprietà barriera, ecosostenibilità. Le caratteristiche del film attivo messo a punto saranno confrontate con differenti materiali bio-based già utilizzati come l'acido polilattico (Avila-Sosa, et al., 2012), pullulano e chitosano. Infine, il film attivo messo a punto sarà accoppiato a imballaggi in cartone ondulato per ortofrutta e sarà valutato l'effetto sulla shelf-life, sicurezza e proprietà organolettiche di un prodotto ortofrutticolo ad alto valore definito insieme al partner industriale.
Tale progetto di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt in **Tabella 1** riportata di seguito:

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1	***Ricerca bibliografica***																		
A2	***Selezione e produzione di film bio-based di cellulosa batterica***																		
	1) Isolamento di batteri acetici da diverse matrici alimentari																		
	2) Screening e caratterizzazione di batteri acetici produttori cellulosa batterica																		
	3) Ottimizzazione delle performance dei ceppi selezionati																		
	4) Definizione dei protocolli ottimali per l'ottenimento di film bio-based																		
A3	***Selezione di antimicrobici naturali per l'attivazione di film bio-based***																		
	1) Screening e caratterizzazione di antimicrobici naturali ottenuti da diverse fonti																		
	2) Valutazione dell'attività antimicrobica e selezione di quelli di maggiore interesse																		
A4	***Attivazione e caratterizzazione del film biobased***																		
	1) Definizione di protocolli per la produzione di biopolimeri integrati con antimicrobici naturali																		
	2) Caratterizzazione delll'attività antimicrobica del film attivo bio-based																		
	3) Caratterizzazione tecnologica del rivestimento bio-based																		
A5	***Applicazione su cartone ondulato e valutazione dell'effetto su prodotto confezionato***																		
	1) Definizione del protocollo ottimale per il rivestimento di cartone ondulato con film biobased																		
	2) Valutazione dell'effetto del nuovo packaging attivo sulla shelf-life, sicurezza e proprietà organolettiche del prodotto confezionato																		
A6	***Preparazione della tesi, di articoli scientifici, poster e presentazione orale***																		

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

4. Bibliografia

- Almasi H, Oskouie MJ, Saleh A (2021) A review on techniques utilized for design of controlled release food active packaging, Crit Rev Food Sci Nutr. 61(15):2601-2621.
- Alshehrei F (2017) Biodegradation of synthetic and natural plastic by microorganisms, J Appl. Environ. Microbiol. 5(1):8-19.
- Avila-Sosa R, Palou E, Munguía MTJ, Nevárez-Moorillón VG, Cruz ARN, López-Malo A (2012) Antifungal activity by vapor contact of essential oils added to amaranth, chitosan, or starch edible films, Int. J. Food Microbiol. 153:66-72.
- Lambert S, Wagner M (2017) Environmental performance of bio-based and biodegradable plastics: the road ahead, Chem. Soc. Rev. 46:6855-6871.
- Meerasri J, Sothornvit R (2020) Characterization of bioactive film from pectin incorporated with gamma-aminobutyric acid, Int. J. Biol. Macromol. 147:1285-1293.
- Rochman CM, Browne MA, Halpern BS, Hentschel BT, Hoh E, Karapanagioti HK, Rios-Mendoza LM, Takada H, Teh S, Thompson RC (2013) Policy: Classify plastic waste as hazardous, Nature 494 (7436):169-71.
- Xu Q, Chen C, Rosswurm K, Yao T, Janaswamy S (2016) A facile route to prepare cellulose-based films, Carbohydr. Polym. 149:274-281.

Sviluppo di organogel contenenti composti bioattivi da sottoprodotti e loro applicazione per la formulazione di alimenti innovativi e sostenibili

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: I
Tutor: Prof.ssa Maria Teresa Rodriguez Estrada; Co-tutor: Dott. Dario Mercatante

1. Curriculum

Istruzione:
A.A. 2022/23-in corso: Dottorato di Ricerca presso l'*Alma Mater Studiorum*-Università di Bologna XXXVIII ciclo, progetto dedicato al tema di ricerca: "Sviluppo di organogel contenenti composti bioattivi da sottoprodotti e loro applicazione per la formulazione di alimenti innovativi e sostenibili"; Tutor: Prof.ssa Maria Teresa Rodriguez Estrada.
A.A. 2020/21: Laurea Magistrale (LM-70) in Scienze e Tecnologie Alimentari presso *Alma Mater Studiorum*-Università di Bologna, Campus Scienze degli Alimenti di Cesena. Titolo tesi sperimentale: "Integrazione delle diete con additivi fitogenici nel pollo: valutazione degli effetti sulla qualità delle carni". Relatore: Prof. Massimiliano Petracci. Votazione: 110/110 *cum Laude.*
A.A. 2017/18: Laurea Triennale (L-26) in Scienze e Tecnologie Agro-Alimentari, Università degli Studi di Perugia, Dipartimento di Scienze Agrarie, Alimentari e Ambientali (DSA3). Titolo tesi sperimentale: "Trattamento con ultrasuoni ad alta frequenza delle paste di oliva: effetti sulla resa di estrazione e sulla qualità degli oli extravergini di oliva". Relatore: Prof. Maurizio Servili. Votazione: 110/110 *cum Laude* e Menzione d'Onore al merito.
A.A. 2014/2015: Diploma di maturità presso l'Istituto Tecnico Agrario ITAS "Garibaldi-da Vinci" di Cesena (FC) - Settore Tecnologico, indirizzo "Agraria, Agroalimentare e Agroindustria", articolazione "Produzioni e Trasformazioni", con votazione di 100/100.

2. Stato dell'arte

Negli ultimi anni, le limitazioni legislative all'utilizzo di lipidi ricchi in acidi grassi *trans* e saturi, la crescente consapevolezza dei consumatori in merito ai loro effetti negativi sulla salute umana e la diffusa preoccupazione degli impatti ambientali legati al vasto impiego dell'olio di palma nelle produzioni alimentari, hanno orientato i ricercatori allo studio di strutture lipidiche alternative che fossero a minore impatto ambientale e complessivamente più salutari (Li et al., 2022). Tuttavia, gli acidi grassi saturi e *trans* svolgono un ruolo importante negli alimenti, perché conferiscono diverse proprietà come plasticità, sapore, *texture*, ecc. Questo comporta una reale necessità di trovare soluzioni per la sostituzione dei grassi ad alto tenore in acidi grassi *trans* e saturi negli alimenti senza però comprometterne le caratteristiche tecnologiche e sensoriali. Una delle principali alternative oggetto di ricerca negli ultimi anni è rappresentata dagli organogel, ovvero una classe di gel in cui una fase liquida (oli vegetali o acqua) viene immobilizzata e intrappolata in una rete tridimensionale termo-reversibile attraverso l'impiego di organogelatori non-trigliceridici (Bascuas et al., 2020). Ricerche recenti, hanno studiato l'impiego di organogel come sostituti dei grassi solidi tradizionali in diversi prodotti alimentari (prodotti da forno, prodotti a base di carne, prodotti lattiero caseari) nei quali tali strutture hanno permesso di diminuire la quantità complessiva di grasso, in particolare di acidi grassi saturi e/o *trans*, dando comunque una *texture* solida. Infatti, il meccanismo di strutturazione degli organogel non modifica la composizione chimica della fase liquida di partenza, né il suo valore nutrizionale (Li et al., 2022), contrariamente ad altri processi di strutturazione dei grassi, ampiamente diffusi nell'industria alimentare, come idrogenazione ed interesterificazione. Molti studi, inoltre, hanno valutato positivamente il loro impiego come "sistemi *carrier*" per il trasporto di composti bioattivi per i quali rappresentano un sistema in grado di aumentare la loro dispersibilità nella matrice alimentare e biodisponibilità nel tratto gastro-intestinale, essendo un modo per controllarne il rilascio, proteggerli dall'ossidazione e dalla perdita di funzionalità (Orhan & Eroglu, 2022). In questo contesto, i sottoprodotti della filiera agro-alimentare, a cui sono attribuiti elevati costi di smaltimento e basso valore di mercato per il loro reimpiego, rappresentano una notevole fonte di composti bioattivi ad elevato valore biologico che, grazie alle loro comprovate proprietà antiossidanti, antimicrobiche e salutistiche, potrebbero essere utilizzati come ingredienti e/o additivi nelle formulazioni alimentari (Fritsch et al., 2017). Pertanto, gli organogel, aderendo ai concetti di economia circolare, *green economy* e sviluppo sostenibile, rappresentano un interessante alternativa per l'inclusione e conseguente valorizzazione di tali sottoprodotti. I principali sottoprodotti incidenti a livello nazionale ed Europeo sono quelli derivanti dalla filiera cerealicola, dell'olio di oliva, del pomodoro, delle patate, ecc. (Fritsch et al., 2017), i quali sono ricchi di composti bioattivi come carotenoidi, composti fenolici, beta-glucani, ecc. Infine, gli organogel potrebbero essere impiegati nella formulazione di prodotti alimentari innovativi. In quest'ottica, una tendenza di mercato emergente riguarda i "*plant-based food*", percepiti dal consumatore come soluzioni alimentari più sostenibili e più aderenti ad aspetti etici, di sostenibilità ambientale e salutistico-nutrizionali (McClements & Grossmann, 2021). Lo

sviluppo e l'impiego di organogel leganti composti bioattivi derivanti da sottoprodotti agro-alimentari nella formulazione di alimenti innovativi (convenzionali e/o *plant-based*), rappresenterebbe un'applicazione in grado di migliorarne la *shelf-life*, rallentando e/o riducendo drasticamente processi ossidativi e idrolitici a carico della frazione lipidica e proteica, con risvolti positivi sulla qualità organolettica e sulle caratteristiche nutrizionali-salutistiche di questi alimenti.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di sviluppare organogel leganti composti bioattivi che forniscano una soluzione innovativa e sostenibile ai grassi saturi e/o *trans* attualmente impiegati nelle formulazioni alimentari, valorizzando i sottoprodotti della filiera agro-alimentare e successivo sviluppo di prodotti alimentari innovativi che siano sicuri, stabili da un punto di vista ossidativo, con almeno le stesse, se non superiori, caratteristiche di conservabilità, organolettiche e nutrizionali dei prodotti finiti.
Il progetto di tesi di Dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt riportato in Tabella 1:

A1) Ricerca bibliografica
A2) Estrazione e caratterizzazione dei composti bioattivi da sottoprodotti agro-alimentari
A3) Sviluppo di organogel con composti bioattivi
A4) Formulazione di alimenti innovativi (convenzionali e/o *plant-based*)
A5) Studio di *shelf-life* dei prodotti selezionati
A6) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazione orale

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Ricerca bibliografica***																		
A2)	***Estrazione e caratterizzazione dei composti bioattivi da sottoprodotti agro-alimentari***																		
	1) Selezione dei sottoprodotti																		
	2) Estrazione e caratterizzazione chimico-fisica dei composti bioattivi																		
A3)	***Sviluppo di organogel con composti bioattivi***																		
	1) Valutazione della stabilità chimico-fisica e ossidativa																		
	2) Valutazione degli organogel più performanti per la veicolazione di composti bioattivi																		
A4)	***Formulazione di alimenti innovativi (convenzionali e/o plant-based)***																		
	1) Definizione della formulazione e inclusione degli organogel pre-selezionati																		
	2) Valutazione della stabilità chimico-fisica e ossidativa, delle caratteristiche organolettiche e nutrizionali-salutistiche (veicolazione/rilascio) dei prodotti innovativi																		
A5)	***Studio di shelf-life dei prodotti selezionati***																		
A6)	***Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazione orale***																		

4. Bibliografia

Bascuas S, Hernando I, Moraga G, Quiles A (2020) Structure and stability of edible oleogels prepared with different unsaturated oils and hydrocolloids. Int. J. Food Sci. Technol. 55(4): 1458-1467.

Fritsch C, Staebler A, Happel A, Cubero Márquez MA, Aguiló-Aguayo I, Abadias M, Gallur M, Cigognini IM, Montanari A, Lopez MJ, Suarez-Estella F, Brunton N, Luengo E, Sisti L, Ferri M, Belotti G (2017) Processing, valorization and application of bio-waste derived compounds from potato, tomato, olive and cereals: A review. Sustainability 9(8): 1492.

Ghebreyesus TA, Frieden TR (2018) REPLACE: a roadmap to make the world *trans* fat free by 2023. The Lancet, 391(10134): 1978-1980.

Li L, Liu G, Bogojevic O, Pedersen JN, Guo Z (2022) Edible oleogels as solid fat alternatives: Composition and oleogelation mechanism implications. Compr. Rev. Food Sci. Food Saf. 21(3): 2077-2104.

McClements DJ, Grossmann L (2021) The science of plant-based foods: Constructing next-generation meat, fish, milk, and egg analogs. Compr. Rev. Food Sci. Food Saf. 20(4): 4049-4100.

Orhan NO, Eroglu Z (2022) Structural characterization and oxidative stability of black cumin oil oleogels prepared with natural waxes. J. Food Process. Preserv. 46(12): e17211.

Novel Algorithms and Software Tools for LR-NMR Applications in Food Science and Technologies

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1. Curriculum

I graduated in physics at the University of Milan. Then, I have been working for three years at Extra Byte, a company whose activities embrace scientific research, mainly in the area of Nuclear Magnetic Resonance, and it specialises in the development of STEM software tools. Here I reached expertise in C++ coding and I acquired basic knowledge of NMR data acquisition and processing. Also, I attended the basic and the advanced courses of the national NMR school in Turin, organised by the GIDRM (Gruppo Italiano Discussione Risonanza Magnetica), and participated at different congresses in Italy and abroad.
My tasks include, between others, the development of software dedicated to the evaluation of NMR relaxometry data, written in C++. It provides support for all common relaxometry sequencies, as well as variable frequency and temperature data. It provides basic processing tools like for phase correction or denoising (smoothing), fitting procedures for time-domain signals or NMR dispersion profiles, algorithms for the Inverse Laplace transform, data simulation, graphical plot and user interface.

2. State of the art

Food technology impacts on all steps of food processing, starting from the production of foodstuffs, to their storage, various transformations, and even cooking. Each step must include proper concurrent quality assessment and safety controls.
Nuclear Magnetic Resonance (NMR) is a very useful method to study and characterize several chemical and physical properties of the soft matter, including all kinds of materials and therefore also foodstuffs. The salient features of NMR include a large penetration depth, a totally non-invasive nature, the capability to discriminate even small variations in chemical composition as well as in molecular aggregation and mobility, an intrinsic quantitative response and good reproducibility. The drawbacks of NMR, in some contexts, are its relatively low sensitivity and the need to apply a relatively strong and very homogeneous magnetic field.
NMR comprises three distinct branches: relaxometry, spectroscopy, and imaging. Relaxometry studies the temporal evolution of nuclear magnetization and the ways it is affected by the molecular dynamics of the sample, spectroscopy is concerned mostly with highly resolved radio spectrum of a sample which reflects its chemical properties (molecular structure and composition), and imaging specializes in obtaining various kinds of visual images of the internal parts of a sample.
NMR have been widely used to solve many problems in the general area of food technology. In particular, high-resolution NMR spectroscopy (HR-NMR) became widely used to study the molecules found in food-related materials, usually focusing on the chemical assignments and quantification of various spectral peaks. This led to the development of a large number of useful spectroscopic HR-NMR applications. In general, however, such applications require very sophisticated equipment, in particular high-field, high-resolution superconducting magnets, and highly skilled operators. This makes them unsuitable for large scale deployment in industrial process applications.
In NMR relaxometry, the situation is significantly different. Low Resolution (LR) NMR equipment is more compact, lighter, and less expensive, features that make it suitable for small industrial and academic labs. While there exist hundreds of publications proposing various LR-NMR applications related to food quality and processing, relatively few of these potential applications were so far actually refined to the stage of practical assessment procedures.
Potential LR-NMR applications cover many recognizable categories, such as the distinction of sample components (muscle/fat, oil/water) or phases (solid/liquid) or inner states (ripe or damaged), melting/freezing processes (margarine melting curves), assessment of particle/droplet sizes in emulsions (milk, cream), ageing of materials (stocked food, cheese ripening), assessment of DOP products authenticity (mozzarella di bufala).
Original Equipment Manufacturers (OEMs) produce LR-NMR instruments equipped with different hardware features from each other and with low software support to any particular application. In general, data formats and even the OEM data evaluation procedures do not follow any universal standard. In this situation application developers struggle and find difficult to guarantee reproducibility of the results. So, there is a great need for a uniform, vendor-agnostic software tool, one sufficiently sophisticated to allow an expert user, once he selects a potential application, to optimize it, to assess its precision and its reproducibility, to automate it, and to make it suitable for practical use in industrial environments.

3. Goals and expected results

An applications developer employs one or more LR-NMR instruments to acquire data and a software tool for the data analysis, in order to optimize and automate the whole process. The goal of this project is the development of high-level software tools for the evaluation of LR-NMR data pertinent to possible applications in food technology and testing. This would provide either basic processing operations and support for specific applications evaluation tasks.
The software will be mostly written in C++ and will be based on either innovative algorithms and on improvements of the existing ones, and it will be focused on obtaining quantitative information about the sample physical and chemical properties, structure, quality. Simulated data will be generated to test the algorithms correct operation; then, real data will be acquired to test the effective robustness and stability of the software routines. The quality of the results achieved during the PhD course will be verified through the feedback received from potential users, who will employ the alpha version of the application software to analyse food products selected for verification.

The doctoral project may be organised in the following activities, resumed in the Gantt chart shown in table 1:

A1) Preparation: bibliographic research about LR-NMR applications in food science and technology.
A2) Software development: research of the currently available software tools for NMR data evaluation, research of innovative algorithms and their implementation and testing.
A3) Experiments: data acquisition and sequence optimization.
A4) Application development: choice of one or two potential applications to optimise in terms of analysis workflow from the data acquisition to the final extrapolation of results.
A5) Writing and publishing: scientific papers, posters, final thesis and oral presentation.

Table 1. Gantt chart of doctorate research activities.

Activities	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Preparation***																		
	1) Bibliographic research																		
A2)	***Software development***																		
	1) Research of currently available software tools and algorithms																		
	2) Software project and implementation																		
	3) Testing algorithms																		
A3)	***Experiments***																		
	1) Data acquisition																		
A4)	***Applications development***																		
A5)	***Preparation of papers and thesis***																		

4. Bibliography

Mannina L, et al (2017) NMR Methodologies in Food Analysis. In Analytical Chemistry: Developments, Applications and Challenges in Food Analysis, Nova Science Publishers, pp. 103-156.

Sobolev AP, et al (2017) NMR applications in Food Analysis. In Analytical Chemistry: Developments, Applications and Challenges in Food Analysis, Nova Science Publishers, pp. 157-254

Kirtil E, Oztop MH (2016) 1H Nuclear Magnetic Resonance Relaxometry and Magnetic Resonance Imaging and Applications in Food Science and Processing. Food Eng Rev, 8:1–22.

Analysis of volatile substances on extra virgin olive oils and flavored oils, development of a predictive system for determining the shelf-life

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1. Curriculum

Rosalba Tucci is currently a first-year student in Agricultural, Environmental and Food Science and Technology PhD course at the Department of Agricultural and Food Sciences (DISTAL) of the University of Bologna. Her project aims specifically on the development of rapid analytical methods for screening volatile compounds in vegetable oils (virgin olive oil and flavored oils) to obtain information useful to support sensory analysis and to study their shelf-life. Rosalba received her bachelor's degree in Science and Food Technology at the University of Foggia in 2019. Her thesis focused on the determination of heavy metals by inductively coupled plasma-mass spectrometry (ICP-MS) in environmental matrices. Her research was conducted at the Regional Agency for environmental protection (ARPA). In 2022, Rosalba obtained her master's degree in Science and Food Technology at the University of Bologna with a thesis developed at a meat and sausage company (Salumificio DelVecchio, Cesena, Italy) that investigated company differentiation strategies for fermented sausages production.

2. State-of-the-art

In the Mediterranean area, olive oil represents one of the main food products with a world production of 2,511,000 t expected for the 2022/23 campaign (DG AGRI dashboard: olive oil, 2023). Almost the 93% of the world production comes from this area, mainly in European countries like Spain, Italy, and Greece (Espadas-Aldana *et al*., 2019).
In the European Union (EU), virgin olive oils (VOOs) can be classified in three commercial categories depending on their quality: extra virgin (EV), virgin (V) and lampante (L) (Reg. EC n. 2022/2104). The EU has established this classification to ensure the quality of European olive oil and to prevent fraudulent practices as mixing EVOO with lower quality oils, protecting consumers from frauds (Violino *et al.,* 2021).
Both physicochemical and sensory parameters are used to classify each product in the correct commercial category. The official methodology for sensory evaluation of VOOs, known as a Panel test, has played a fundamental role in their classification (Barbieri *et al*., 2020) since 1991 (Reg. EEC 2568/91). However, despite several modifications occurred over the years, this method still shows some weaknesses as is time-consuming and, in case of non-correct training of assessors (Casadei *et al.* 2021), can be affected by a not satisfactory reproducibility of results among different panels. For this reason, the identification and quantification of volatile compounds of VOOs are of great importance for assessing their quality and instrumental methods, based on the analysis of volatile compounds, can be considered as an interesting tool useful to support the Panel test (Cavalli *et al.,* 2004). In fact, specific volatile compounds have been proposed as markers to detect positive (e.g. fruity) and negative sensory attributes according to their concentrations (Valli *et al.,* 2020). At this purpose, targeted methods based on headspace solid phase microextraction (SPME) with the use of two possible detectors (flame ionization detector and mass spectrometry) are being recently validated (Aparicio-Ruiz *et al*., 2023). Rapid instrumental methods concern gas-chromatographic techniques, such as Flash-GC and Ion Mobility Spectrometry (HS-GC-IMS) can be also useful for this aim permitting a fast pre-classification of samples and increasing the efficiency of quality control analyses (Valli *et al*., 2020).

3. Goals and expected results

The main aim of this research project will be to build chemometrics models to estimate olive oil commercial category, discriminating specifically V and EV olive oils. The samples considered in this doctoral project, will be sensory evaluated by five different panels and classified in a robust way applying a decision tree based on the agreement among panels for commercial category and main perceived defect. In parallel, head-space volatile compounds of oils will be screened by HS-GC-IMS technique (five laboratories having the same kind of instrument will be involved in the trial). The dataset built analysing at least 150 samples will be used to develop estimation models, useful to companies for a rapid screening, particularly in case of samples defining as border line between EV and V. This PhD thesis project can be subdivided into the following activities according to the Gantt diagram reported in Table 1.
A1) Bibliographic research.
A2) Sampling: sample preparation, anonymization by sample coding and shipment of oils to the laboratories involved.
A3) Definition of instrumental and sensory protocols: setting up of analytical protocols for GC-IMS and sensory analysis to be applied in a shared mode by the five different laboratories.
A4) Sensory and instrumental alignment tests:

A4.1) Instrumental alignment: verification of the degree of analytical alignment of the five GC-IMS instruments using specific standards prepared ad hoc (the same analytical protocol has to be applied by the five laboratories participating in the trial).
A4.2) Sensory alignment: check of sensory alignment among the five panels participating in the trial by application of a specific decision tree scheme.
A5) Creation of sensory and instrumental datasets: the dataset will consist of at least 150 samples analysed by both sensory and instrumental analysis (GC-IMS).
A6) Development of chemometric models: estimation models (EV vs V) will be built using the dataset (A5).
A7) Shelf-life study: evaluation of the sensory characteristics and volatile profiles of selected samples (EV and V olive oils) monitored during the shelf-life (0, 6, 12 months) by SPME-GC-MS/FID and sensory descriptive analysis.
A8) Volatile analysis on flavored oils: flavored oils of particular interest for the company will be selected and analysis of volatile profiling and sensory characteristics will be carried out.
A9) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	**Bibliographic research**																		
A2)	**Sampling**																		
A3)	**Definition of sampling, analytical and sensory protocols**																		
A4)	**Sensory and instrumental alignment tests**																		
	A4.1) Instrumental alignment																		
	A4.1) Sensory alignment																		
A5)	**Creation of sensory and instrumental datasets**																		
A6)	**Development of chemometric models**																		
A7)	**Shelf-life study**																		
A8)	**Volatile analysis on flavored oils**																		
A9)	**Writing and editing**																		

4. References

Agriculture and Rural Development (DG AGRI). DASHBOARD: OLIVE OIL, last update 17.03.2023: https://agriculture.ec.europa.eu/system/files/2023-04/olive-oil-dashboard_en.pdf

Aparicio-Ruiz R, Ortiz-Romero C, Casadei E, García-González DL, Servili M, Selvaggini R, Lacoste F, Escobessa J, Vichi S, Quintanilla-Casas B, Pierre-Alain G, Lucci P, Moret, E, Valli E, Bendini A, Gallina Toschi T (2022) Collaborative peer validation of a harmonized SPME-GC-MS method for analysis of selected volatile compounds in virgin olive oils, Food Control 135: 108756.

Barbieri S, Cevoli C, Bendini A, Quintanilla-Casas B, García-González DL, Gallina Toschi T (2020) Flash gas chromatography in tandem with chemometrics: A rapid screening tool for quality grades of virgin olive oils, Foods 9: 862.

Casadei E, Valli E, Aparicio-Ruiz R, Ortiz-Romero C, García-González DL, Vichi S, Quintanilla-Casas B, Tres A, Bendini A, Gallina Toschi T (2021) Peer inter-laboratory validation study of a harmonized SPME-GC-FID method for the analysis of selected volatile compounds in virgin olive oils, Food Control, 123: 107823.

Cavalli JF, Fernandez X, Lizzani-Cuvelier L, Loiseau AM (2004) Characterization of volatile compounds of French and Spanish virgin olive oils by HS-SPME: Identification of quality-freshness markers, Food Chem. 88: 151-157.

EEC 2568791 (1991): Commission regulation (EEC) No. 2568/91 of 11 July 1991 on the characteristic of olive oil and olive-residue oil on the relevant methods of analysis. Brussels, Belgium.

Espadas-Aldana G, Vialle C, Belaud JP, Vaca-Garcia C, Sablayrolles C (2019) Analysis and trends for Life Cycle Assessment of olive oil production, Sustain. Prod. Consum. 19: 216-230.

European Union Commission. Commission Delegated Regulation (EU) 2022/2104 of 29 July 2022 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as Regards Marketing Standards for Olive Oil, and Repealing Commission Regulation (EEC) No 2568/91 and Commission Implementing Regulation (EU) No 29/2012; European Union Commission: Brussels, Belgium, 2012.

Valli E, Panni F, Casadei E, Barbieri S, Cevoli C, Bendini A, García-González DL, Gallina Toschi T (2020) An HS-GC-IMS method for the quality classification of virgin olive oils as screening support for the panel test, Foods 9: 657.

Violino S, Benincasa C, Taiti C, Ortenzi L, Pallottino F, Marone E, Mancuso S, Costa C (2021) AI-based hyperspectral and VOCs assessment approach to identify adulterated extra virgin olive oil, Eur. Food Res. Technol. 247: 1013-1022.

Miglioramento della sostenibilità della filiera olivicolo-olearia: implementazione di metodi innovativi per il controllo qualità dell'olio di oliva e la valorizzazione dei sottoprodotti del frantoio

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Tutor: Prof.ssa Tullia Gallina Toschi; co-tutor: Prof. Enrico Valli e Dott.ssa Matilde Tura

1. Curriculum

Nov 2022 - in corso: Dottorato di Ricerca presso l'Alma Mater Studiorum - Università di Bologna, Dipartimento di Scienze e Tecnologie Agro-Alimentari. Tutor: Prof.ssa Tullia Gallina Toschi; co-tutor: Prof. Enrico Valli e Dott.ssa Matilde Tura.
Set 2020 – Ott 2022: Laurea magistrale in Scienze e Gestione della Natura – curriculum "Global Change Ecology and Sustainable Development Goals" presso l'Alma Mater Studiorum - Università di Bologna. Tesi sperimentale "*Improving water use efficiency of nutrient-dense food cultivation as a part of integrated sustainable agriculture management to promote food security in Huila department, Colombia*", svolta in collaborazione con l'Istituto Italo Latino Americano (IILA) e il Servicio Nacional de Aprendizaje (SENA). Relatore: Prof. Giorgio Prosdocimi Gianquinto; correlatore: Dott. Nicola Michelon.
Set 2017 – Set 2020: Laurea Triennale in Biologia Molecolare presso l'Università di Padova. Tesi compilativa "Biodiversità delle comunità di funghi micorrizici arbuscolari in ecosistemi tropicali". Relatore: Prof. Alessandro Vezzi.
Set 2019 – Ago 2020: Erasmus studio presso l'Università di Coimbra, Dipartimento di Scienze della Vita.

2. Stato dell'arte

La produzione di olio di oliva è in continua crescita a livello mondiale, in particolare nell'area del Mediterraneo. Ciò è importante per l'elevato valore economico del prodotto e la sua rilevanza nella dieta mediterranea, nella quale rappresenta la principale fonte di acidi grassi monoinsaturi (Guasch-Ferré et al., 2014). L'olio di oliva è soggetto al fenomeno dell'ossidazione, che, nel caso degli oli e dei grassi, determina una riduzione della *shelf-life* a causa della formazione di radicali liberi e di *off-flavors*, quale per esempio il rancido (Diaz-Montana et al., 2023). La reazione di ossidazione determina la formazione di prodotti primari e secondari che possono essere determinati, rispettivamente, da una titolazione iodometrica e dalla misura spettrofotometrica delle estinzioni specifiche nell'ultravioletto di dieni e trieni coniugati. Tali determinazioni, riportate nel Reg. (EU) 2022/2105, rappresentano alcuni dei parametri di qualità per poter stabilire la categoria merceologica dell'olio di oliva. Infatti, un olio di oliva può essere classificato extra vergine se vengono rispettati i limiti, stabiliti dall'Unione Europea, relativi a: acidità libera, numero di perossidi, estinzioni specifiche nell'ultravioletto, valutazione organolettica (mediante panel test, in relazione all'intensità dell'attributo fruttato ed alla presenza di difetti) e contenuto in etil esteri degli acidi grassi. Risulta necessario sviluppare nuovi metodi analitici più sostenibili, in particolare con un minor utilizzo di prodotti chimici e solventi. Infatti, ad esempio, la determinazione del numero di perossidi per titolazione presenta numerosi svantaggi quali i tempi lunghi di esecuzione, la quantità di campione necessaria, l'ampio utilizzo di solventi e la produzione di scarti (Longobardi et al., 2021). La risonanza di spin elettronico rappresenta una possibile e promettente alternativa per la valutazione dello stato di ossidazione dell'olio d'oliva, in quanto è in grado di rilevare la presenza di radicali liberi. In letteratura si trovano studi sull'applicazione della risonanza di spin elettronico per valutare la stabilità ossidativa in diversi alimenti e bevande, come caffè, farina di grano, pane, arachidi, latte in polvere, carne di pollo (Andersen et al., 2018) e olio di canapa (Tura et al., 2019).
Per rendere la produzione di olio d'oliva sostenibile è necessario intervenire su tutte le fasi della filiera. Un aspetto molto importante, oltre alle valutazioni di qualità post-produzione, è sicuramente l'elevata quantità di sottoprodotti e scarti che si generano in frantoio. In particolare, viene prodotta una ingente quantità di acque di vegetazione, che presentano un problema a livello ambientale, in quanto il loro potere inquinante causa danni al suolo e alle falde acquifere (Gómez-Caravaca et al., 2014). Tuttavia, essendo ricche di composti bioattivi possono essere reimpiegate: ad esempio è possibile estrarre composti fenolici che, successivamente, possono essere impiegati come componenti funzionali in alimenti, cosmetici o mangimi (Comandini et al., 2015). Inoltre, le foglie d'ulivo rappresentano un altro scarto raramente valorizzato. Un possibile scenario vede impiegati entrambi i sottoprodotti per la produzione di biogas (Romero-Garcia et al., 2014). Tuttavia, l'alto contenuto di lignina nelle foglie presenta un problema per la loro conversione in biogas (Espeso et al., 2021). Data la grande importanza dello sviluppo di una filiera dell'olio d'oliva sostenibile e di frantoi virtuosi, risulta necessario investigare possibili pretrattamenti per rendere anche le foglie d'ulivo un substrato efficiente da poter inserire in un biodigestore insieme alle acque di vegetazione.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di migliorare la sostenibilità della filiera olivicolo-olearia tramite i seguenti obiettivi:
Utilizzo del MicroESR per la valutazione dello stato di ossidazione dell'olio di oliva. Questo metodo è più sostenibile e veloce rispetto ai metodi tradizionali (titolazione iodometrica e determinazione delle estinzioni specifiche nell'ultravioletto di dieni e trieni coniugati).
Riutilizzo dei sottoprodotti della filiera olivicolo-olearia, in particolare utilizzo di foglie d'ulivo e acque di vegetazione per la produzione di biogas.
Il progetto di tesi di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt riportato in tabella 1:
A1) Ricerca bibliografica sulle metodologie ESR e il loro possibile utilizzo per valutare lo stato di ossidazione dell'olio di oliva e sulla valorizzazione dei sottoprodotti dei frantoi;
A2) Messa a punto del sistema modello per MicroESR: correlazione tra risultati delle determinazioni analitiche, strumentali e sensoriali, e del MicroESR. Successivamente, se possibile, si prevede la costruzione di un modello statistico di stima dello stato ossidativo.
A3) Studio dello stato ossidativo: determinazione del numero di perossidi, estinzioni specifiche nell'ultravioletto di dieni e trieni, OSI time, acidi grassi, profilo dei volatili, fenoli totali e analisi sensoriale, effettuate su almeno 100 campioni di oli di oliva; analisidei risultati ottenuti in relazione alla determinazione dei radicali effettuata mediante MicroESR.
A4) Impiego delle foglie di ulivo e acque di vegetazione per la produzione di biogas: prove sperimentali per la digestione della lignina delle foglie d'ulivo e il loro possibile utilizzo per la produzione di biogas.
A5) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazioni orali

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Ricerca bibliografica***																		
A2)	***Messa a punto del sistema modello per MicroESR***																		
	1) Preparazione campioni di olio di oliva per la messa a punto di un sistema modello per il MicroESR																		
	2) Analisi dei campioni di olio d'oliva ossidati al MicroESR, perossidi, analisi spettrofotometrica nell'ultravioletto, OSI time, acidi grassi, profilo dei volatili, fenoli totali, e analisi sensoriale																		
A3)	***Studio dello stato ossidativo***																		
	1) Determinazioni analitiche strumentali e sensoriali																		
	2) Analisi dei risultati ottenuti																		
A4)	***Impiego delle foglie di ulivo e acque di vegetazione per la produzione di biogas***																		
	1) Prove sperimentali per digestione della lignina																		
	2) Prove sperimentali per produzione di biogas																		
A5)	***Preparazione della tesi e di altri contributi***																		

4. Bibliografia

Andersen ML, Skibsted LH (2006) ESR spectroscopy for the study of oxidative processes in food and beverages. Modern magnetic resonance, 1861-1866.

Comandini P, Lerma-García MJ, Massanova P, Simó-Alfonso EF, Gallina Toschi T (2015) Phenolic profiles of olive mill wastewaters treated by membrane filtration systems, Journal of Chemical Technology & Biotechnology, 90(6), 1086-1093.

Díaz-Montaña EJ, Aparicio-Ruiz R, Morales MT (2023) Effect of flavorization on virgin olive oil oxidation and volatile profile. Antioxidants, 12(2), 242.

Gómez-Caravaca AM, Verardo V, Bendini A, Gallina Toschi T (2014) From wastes to added value by-products: An overview on chemical composition and healthy properties of bioactive compounds of olive oil chain by-products. In Virgin Olive Oil: Production, Composition, Uses and Benefits for Man; De Leonardis, A., Ed, pp. 301-334.

Guasch-Ferré M, Hu F B, Martínez-González MA, Fitó M, Bulló M, Estruch R, Longobardi F, Contillo F, Catucci L, Tommasi L, Caponio F, Paradiso VM (2021) Analysis of peroxide value in olive oils with an easy and green method, Food Control, 130, 108295.

Romero-García JM, Niño L, Martínez-Patiño C, Álvarez C, Castro E, Negro M J (2014) Biorefinery based on olive biomass. State of the art and future trends, Bioresource technology, 159, 421-432.

DOTTORANDI ISCRITTI AL II ANNO (XXXVII CICLO)

Investigation on oxidative behaviour of rosé wines as affected by phenolic composition and tannins addition

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Tutor: Fabio Chinnici

1. State-of-the-art

Rosé wine gets its characteristic pink nuances from limited contact with the red grape skins during fermentation. This contact allows the wine to acquire some of the colour and flavour of the grapes, but not enough to make it a full-bodied red wine. The color of rosè wines can be considered the result of a balance between their anthocyanins (pigments) content and other phenolic compounds which can interact each other decreasing or enhancing the overall stability all along the productive and commercial product life. Copigmentation is a phenomenon where colourless compounds interact with anthocyanins to form a more stable and intense colour. It has been shown to play a significant role in the colour stability of red wines (Boulton, 2001) but there is a lack of information on its importance in rosé wines. This association between the pigments and their copigmentation cofactors (also referred to as "copigments" by some authors) involves the anthocyanin glucosides, and certain phenolic acids, flavonoids, and, in particular, derivatives of the flavonol and flavone subgroups. Indeed, copigmentation generally results in an increase in absorbance and, in some cases, a wavelength shift of the pigment's maximum absorbance.
Bottled rosé wine may be exposed to UV-visible light for relatively long periods in retail shops, restaurants, homes and elsewhere. Exposure to light can induce the production of unpleasant aromas (Dozon & Noble, 1989), colour and thus lower the quality and reduce the shelf life of bottled rosé wine. The Fenton reaction involves the presence of hydrogen peroxide (H_2O_2) and a catalyst such as iron (Fe2+) or copper (Cu+) to produce hydroxyl radicals (OH°). Hydroxyl radicals are highly reactive and can oxidize organic compounds, generating oxidation-related decay of foods and beverages (Brillas et al., 2009). The reduced metal can activate oxygen by donating a single electron. Carboxylate anions have a high charge density and generally bind more strongly to iron(III) than to iron(II). It is believed that the formation of iron(III) complexes in wine shifts the iron(III)/iron(II) balance in favour of the iron(III) form, which in turn promotes the reduction of oxygen and its derivatives by iron(II) (Danilewicz, 2003). Photolysis, on the other hand, involves the use of light to break down organic compounds. When light of a certain wavelength is absorbed by a molecule, it can cause it to break down into smaller, less complex molecules. This process is particularly effective for breaking down large, complex organic molecules that are resistant to other forms of degradation. When photolysis is combined with the Fenton reaction, the result, that was called Photofenton, is a process that involves the use of iron and hydrogen peroxide to create hydroxyl radicals, which can oxidize and degrade organic compounds. These hydroxyl radicals can react with various wine components, including phenolic compounds and aminoacids, resulting in a series of chemical reactions that can adversely affect wine quality. For example, hydroxyl radicals can cause oxidation of phenolic compounds in wine, resulting in loss of colour and aroma and alteration of taste. Aminoacids in wine can also be oxidized, resulting in unpleasant flavours and aromas (Santos et al., 2012).
Irradiation conditions and the duration of exposure to light can cause more rapid changes. Bottles are usually exposed to light under different conditions and for different periods of time, which may contribute to variations in sensory attributes and shelf life between bottles. Dark-coloured bottles, such as green and amber bottles, protect more from light than clear and transparent ones, but the latter are sometimes preferred for marketing reasons. Although it is known that bottle colour plays an important role in the storage of rosé wine in bottles that may be exposed to light, the contribution of bottle weight and other factors, such as the degree of UV protection of transparent bottles, are not as well understood (Grant-Preece et al., 2018).
Overall, the chemistry of rosé wines is complex and varied and there is still much to learn about how different grape varieties and winemaking techniques influence their flavour and health properties.

2. Bibliography

Boulton R (2001) The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: a critical review, Am. J. Enol. Vitic. 52: 67-87.

Brillas E, Sirés I, Oturan MA (2009) Electro-fenton process and related electrochemical technologies based on fenton's reaction chemistry, Chem. Rev. 109: 6570–6631.

Danilewicz JC (2003) Review of Reaction Mechanisms of Oxygen and Proposed Intermediate Reduction Products in Wine: Central Role of Iron and Copper, Am. J. Enol. Vit. 54: 123-127.

Dozon NM, Noble AC (1989) Sensory Study of the Effect of Fluorescent Light on a Sparkling Wine and Its Base Wine, Am. J. Enol. Vit. 4: 265–271.
Grant-Preece P, Barril C, Clark AC (2018) Impact of fluorescent lighting on the browning potential of model wine solutions containing organic acids and iron, Food Chem. 243: 239–248.
Santos MC, Nunes C, Saraiva JA, Coimbra MA (2012) Chemical and physical methodologies for the replacement/reduction of sulfur dioxide use during winemaking: Review of their potentialities and limitations, Eur. Food Res. Tech. 234:1–12.

3. Objectives

This research project aims to investigate the mechanisms of formation, evolution and stabilisation of colour in rosé wines. It is also intended to assess the effectiveness of different cellar strategies, capable of enabling the management of this component of the quality of the wines produced, and its maintenance during shelf-life.
In order to achieve the objectives of the dissertation project, the work was divided into the following activities according to the Gantt diagram shown in Table 1:
A1) Bibliographic research on the different distinct and established analytical methodologies for the determination of colour, anthocyanins and tannins in rosé wines.
A2) Evaluation of analytical techniques for colour and tannins that are easy to apply in the production environment, based on spectrophotometric determinations, including: a) acquisition of UV/Vis spectra in absorbance and reflectance; b) vanillin-reactive phenols; c) p-DAC-reactive phenols; d) gelatine-reactive tannin index (astringent power); e) Bate-Smith method; f) methylcellulose method
A3) Anthocyanins and tannins interactions in a model matrix to model the phenomena under study; it is considered important to be able to work under laboratory conditions, using synthetic matrices or white and red musts that have been previously characterised and subsequently tested at different combinations to vary the ratio of pigments, tannins and phenolic acids.
A4) Development of a colour and tannic profile map through the execution of an analytical-sensorial screening of Italian rosé wines, acquired on the market and coming from national territories historically vocated to these products (Bardolino, Valtenesi, Abruzzo, Apulia), but also emerging districts (Tuscany, Sicily) or dedicated to sparkling rosé wines (Lambrusco base in Emilia, Pinot noir base in Franciacorta or Trento).
A5) Evaluation of the influence of maceration time and the presence of oenological tannins of different origins on the evolution of colour over time (spectrophotometry, CIELab profile) and on the phenolic (HPLC-DAD-TOF) and volatile (GC-MS) component of laboratory vinified rosé wines. The study will focus on the phenomenon of copigmentation of wine components with added tannins and the photofenton reaction due to the influence of light on bottled wine.
A6) Meso-scale vinification in at least one of the two vintages following the first year, to study two different oenological techniques applied to rosé wines: sparkling and macro/micro-oxygenation.
A7) Writing and publication of PhD thesis, posters, scientific articles and oral presentation with the publication of at least 2 articles at the end of the project, published in national and international peer-reviewed journals, in the field of food science and technology. At least 1 oral presentation and 1 poster will be published, at the end of the project, concerning the topic of the project carried out at national and international congresses in the field of food science and technology.

Table 1. Gantt chart of PhD research activity

Activity	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***Evaluation of analytical techniques for colour and tannicity***																		
A3)	***Anthocyanin and tannin interaction in model matrix***																		
A4)	***Development of a colour and tannin profile map***																		
A5)	***Evaluation of influence of maceration time and tannin presence***																		
	1) Analysis of the role of copigmentation and photofenton																		
	2) Study shelf life/evolution of the above parameters																		
A6)	***Meso-scale vinification***																		
	1) study of sparkling wine and macro/micro-oxygenation																		
	2) colour, phenols, volatiles analysis of wines and their evolution																		
A7)	***Publications - communications and writing of final thesis***																		

4. Research status and main results

The research so far has yielded interesting results with regard to thermophenton analysis on model matrix samples. These were prepared attempting to obtain a rosé wine realistic colour palette, based on bibliographic data, by blending tannin and oenocyanin stock solutions, obtaining distinct colour intensities spanning from very dark to very light rosé nuances. The

evolution of the phenolic component and the anthocyanin content was studied over time, although particular attention was paid to the oxidation evolution of the solutions, as these were subjected to the Fenton reaction speeded up by adding hydrogen peroxide. Samples were later kept in a cool and dark place during storage to avoid the influence of temperature and light and four sampling points were done. The percentage change of the samples at the end of the test compared to time zero was calculated (Table 2). The evolution of anthocyanins in the different samples yielded interesting results. For example, it has been observed that specific anthocyanins degrade much faster than others and differently from one sample to another. This is probably due to different starting concentrations in the samples. The creation of the colour and tannin profile map is in development at the moment, with the analyses of about forty different rosé from different parts of Italy. The evaluation of influence of tannin presence is taking place in Sevilla (Spain); eight tannins of different origins were separately added on a rosé wine made in laboratory from grape of Sangiovese and Merlot. At the moment, their consume of oxygen is being studied and their capacity to stabilize the colour of the wine. Afterwards, photofenton reaction will be studied on three samples of model wine made from different dilutions of pure oenocyanin and tannin stock solution. The effect of light on the bottled sample will be studied depending on the glass colour of the bottles and the lamp emission spectrum (natural illumination or fluorescent lamp).

	VD		D		I		L		VL	
Colour T480▲										
Hue	39.92 ± 2.71	a	71.89 ± 2.43	b	66.98 ± 0.88	b	60.65 ± 1.07	b	33.20 ± 7.15	a
CD	22.30 ± 5.68	a	31.47 ± 8.33	a	68.96 ± 13.88	b	77.9 ± 14.43	b	121.95 ± 11.62	c
dA%	-26.53 ± 2.83	a	-50.94 ± 2.96	ab	-73.46 ± 2.89	ab	-100.00 ± 0.00	b	-100.00 ± 0.00	b
Polymers contribution%	26.43 ± 0.57	a	19.34 ± 0.47	b	16.38 ± 0.06	c	8.95 ± 0.14	d	2.61 ± 0.27	e
L*	11.59 ± 2.29	a	4.20 ± 3.12	b	0.37 ± 2.47	b	-1.29 ± 2.16	b	-2.09 ± 3.84	b
a*	-23.18 ± 1.98	a	-26.63 ± 1.42	a	-18.98 ± 2.31	ab	-20.79 ± 1.68	b	-4.38 ± 1.62	c
b*	311.96 ± 5.24	a	365.42 ± 4.81	b	250.36 ± 4.56	c	228.91 ± 4.28	cd	213.29 ± 4.12	d
C	-11.21 ± 2.03	a	8.02 ± 2.54	b	79.19 ± 2.35	c	122.62 ± 3.52	d	175.48 ± 3.27	e
H	-69.33 ± 0.81	a	-52.01 ± 0.42	b	-34.85 ± 0.76	c	-25.68 ± 0.48	d	-19.98 ± 1.02	e
TPI	-10.80 ± 3.86	a	-13.59 ± 3.46	a	-17.89 ± 0.61	b	-22.72 ± 0.73	c	-13.77 ± 1.42	a
VRF	-15.69 ± 0.96	a	-19.38 ± 1.43	a	-18.30 ± 0.67	a	-13.46 ± 1.58	a	-16.16 ± 4.59	a
Total anthocyans mg/L	-51.67 ± 2.81	a	-50.57 ± 4.12	a	-50.63 ± 5.01	a	-41.70 ± 3.05	ab	-32.46 ± 5.59	b

Table 2. Changes in colour and phenolic parameters (as percentages) at T480 compared to T0. ▲Colour simulation based on CIELAB parameters was obtained by using the software https://convertingcolors.com/

5. List of publications produced as part of the PhD activity

Baris F., Castro Marín A., De Aguiar Saldanha Pinheiro A.C., Tappi S., Chinnici F. (2023) Efficacy of fungoid chitosan from Aspergillus niger and Agaricus bisporus in controlling the oxidative browning of model white wines. Submitted to: Innovative Food Science and Emerging technologies.

Baris F., Chinnici F. (2023) Oxidative evolution of different model rosé wines as affected by distinct anthocyanin/phenolics ratios. Poster submitted to: Enoforum Italia 2023, Congress to be held in Vicenza (Italy) on May 16/18, 2023.

Applications of Cold Atmospheric Plasma as *Green* Technology for Food Shelf-life Extension

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVII; Anno di frequenza: I
Tutor: Pietro Rocculi;Co-tutor: Filippo Capelli, Silvia Tappi, Romolo Laurita, Santina Romani

1. State-of-the-Art

Starch is a biopolymer that contains amylose and amylopectin chains, and other minor components, and is often used as multipurpose food ingredient (de Oliveira et al., 2018); however, native starch presents several drawbacks such as insoluble granules, tendency to syneresis, gel unstability to heat, acid and shear forces and weak structure (Singh et al., 2011). As a result, starch modification became important to change functionality. Various modification methods have been applied (Zia-ud-Din et al., 2017); however, these methods pose problems related to environmental pollution, food safety, chemical residues, waste disposal, longer treatment time, and costs. To overcome these issues, physical methods, and in particular emerging technologies such as cold plasma (CP) and plasma-activated water (PAW) are also being explored (Ganesan et al., 2021; Laurita et al., 2021). CP is defined as the fourth state of matter, contains a mixture of reactive species that play a key role with promising results in various food applications (Luo et al., 2020). PAW, generated by exposure of water to CP, is characterized by acidic pH, changes in the redox potential, electrical conductivity, and the presence of reactive oxygen and nitrogen species (Laurita et al., 2021), and is used for different applications (Guo et al., 2021). Cold plasma (CP and PAW) technology is considered a smart green technology, that does not involve the generation of hazardous, chemical waste, it has low-costs and therefore is promising for sustainable food consumption patterns and ensuring global food security (Misra et al., 2016). However, limited research works have been reported on starch modification. Therefore, this study aimed to evaluate the effect of cold plasma (gas) and PAW to food modifications for more industrial application.

2. Bibliography

de Oliveira CS, Bet CD, Bisinella RZB, Waiga LH, Colman TAD, Schnitzler E (2018) Heat-moisture treatment (HMT) on blends from potato starch (PS) and sweet potato starch (SPS), J. Therm. Anal. 133:1491-1498.

Ganesan AR, Tiwari U, Ezhilarasi P, Rajauria G (2021) Application of cold plasma on food matrices: A review on current and future prospects, J. Food Process. Preserv. 45:e15070.

Guo D, Liu H, Zhou L, Xie J, He C (2021) Plasma-activated water production and its application in agriculture, J. Sci. Food Agric. 101:4891-4899.

Laurita R, Gozzi G, Tappi S, Capelli F, Bisag A, Laghi G, Gherardi M, Cellini B, Abouelenein D, Vittori S (2021) Effect of plasma activated water (PAW) on rocket leaves decontamination and nutritional value, Innov. Food Sci. Emerg. Technol. 73:102805.

Luo J, Nasiru MM, Yan W, Zhuang H, Zhou G, Zhang J (2020) Effects of dielectric barrier discharge cold plasma treatment on the structure and binding capacity of aroma compounds of myofibrillar proteins from dry-cured bacon, LWT 117:108606.

Misra N, Schlüter O, Cullen PJ (2016) Cold plasma in food and agriculture: fundamentals and applications: Academic Press.

Singh S, Singh N, Ezekiel R, Kaur A (2011) Effects of gamma-irradiation on the morphological, structural, thermal and rheological properties of potato starches, Carbohydr. Polym. 83:1521-1528.

Zia-ud-Din, Xiong H, Fei P (2017) Physical and chemical modification of starches: A review, Crit. Rev. Food Sci. Nutr. 57:2691-2705.

3. Objectives

Within the overall objective mentioned above, this PhD research project can be subdivided into the following activities, according to the Gantt diagram given in Table 1:

A1) **Literature review** of previous studies on the application of cold plasma in food (A1.1), properties and types of plasma (configuration, diagnostic methods, and characterization of plasma) (A1.2);

A2) **Effects of Plasma Activated Water (PAW)** on rheological, thermal, hydration, and pasting properties of three types of starches (A2);

A3) **Effect of plasma-activated Water (PAW)** on the rheological, thermal, and pasting properties of potato starch during annealing treatment (A3);

A4) **Application of cold plasma (CP) to modify the flour technological porperties**, through the set up of a plasma devie that allows constant mixing of the product (A4);

A5**) Development of food product obtained with ingredient modified by plasma,** to understand how observed physical modification affect the behaviour of the ingredient in the formulation (A5):

A6) **Evaluate the safety/toxicity, scalability, and environmental impact of cold plasma,** contributing to the approval and acceptance of cold plasma for commercial purposes (A5):

A7) Writing and editing of PhD thesis, posters, scientific papers, and oral and/or poster communications

Table 1 Gantt diagram for this PhD thesis project.

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Literature Review***																		
	1) Application of Cold Plasma																		
	2) Types and Diagnostic of Plasma																		
A2)	***Application of PAW for Starch Modification***																		
	1) Rhelogical and Pasting Properties																		
	2) Thermal and Functional Properties																		
A3)	***Application of PAW on potato starch modification***																		
	1) Without annealing																		
	2) With annealing																		
A4)	***Application of Atmospheric cold plasma (gas) to modify the whole flour powder***																		
A5)	***Development of product modified with cold plasma***																		
A6)	***Evaluation of Safety, Scale-up, and Environmental Impact of Cold Plasma***																		
A7)	***Thesis and Paper Preparation***																		

4. Progress of research and main results

Plasma-activated water (PAW), generated with a corona discharge at 15kV, 5 kHz for 1 min, and was applied as a strategy to modify the structure and functionality of potato, normal maize, and waxy maize starches. After the PAW was generated, it was mixed with the starches (1:2; starch:PAW), for 20 min. Starches were then evaluated for various charcateristics (rheological, thermal, and functional properties) and by Fourier-transform infrared (FTIR) spectroscopy. The average discharge power was ~167.12W, and the concentrations of the main long-lived species highlighted the production of hydrogen peroxide (1.18 mg/l), nitrites (13.98 mg/l), and a reduction of pH from 6.12 (distilled water) to 3.48. The pasting properties, gel hydration, gel hardness, and viscosity of the properties of potato (P), normal (NM), and waxy maize (WM) native and PAW treated are shown in Tables 1 and 2, respectively. The results for the pasting properties for the P sample, and statistical analysis revealed that PAW-treated samples are characterized by significantly higher values for all parameters compared to the native one, except for the setback viscosity (SBV). Similarly, except for pasting temperature (PT), other values were increased for the NM sample, although differences were found significant only for breakdown viscosity (BV). On the other hand, PAW treatment significantly ($P< 0.05$) reduced the value of peak viscosity (PV), holding strength viscosity (HSV), SBV, and final viscosity (FV) in WM compared to the native one.

Table 2. *Pasting parameters of native and PAW-treated potato (P), normal maize (NM), and waxy maize (WM)*

Starches		PT(°C)	PV(Pa.s)	HSV(Pa.s)	BV(Pa.s)	SBV(Pa.s)	FV(Pa.s)
P	Native	64.19±0.68^{b}	12.34±0.19^{b}	2.73±0.01^{b}	9.61±0.20^{b}	1.73±0.14	4.46±0.14^{b}
	PAW	65.05±0.60^{a}	15.78±0.9^{a}	3.26±0.10^{a}	12.52±0.03^{a}	1.9±0.07	5.16±0.03^{a}
NM	Native	75.54±0.95^{a}	3.67±0.19	1.78±0.04	1.88±0.15^{b}	1.78±0.13	3.57±0.17
	PAW	71.72±0.22^{b}	3.85±0.17	1.83±0.02	2.02±0.15^{a}	1.92±0.14	3.75±0.16
WM	Native	70.72±0.20	4.25±0.12^{a}	1.37±0.03^{a}	2.87±0.10	0.55±0.02^{a}	1.93±0.04^{a}
	PAW	70.63±0.25	3.22±0.18^{b}	0.46±0.07^{b}	2.75±0.13	0.24±0.03^{b}	0.71±0.10^{b}

The effect of PAW treatment on the gel hydration properties namely Swelling Power (SP) and Water Solubility Index (WSI) at 90°C and on the gel hardness is reported in Table 2. PAW treatment is found to significantly ($p<0.05$) change the SP value of starches, from 11.98±1.80, 15.07±0.91 and 12.98±0.23 for native WM, NM, and P, to 22.49±6.86, 16.52±0.23 and 18.07±0.61 in PAW treated samples, respectively. The WSI of starches treated with PAW was higher than the native ones, but it a significant only for NM and WM. Concerning gel hardness, similarly to other parameters, a different behavior was observed in relation to the types of starch. Increased hardness was detected for P and NM starches after PAW treatment, while PAW applied on WM starch caused a decrease. In general, even if the consistency coefficient (k) and the flow index (n) were different for the three analyzed starches, the flow behaviors of all three samples were typical of pseudo-plastic fluids (shear thinning region) because $n<1$. As depicted in Table 3, the correlation coefficients (R^2) was 0.95–0.99 for all samples, indicating a satisfying fit. PAW treatment resulted in a substantial and significant ($P<0.05$) increase in the viscosity value (k) and a decrease of the flow behavior index (n) between native and PAW-treated samples for P and NM samples. On the contrary, the viscosity of WM starch was significantly decreased by the PAW.

Table 3. *Influences of PAW treatment on gel hydration, gel hardness, and flow behavior of native and PAW treated potato (P), normal maize (NM), and waxy maize (WM)*

Starches		SP (g/g)	WSI (g/g)	Hardness (N)	K (Pa.sn)	n	R^2
P	Native	12.98±0.23^b	6.23±0.53	4.26±0.51^b	47.91±0.12^b	0.50±0.03^a	0.99
	PAW	18.07±0.61^a	6.45±0.37	6.90±0.44^a	88.74±1.14^a	0.44±0.02^b	0.99
NM	Native	15.07±0.91^b	10.85±1.29^b	4.67±0.10^b	11.7±0.64^b	0.46±0.01^a	0.99
	PAW	16.52±0.23^a	12.78±1.74^a	5.25±0.23^a	58.56±2.42^a	0.24±0.01^b	0.95
WM	Native	11.98±1.80^b	22.52±3.86^b	0.57±0.05^a	6.85±0.59^a	0.38±0.01	0.99
	PAW	22.49±6.86^a	44.88±2.85^a	0.44±0.02^b	4.63±0.18^b	0.39±0.01	0.99

A research article was submitted, and a second is under preparation. In addition, few experiments are undergoing on the effect of plasma-activated (PAW) water on the rheological, pasting, thermal properties, chemical composition, sorption isotherm, pasting clarity, and starch damage of potato starch with and without annealing treatment.

5. List of publications produced as part of the doctoral study

Gebremedhin G.G, Silvia S, Romolo L, Filippo C, Federico D, Pietro R, Chiara C, Santina R (2023) Effects of Plasma Activated Water (PAW) on Rheological, Thermal, Hydration and Pasting Properties of Normal Maize, Waxy Maize and Potato starches (Submitted and Under Review)

do Amaral Sobral PJ, Gebremariam G, Drudi F, De Aguiar Saldanha Pinheiro AC, Romani S, Rocculi P, Dalla Rosa M (2022) Rheological and Viscoelastic Properties of Chitosan Solutions Prepared with Different Chitosan or Acetic Acid Concentrations, Foods. 2022 Sep 3;11(17):2692.

Gebremedhin G.G (2022) Applications of Cold Atmospheric Plasma as Green Technology for Food Shelf-life Extension. In Proceedings of the XXVI Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, Asti (Italy), 19-21, September, 2022, pp. 89–90.

Production, composition and sensory characterization of new flavoured oils: focus on sustainability

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1. State of the art

Malnutrition is a matter of major concern globally, and it is also addressed by the United Nations in its Agenda 2030 within the Sustainable Development Goals (SDG 2) through the promotion of practices voted to eradicate all its forms (FAO, 2020a).
It is well known that around 1.3 billion tonnes of food are lost or wasted every year globally, nearly one-third being edible parts, mostly from fruits, vegetables and cereals. As a matter of fact, to increase the affordability of healthy diets, the costs of nutritious foods must come down, as their accessibility has to rise (FAO, 2020). One of the strategies to reach this goal is reducing pre-harvest and post-harvest losses and wastes, both in terms of quantity and quality and in each food supply chain, through their valorization, with a resulting increase in sustainability and circularity of the whole food sector (FAO, 2020a).
According to Garn and Leonard (1989), more than 7'000 crop species have been cultivated and domesticated, but no more than 150 species are intensively cultivated for commercial purposes and just three main crops provide 60% of the world's food energy intake (Garn & Leonard 1989). Moreover, the use of local and traditional species can increase agricultural sustainability by reducing the need for external inputs, such as pesticides and fertilizers, and, depending on their species, can also improve soil fertility and the resilience of the entire system against climate change (Imathiu 2021).
One example is hemp cultivation, which produces not only a various end-use product, but it is also carbon-sequestering and highly productive. Thanks to its phytoremediation properties, hemp can be considered a cover crop, being also able to grow without the use of pesticides and with low water demand (Ahmed et al., 2022). The hemp crop is natively from Asia, but due to its characteristics, resistance and resilience, it has been adapted also in Europe and America (Yano and Fu, 2023).
One of the major causes of food insecurity is climate change: the increase in the severity of natural disaster events in such a way it has become fundamental to discover strategies to cope with that. Agroecology is an integrated approach that applies ecological and social concepts for the management of the food sector and the agricultural system, optimizing the interactions between plants, animals, humans and the environment taking into consideration the social aspects for sustainable production (FAO, 2020b). The agroecology approach is coherent with the use of by-products for the formulation of new enriched food and the promotion of knowledge on local biodiversity and raw material; in addition, the improvement of food production processing techniques is another driver towards more efficient and sustainable food-making.
Consumers are more and more attracted by healthy foods; markets are following this trend by highlighting these kinds of products and adding functional ingredients to regular foods (Meyerding et al. 2018).
In this framework, the enrichment of local foods with bioactive compounds would meet the market demand for healthy products, and proper labelling focusing on geographical origin and health and sustainability claims would be a possible driver of economic growth for developing countries (Bradley et al., 2011). The recovery of important compounds, especially using innovative strategies, focused on the valorization of agro-industrial by-products is a growing sector nowadays (Spaggiari et al., 2021). An example is the recovery of lycopene from tomato by-product, which is a powerful antioxidant with several beneficial properties for human health, such as the reduction of heart-related chronic diseases, it can lower the risk of some kinds of cancer and neurological degenerative diseases. Moreover, the extraction of valuable compounds can involve cutin, for pharmaceutical and cosmetics purposes, pectin, for the production of packages and oil production from tomato seeds (Eslami et al., 2023). Sustainable food technologies play a relevant role in determining the overall sustainability of food systems; some of them work without the direct application of thermal energy and use of chemicals such as the cold pressing for the production of unconventional and speciality edible oils, which recently have gained a lot of attention due to their useful and beneficial properties (Vladic et al., 2020). Besides the use of non-thermal technologies, another example of sustainable extraction approach is vacuum distillation which is used on mixtures with thermolabile compounds. It allows operating at low temperatures, avoiding the degradation of compounds in the extract. This technique can be applied to obtain natural products (e.g. essential oils) (Falcao et al. 2012). Different techniques could be applied for the separation of oil fraction from the seeds such as the more sophisticated supercritical fluid extraction and the simpler cold pressing (Vladic et al., 2020).
Spices, herbs but also vegetables, fruits and essential oils can be added to olive oils to produce flavoured oils, in order to improve health properties and sensory characteristics (Sacchi et al., 2017). The addition of such vegetable matrices to

the oil may also promote its oxidative stability thanks to the antioxidants, such as lycopene, which can derive from tomato by-products (Lamas et al., 2022).

2. Bibliography

Ahmed ATMF, Islam MZ, Mahmud MS, Sarker ME, Islam MR (2022) Hemp as a potential raw material toward a sustainable world, A review. Heliyon. 8(30): e08753

Bradley EL, Castel L, Chaudry Q (2011) Applications of nanomaterials in food packaging with the consideration of opportunities for developing countries, Trends Food Sci. Technol. 22: 604-610.

Eslami E, Carpentieri S, Pataro G, Ferrari G (2023) A Comprehensive Overview of Tomato Processing By-Product Valorization by Conventional Methods versus Emerging Technologies.,Foods 12(1): 166.

Falcao MA, Fianco AB, Luca AM, Pereira MAA, Torres FC, Vargas RMF, Cassel E (2012) Determination of antibacterial activity of vacuum distillation fractions of lemongrass essential oil, Pytochem Rev 11: 405-412.

FAO, Food and Agriculture Organization of the United Nations (2020b) Biodiversity for food and agriculture and ecosystem services. Thematic study for the State of the World's Biodiversity for Food and Agriculture. Rome.

FAO, Food and Agriculture Organization of the United Nations, IFAD, UNICEF, WPF, and WHO (2020a). 2020 The state of food security and nutrition in the world. Transforming food systems for affordable healthy diets. Rome.

Garn SM and Leonard WR (1989) What did our ancestors eat?, Nutr. Rev. 47:337- 345.

Imathiu S. (2021) Neglected and Underutilized Cultivated Crops with Respect to Indigenous African Leafy Vegetables for Food and Nutrition Security, J. Food Secur. 9(3): 115-125.

Lamas S, Rodrigues N, Peres AP, Pereira JA (2022) Flavoured and fortified olive oils – Pros and cons. Trends Food Sci. Technol, 124: 108-127.

Meyerding SGH, Kurzdorfer A, Gassler B (2018) Consumer preferences for superfood ingredients. The case of bread in Germany. Sustainability 10: 4667.

Sacchi R, Della Medaglia D, Paduano A, Caporaso N (2017) Characterization of lemon-flavoured olive oil, LWT Food Sci. Technol. 79: 326-332.

Spaggiari M, Dall'Asta C, Galaverna G, Dolores del Castillo Bilbao M (2021) Rice Bran By-Product: From Valorization Strategies to Nutritional Perspectives, Foods 10: 85.

Tran D, Broeckhoven I, Hung Y, Diem My NH, De Steur H, Verbeke W (2022) Willingness to pay for food labelling schemes in Vietnam: a choice experiment on water spinach, Foods 11:722.

Vladic J, Gavaric A, Jokic S, Pavlovic N, Moslavac T, Popovic L, Matias A, Agostinho A, Banozic M, Vidovic S (2020) Alternative to conventional edible oil sources: cold pressing and supercritical CO2 extraction of plum (Prunus domestica L.) Kernel seed, Acta. Chim. Slov. 67: 778-784.

Yano H and Fu W (2023).Hemp: A Sustainable Plant with High Industrial Value in Food Processing, Foods 12(3): 651.

3. Objectives

The PhD research project pursues the main objective to enhance the diversity of food production through the application of sustainable technologies for the development of flavoured oils. This kind of product would be a strategy for food by-products and waste valorization to recover high-added value bioactive compounds, which have positive effects on human health and the stability of oils. At first, the production is at a lab scale, with the opportunity to be transferred to pilot and industrial scale towards a circular economy approach.

A1) **Bibliographic research.**

A2) **Sustainable food technologies:** new flavored oils are prepared using local raw materials, ingredients and treated food by-products and waste. The bioactive compounds, to be incorporated within the new formulations of flavoured oils, are extracted using sustainable techniques (e.g. vacuum distillation, non-thermal drying, …).

A3) **Composition of by-products and flavoured oils** : instrumental analyses of the new products are carried out to characterize them, as well as the by-products (e.g from tomato, hemp and orange) after proper treatments, used as ingredients and flavouring agents.

A4) **Sensory analysis of flavoured oils:** the flavoured oils (hemp and olive oils) are sensory tested to assess the liking and the intensity of some particular attributes; consumer tests are carried out to assess neophobia and sustainability aspects communication.

A5) **Writing of research papers and final thesis.**

Table 1. Gantt diagram related to this PhD project

Activity	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***Sustainable food technologies***																		
	1) Vacuum distillation																		
	2) Non-thermal drying																		
	3) Other technologies																		
A3)	***Composition characterization of food products***																		

	1) Characterization of by-products																	
	2) Characterization of oils																	
	3) Characterization of flavoured oils																	
A4)	***Sensory analysis***																	
	1) Sensory analysis of hemp-flavoured oil																	
	2) Sensory analysis of flavoured olive oils																	
	3) Joint sensory and biometric analysis (research period abroad)																	
A5)	***Writing of research papers and final thesis***																	

4. Research progress and main results

This research is focused on sustainability aspects, related to the valorization of both food and agricultural by-products, which, thanks to their beneficial properties related to the presence of bioactive compounds, can be added as functional ingredients for the preparation of new flavoured oils.

As starting point, the by-products have been characterized: in the case of tomato pomace, obtained from the production of tomato puree, water activity, fat content and humidity have been determined. Tomato pomace has been dried out using a non-thermal technology (by using an air-drier prototype) to improve its stability. Characterized tomato pomaces, one collected from the market and the others dried in the lab, have been powdered and added to olives. The flavouring has been carried out during the malaxation producing flavoured olive oils, both at laboratory and at semi-industrial scale. The produced oils have been sensory evaluated by a trained panel and quality and compositional analyses have been carried out, free acidity, determination of the phenols content, content, semi quali-quantitative analysis of volatile compounds. Tocopherols and carotenoids contents will be determined, during the next moths, by HPLC.

Flavoured olive oils with orange pomace and black pepper have been also produced with the co-milling technique and quality/compositional parameters have been assessed with promising results: total phenols content has resulted higher for the flavoured ones with respect to the related test samples (Figure 1). The descriptive sensory analysis confirmed the presence of secondary positive attributes related to the vegetable matrices added. To scale up the process, a commercial orange by-product powder, obtained by freeze drying, has been collected from the market, to be added during the malaxation of the olives. The recipe has been previously tested at a lab scale and then at a semi-industrial one; quality and compositional parameters, including volatile compounds profile, and descriptive sensory analysis have been carried out, while total phenols compounds quantification is ongoing.

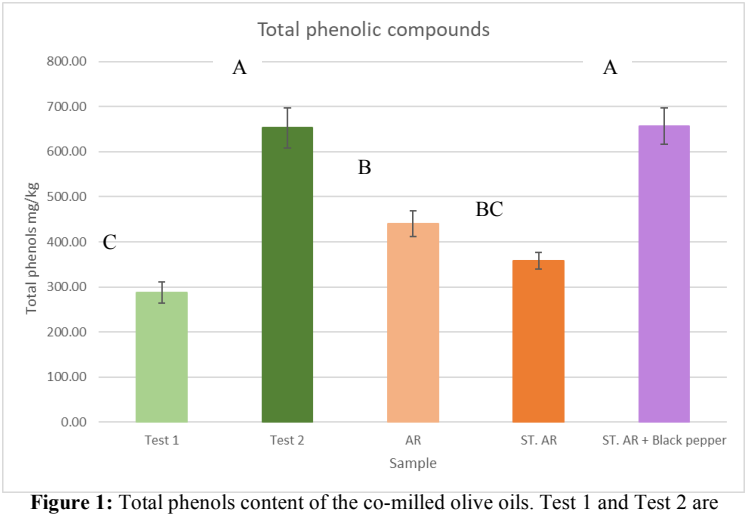

Figure 1: Total phenols content of the co-milled olive oils. Test 1 and Test 2 are prepared with just olives, AR entire oranges + olives, ST AR orange by-product + olives and ST AR + Black pepper with orange by-products, black pepper + olives. Test 1 is the control sample of both AR and ST.AR, while Test 2 refers to ST. AR + Black pepper. Variance analysis ANOVA (tests HSD-Tuckey, α=0.05).

For what concerns the hemp by-product, has been collected from an Italian company that extracts CBD from industrial hemp. It has been hydrodistilled, producing hemp essential oils (EO). EO has been characterized by identifying the main volatile compounds and it has been added to commercial hemp seed oil to produce new flavoured oil. The obtained flavoured hemp seed oil has been used for sensory analysis and consumer tests and its oxidative stability has been determined (Rancimat®).

During the period abroad, biometric tests will be carried out to assess how much consumers like the produced flavoured oils and which emotions may recall, using eye-tracking measurements.

5. Publications produced during the PhD activities

Barlangane C, Gallina Toschi T, Piras S, Suurmets S, Clement J, Tura M, Lazzarini C, Mokhtari N, Elkassia AA, Thabet C, Souissi A, Bendini A (2022) Protocol for a joint auction-eye tracking measurement of consumers preferences for extra virgin oil in Tunisia and Morocco. Proc. of EuroSense 2022, 13-15 September, Turku, Finland.

Lazzarini C, Iacono A, Cellini B, Vannini L, Gallina Toschi T, Valli E, Bendini A (2022) Sustainable drying applied to tomato pomace for preserving its quality during storage. Proc. of the 6th International Conference on Food and Wine Supply Chain (FWSCC), 7-10 June, Bologna.

Lazzarini C, Bendini A, Valli E, Mokhtari N, Elfazizi K, Gallina Toschi T, Nchimbi Msolla S, Setti M (2022). Oli d'oliva aromatizzati mediante co-molitura di olive, pepe nero e polpa di frutti d'arancia: caratterizzazione della composizione, proprietà sensoriali ed aspetti di sostenibilità. Proc. Of SISSG Congress "Edible fats and oils innovation and sustainability in production and control", 15-17 June, Perugia.

Technological, sensory, and nutritional assessment of eco-friendly food lipids

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1. State-of-the-art

The food industry makes intensive use of oils, fats and derivatives of both animal and vegetable origin for a myriad of different products. Worldwide, in 2014, 165 million tons of fatty substances were consumed, and the estimate is that this quantity will double over the next 30 years. After the establishment of the European Regulation 2019/649 (in modification of the previous 1925/2006), the food industry had to pay particular attention to the use of hydrogenated fats to avoid the use of trans fatty acids. Thus, has grown the consumption of palm oil. In 2011, 77,000 tons of palm oil for food use were imported into Italy, which corresponded to 8.4% of the total imported, while over 90% was destined to the production of biofuels. The import of this fat into our country has progressively increased from 40,000 tons / year in the period 2005-2008 to 75,000 in 2009 and 76,000 in 2010 (ISTAT data). This high consumption is linked both to its productivity (4.5 tons of oil / hectare against 1 ton of oil / hectare of the sunflower oil) and to its technological characteristics. This oil has been the subject of criticism both for its nutritional and environmental aspects: the former linked to the presence of toxic newly formed molecules, have disappeared, thanks to the improvement of production and refining techniques which have led to comparable MCPD values for all refined oils (Arris et al., 2020; Chain (CONTAM) et al., 2018). The environmental aspects were addressed with the establishment of the Roundtable on Sustainable Palm Oil (RSPO) which is a multi-stakeholder capable of supplying and certifying sustainable palm oil. Both because certified palm oil is available in fixed quantities and at increased prices, and because the consumer has remained a certain mistrust towards this fat, a new page has opened in the procurement of fatty substances to increase the safety and sustainability of this supply chain and those connected to it. Net of these considerations, and to reduce the ecological, economic, and ethical impact of the industry, it is necessary to enhance the by-products from other supply chains (wine, cereal, and tomato) increasing the sustainability of the recovery of fatty substances also by virtue of of the content in bioactive compounds (tocopherols and sterols in particular). It is therefore important to develop mixtures of oils and fats from by-products, also with the addition of other fats as such or fractionated, suitable for giving the formulations the overall safety characteristics and the required shelf life, with particular attention to lipid oxidation. Particularly interesting, is the possibility of enhancing the by-products of the Italian cereal sector such as rice germ and soft and hard wheat germ, historically an integral part of Italian agricultural production. ISTAT data tell us that in 2020 the production of durum wheat in Italy was about 4 million tons, which must be degeminated in the early stages of milling. Wheat germ represents about 2% of the weight of the caryopsis and contains a quantity of valuable extractable fat (about 15%), with molecules of high organoleptic and industrial interest, such as cis-linoleic acid (C18: 2 cis, about 59.7% of total fats) and the set of monounsaturated fatty acids (MUFAs, about 29% of total fats) (Orsavova et al., 2015). The same consideration can be made for the rice germ, residue of rice husking, which currently amounts to about 2.5 million tons per year in Italy (ISTAT data); rice germ, just like its wheat analogue, has a high quantity of linoleic acid, but also a valuable quantity of palmitic acid (C16: 0, about 20%) (Orsavova, 2015 et al.). Furthermore, Italian agriculture also produces large quantities of grape seeds, from the wine and tomato seed supply chain, from the canning industry. The first is very rich in unsaturated fatty acids, as well as antioxidants and is therefore rather resistant to the thermal stresses typical of frying; the second, on the other hand, in addition to lycopene and beta carotene, contains 25% palmitic acid which, being saturated, has excellent stability. The study of these matrices could lead to the development of mixtures useful for industry, capable of reducing the waste of "good" fats with a consequent increase in the sustainability of the supply chain, also thanks to the reduction of pollution caused by transport.
However, to achieve this goal, it is necessary to study the raw materials, individual oils, extraction and / or co-extraction and possible refining methods at a chemical, sensorial and industrial level, as well as their evaluation in model products compared with food industry standards. Another important part of the research in this area would also be represented by the development of rapid screening methods, possibly non-destructive, and therefore in the construction of reference systems for the evaluation of the quality of fats, with the aim of avoiding or minimizing the use of solvents and reagents.

2. Bibliography

Arris, F. A., Thai, V. T. S., Manan, W. N., & Sajab, M. S. (2020). A Revisit to the Formation and Mitigation of 3-Chloropropane-1,2-Diol in Palm Oil Production. Foods (Basel, Switzerland), 9(12), 1769.

Chain (CONTAM), E. P. on C. in the F., Knutsen, H. K., Alexander, J., Barregård, L., Bignami, M., Brüschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hoogenboom, L. (Ron), Nebbia, C. S., Oswald,

I. P., Petersen, A., Rose, M., Roudot, A.-C., Schwerdtle, T., Vleminckx, C., … Hogstrand, C. (2018). Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. EFSA Journal, 16(1), e05083.

Niu, L.-Y., Jiang, S.-T., Pan, L.-J., & Pang, M. (2013). Characterization of Wheat Germ Oil in Terms of Volatile Compounds, Lipid Composition, Thermal Behavior, and Structure. International Journal of Food Properties, 16(8), 1740–1749.

Orsavova, J., Misurcova, L., Ambrozova, J. V., Vicha, R., & Mlcek, J. (2015). Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. International Journal of Molecular Sciences, 16(6), 12871–12890.

Schuler, P. (1990). Natural Antioxidants Exploited Commercially. In B. J. F. Hudson (Ed.), Food Antioxidants (pp. 99–170). Springer Netherlands.

3. Objectives

This research project aims to study innovative vegetable oil blends to be used in baked products (both sweet and salty) to replace the oils commonly used today by the food industry (palm oil and olive oil). The PhD thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1.

Table 1: Gantt chart of the PhD research activity

Activity	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Study of innovative vegetable oil blends***																		
	1) Compositional and qualitative characterization of oils obtainable from the cereal, wine, and tomato supply chains																		
	2) Formulation of blends of previously characterized oils, also, by fractioning the starting samples																		
A2)	***Production of bakery products with the oil blends developed***																		
	1) Study of oil mixtures replacing palm oil																		
	2) Production of bakery products with the aforesaid oils																		
A3)	***Characterization of the obtained products***																		
A4)	***Economic and ecological evaluation of the alternative oil blends***																		
A5)	***Evaluation of innovative techniques for the analysis of the products and mixtures obtained***																		
A6)	***Bibliographic research***																		

4. Advancements and Results

The PhD project started with the analysis of different types of oils extracted from Italian industrial by-products, such as, wheat germ, rice germ, grapeseed, and tomato seed; said matrices were compared with the data obtained from samples of the most used oils and fats in the baking industry, palm oil, sunflower oil and coconut oil. The oils, obtained from both solvent and mechanical extraction, were characterized by analysing, the methyl-esters of the fatty acids (FAME) by chromatography coupled with revelation using a flame ionization detector (FID) and analysis of the tocopherol content by High-pressure liquid chromatography (HPLC) coupled with detection by fluorescence detector (FLD), to these analysis an extensive bibliographic research on said oils was conducted in order to confirm the data obtained. Wheat germ oil (WGO) is reported to have very high nutritional value. It has the highest tocopherol content of all vegetable oils, up to about 2500 mg/kg (Schuler, 1990), in which α-tocopherol represents around 60%. In addition, it is rich in unsaturated fatty acids, mainly linoleic (18:2) and linolenic (18:3), it is rich in unsaturated ones (83.45%), especially in linoleic acid (64.82%) and oleic acid (13.19%); and it also has a really high content of sterols, squalene, cholesterol, campesterol, β-sitosterol, and fucosterol (Niu et al., 2013). The oil extracted from rice germ (RGO) (*Oryza Sativa L.*) contains about 40% oleic acid and 40% linoleic acid, followed by palmitic acid with 17%. An important feature of rice oil is the high concentration of vitamin E in a 1: 1 ratio between tocotrienols and tocopherols and the presence of gamma oryzanol, consisting of a mixture of ferulic acid esters with plant sterols and triterpene alcohols, it also exhibits a delicate flavor and remarkable stability at elevated temperatures which makes it suitable for cooking methods such as stir-fried and fried foods. Sunflower oil (SO) is characterized by high percentages of unsaturated fatty acids, in particular linolenic acid, which can reach percentages of up to 55-69%. However, precisely because of this high concentration in polyunsaturated acids, sunflower oil is more susceptible to rancidity and lipid peroxidation phenomena during cooking and above all frying processes, making it usable mostly as a raw condiment in foods, for this reason the medium oleic sunflower oil (SOMO) and high oleic sunflower oil (SOHO) variants were also subjected to study. The first is characterized by an oleic acid content of 60-75%, while the second exceeds 75% up to a maximum of 90%, compared to 20% of oleic acid present in sunflower oil as it is. These oils are more resistant to heat and therefore could be suitable substitutes for saturated fats in the preparation of foods that require a fat in liquid form but with high oxidative stability. High oleic sunflower oil is therefore an excellent oil for frying as it is declared stable at temperatures above 200 ° C, unlike traditional sunflower oil. Grape seed oil is composed on average of 90% of monounsaturated and polyunsaturated fatty acids, especially linoleic acid (58-78%), followed by oleic acid and a

smaller amount of saturated fatty acids, grapeseed oil is also rich in tocopherols, which, as already mentioned above, are among the most important natural antioxidants introduced with the diet. The unsaponifiable fraction of grape seed oil contains high phytosterol levels. After the characterization of said oils was conducted, a phase of product formulation came after, using samples normally buyable in convenience stores; said decision was made consequently the delays in the company experience. Five types of "tarallini" and six types of "frollini" biscuits were produced using the following recipes:

Table 2: "tarallini" formulations

Sample	0 flour %	White wine %	Salt %	Fat %
T0	59	24	1	16 (EVO)
T1	59	24	1	16 (SOHO)
T2	59	24	1	14 (EVO) 2 (SO)
T3	59	24	1	12 (EVO) 4 (Rice oil)
T4	59	24	1	14 (SOHO) 2 (Coconut oil)

Table 3: "frollini" biscuits formulation

Sample	00 flour %	Sugar %	Whole eggs %	Chemical yeast %	Fat %
B0	52	16	15	1	16 (PO)
B1	52	16	15	1	16 (butter)
B2	52	16	15	1	8 (butter) 8 (EVO)
B3	52	16	15	1	8 (butter) 8 SOHO)
B4	52	16	15	1	16 (SOHO)
B5	52	16	15	1	14 (coconut oil) 2 (SO)

After product formulation, all the samples were prepared and analysed to check the oxidation ratio of the lipid matrix and the relative content of antioxidants using respectively FAST GC-FID and HPLC-FLD analysis, using the same apparatus as for the crude oils and fats. The results highlighted a major aptitude of the fat matrix for baking products in the T3 and T4 formulation, and B4 and B5. On said products, a conservation monitoring was also applied, using a thermostatic cell, set at 20°C, the samples were stored for different times in ranges varying from 0 to 75 days for "tarallini" and 0 to 355 days for "frollini" biscuits. During these storage periods, peroxide value analysis, volatile compound analysis, oxidated fatty acids (OFA) analysis and general oxidation resistance test, using OXItest were conducted. The next step for the project is the fractioning and study of the lipids which demonstrated sufficient aptitude during the company recruitment period, following that, a comparison between products obtained with the new, fractioned fats, and the previously listed ones is foreseen. It is estimated that during the subsequent year another batch of products will be made to compare the results obtained and eventually get an improvement on the parameters evaluated.

5. List of publications produced in the context of the PhD activity

Ravagli C., Pasini F., Marzocchi S., Caboni M.F., Volatile compounds evolution in vegetable oils subjected to mild thermal stress. In: Proceedings of the 7 MS FOOD DAY Conference. Florence (Italy), 5-7- October 2022 (p.131).

Pasini F., Marzocchi S., Ravagli C., Messia M. C., Caboni M. F., Studio di shelf-life di biscotti con miscele lipidiche diverse, In: proceedings of 12° Convegno AISTEC 2022, Naples (Italy), 15-17 June 2022 (p.96-100 IBSN: 978-88-906680-7-4).

Pasini F., Marzocchi S., Ravagli C., Messia M. C., Caboni M. F., Studio di shelf-life di taralli con miscele lipidiche diverse, In: proceedings of 12° Convegno AISTEC 2022, Naples (Italy), 15-17 June 2022 (p.348-352 IBSN: 978-88-906680-7-4).

Exploring the influence of redox chemistry as driver in precision winemaking

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVII; Anno di frequenza: II
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1. State-of-the-art

Redox potential reflects the oxidation-reduction reactions happening throughout winemaking and aging period. Redox reactions play an important role in the production and aging of wine, and it has quite different consequences for red and white varieties. Red wine can benefit from a certain amount of oxygen exposure by having its bitterness and astringency reduced, but overexposure can be quite harmful(Li and Duan, 2019). However, white wines need to be protected by SO_2 or inert gas from oxidation in order to maintain their fresh, fruity flavor(Gabrielli et al., 2021).

The redox potential is determined by the ratio of oxidative state to reductive state at a fixed temperature, and its values can be modified by many redox active chemicals supplemented during wine fermentation or storage(Liu et al., 2017), which effect on the redox potential and wine quality need to be further investigated and explained. The reduction potential difference(ΔE) can be used to determine whether a redox reaction is thermodynamically favourable or not. A positive ΔE value would favor reaction to the right and a negative value to the left. In wine condition, the oxidation of (+)-catechin by Fe(III) is thermodynamically disfavored since $E_{(+)\text{-catechin/quinone}}$=0.59V and $E_{Fe(III)/Fe(II)}$=0.36V(Danilewicz et al., 2019)., even though tartaric acid in wine lowers the reduction potential of Fe couple(Danilewicz, 2014).

The initial reaction mechanism of redox in wine has been widely accepted. In the presence of transition metals (iron and copper), polyphenols are oxidised in the wine to form quinones and oxygen is reduced to form hydrogen peroxide. The quinone binds to the thiol and reduces the aroma of the wine. Hydrogen peroxide undergoes the Fenton reaction, producing OH·, which oxidizes the first substance encountered at a diffusion rate. Antioxidants such as SO_2 eliminate quinone and hydrogen peroxide and prevent the wine from oxidizing. A better understanding of wine redox may enable control of wine oxidation with the aim of reducing SO_2 utilization.

Polyphenols are the primary substances involved in the oxidation of wine and their quantity and type influence the redox of the wine(Gutiérrez-Escobar et al., 2021). From a structural point of view, polyphenols containing pyrogallol and catechol groups are susceptible to oxidation. The amount of polyphenols that are easily oxidised was determined by cyclic voltammetry to be 112 mg CE/L in white wines and 2 045 mg CE/L in red wines(Kilmartin et al., 2001). The amount of reversible phenols could also have an impact on the antioxidant capacity of the wine(Makhotkina and Kilmartin, 2010).

2. Bibliography

Danilewicz JC (2014) Role of Tartaric and Malic Acids in Wine Oxidation, J. Agric. Food Chem 62: 5149-5155.
Danilewicz JC, Tunbridge P, Kilmartin PA (2019) Wine Reduction Potentials: Are These Measured Values Really Reduction Potentials?, J. Agric. Food Chem 67: 4145-4153.
Gabrielli M, Fracassetti D, Romanini E, Colangelo D, Tirelli A, Lambri M (2021) Oxygen-induced faults in bottled white wine: A review of technological and chemical characteristics, Food Chem 348: 128922.
Gutiérrez ER, Aliaño GMJ, Cantos VE (2021) Wine Polyphenol Content and Its Influence on Wine Quality and Properties: A Review, Molecules 26: 718.
Kilmartin PA, Zou H, Waterhouse AL (2001) A Cyclic Voltammetry Method Suitable for Characterizing Antioxidant Properties of Wine and Wine Phenolics, J. Agric. Food Chem 49: 1957-1965.
Li SY, Duan CQ (2019) Astringency, bitterness and color changes in dry red wines before and during oak barrel aging: An updated phenolic perspective review, Crit Rev Food Sci Nutr 59: 1840-1867.
Liu, CG, Qin JC, Lin YH (2017). Fermentation and Redox Potential. Intech 1-21.
Makhotkina O, Kilmartin PA (2010) The use of cyclic voltammetry for wine analysis: Determination of polyphenols and free sulfur dioxide, Anal Chim Acta 668: 155-165.

3. Objectives and Milestones

The overall objective of this PhD project is to investigate the effect of redox reactions on wine composition in terms of colour components, other non-volatiles and volatiles compounds. Searching optimal redox potential setting is needed for more efficient wine fermentation and driving winemaking operations to enhance the quality and shelf-life of wine.

In this view, the current PhD project includes several experimental trials with the specific aims (i) to better understand redox reactions in wine, either in terms of mechanism and rate; (ii) to identify the effects of redox potential on wine composition, and ultimately (iii) to exploit the application of redox potential measurement and control for enhancing wine ageability, most probably due to a balanced equilibrium between redox moieties at molecular level.

The doctoral thesis project can be divided in the following activities, summarized in the Gantt chart shown in table 1:

A1) Lab training and Literature review about thermodynamics and kinetics of redox reaction in wine.
A2) Design of Experiment (DoE) as scientific approach to optimize the number of trials that later will be evaluated by statistical methods.
A3) Experimental trials on wine model solution to assess the electrochemical properties of individual reactants, e.g. tannin, yeast lees, mannoprotein, etc.
A4) Experimental trials on real white wines - white, rosé and red - to improve their shelf-life depending on the type of antioxidant, packaging and storage conditions, assessed by physico-chemical and sensory analysis.
A5) Statistical analysis of results, both univariate and multivariate, to find optimal redox potential values where shelf-life of wines is favoured.
A6) Writing and publication of doctoral theses, posters, scientific papers and oral presentations.

Table 1. Gantt Chart for the research activities in scope of doctoral study

Attività	Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	**Lab training and literature review**																		
	Laboratory Training																		
	Literature review in wine redox																		
A2)	**Design of Experiment**																		
	Selection of variables and levels to develop model system approach																		
A3)	**Experimental trials on wine model**																		
	1) assess the electrochemical properties of individual reactants																		
	2) interactions between reactants																		
A4)	**Experimental trials on real wines**																		
	improve wine shelf-life depending on the type of antioxidant																		
A5)	**Statistical analysis**																		
	Univariate and multivariate approaches																		
A6)	**Publication of manuscripts and thesis**																		

4. Research progress and main results

Literature research

Understand the application of thermodynamic principles such as reduction potential, equilibrium constant, Gibbs free energy, Nernst equation in solving redox problems in wine and the rate of consumption of redox substances such as oxygen O_2, SO_2 under different conditions.

The amount and type of phenolics in a wine affects its antioxidant capacity. Different amount and type of phenolic content of white wines(in the case of Sauvignon Blanc) in the literature was surmised in Table 2, providing a basis for future electrochemical assessment of phenolics.

Table 2. The nine most abundant phenolics in Sauvignon Blanc white wines

Type	Average Value(mg/L)
(+)-catechin	4.5
(-)-epicatechin	7.34
(+)gallocatechin	23.07
(-)-epigallocatechin	5.45
gallic acid	4.5
coutaric acid	12.55
caftaric acid	27.65
protocatechuic acid	6.17
tyrosol	19.36

Reduction potentials of common redox pairs was listed in Table 3.

Table 3 Reduction potentials of redox pairs in wine and model wine

Couple	**E_0**	**$E_{3.5}$**	**$E_{3.6}$**
caffeic acid	816mV	620mV	604mV
(+)-catechin	789mV	590mV	577mV
(-)-epicatechin	780mV		568mV
(+)-gallocatechin			463mV
(-)-epigallocatechin			463mV
gallic acid	794mV		582mV
pyrogallol		470mV	468mV
catechol	790mV	580mV	
ascorbic acid		190mV	
$O_2/H2O_2$		570mV	565mV
quercetin	779mV		567mV
4-methylphenol			541mV
1,2-benzenediol	793mV	587mV	582mV
$HO\cdot/H_2O$	2.7V		
$SO_5^{-\cdot}/HSO_5^-$		1.17V	
caftaric acid			very similar to catechin
Fe^{3+}/Fe^{2+} (in tartarate)		360mV	345mV
Fe^{3+}/Fe^{2+} hexa-aqua ions	770mV		604mV
1,4-benzenediol	704mV	497 mV	577mV

DOTTORANDI ISCRITTI AL III ANNO (XXXVI CICLO)

Biotechnological valorization of residues and by-products from agro-food industries

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1. Stato dell'arte

Ad oggi, secondo Eurostat (Agriculture, foresty and fisheries statistics, 2020), nella sola Europa vengono generati oltre 22,4 milioni di tonnellate di rifiuti dalla produzione di alimenti e bevande. Questo è dovuto al fatto che gli scarti e sottoprodotti di lavorazione di diversi alimenti possono rappresentare anche oltre il 50% della materia prima. Tra quelli più importanti vi sono i semi, le bucce, i baccelli, la pula, la sansa, il mallo, le bucce, i gambi. Ad oggi le maggiori destinazioni di uso possono essere il riutilizzo per la mangimistica o per la produzione di energia, oltre all'estrazione di alcuni prodotti ad alto valore aggiunto; tuttavia, la maggior parte dei sottoprodotti è inutilizzata e scartata (Maurya *et al*., 2015), nonostante siano ricchi di sostanze che possono essere recuperate o utilizzate, come zuccheri semplici e complessi, lipidi, proteine, micronutrienti, oli essenziali, fibre alimentari (Mejri *et al.*, 2018). Negli ultimi decenni l'attenzione alla dieta e alla salute è diventata il focus della vita di tutti i giorni, e il consumo di alimenti a basso contenuto di grassi, con minor apporto calorico e ricchi di fibre e antiossidanti ha attirato l'attenzione di numerosi consumatori in quanto contribuiscono in modo significativo alla riduzione del colesterolo e alla prevenzione di malattie cardiovascolari e la stipsi (Aslam *et al.,* 2014). Nonostante la loro ricchezza in fattori nutrizionali, essi presentano delle problematiche legate alla poca biodisponibilità delle diverse componenti. Per questo motivo, l'utilizzo di fermentazioni di precisione con ceppi che presentano delle caratteristiche metaboliche specifiche in funzione di quelle chimico-fisiche e compositive dei sottoprodotti, quali attività amilasica, proteolitica, lipolitica o capacità di produrre fenoli (Aruna, 2019), può essere sfruttata come strategia per migliorare le proprietà del sottoprodotto e, conseguentemente, del prodotto finito (Hu *et al.,* 2022). Inoltre, l'utilizzo in alimenti convenzionali di questi sottoprodotti, sia tal quali che dopo fermentazione, può migliorarne le proprietà funzionali, ad esempio favorendo un aumento del contenuto in fibre, dell'attività antiossidante o prebiotica, oltre che prolungarne la shelf-life. Infatti, l'industria alimentare è alla continua ricerca di nuove strategie, anche attraverso la sostituzione dei conservanti tradizionali ad esempio con sostanze naturali, che vadano a soddisfare il desiderio del consumatore di prodotti freschi e "naturali", mantenendo sempre gli standard di sicurezza, qualità e stabilità dell'alimento.
In quest'ottica sono stati scelti 2 diversi di sottoprodotti - quelli dell'industria agrumaria (buccia e semi di arancio e clementine) e quelli del melograno (bucce e arilli). Per quanto riguarda gli agrumi, questi sono colture frutticole ampiamente coltivate con 14,49 milioni di tonnellate prodotte nel mondo nel 2019 (FAOSTAT 2020). A differenza di altri frutti, la porzione commestibile degli agrumi è bassa rispetto a quella non commestibile: infatti, quest'ultima risulta essere circa il 50-60% e comprende principalmente semi, buccia e residui dell'estrazione del succo. Per quanto riguarda la produzione mondiale di melograno, questa risulta essere di circa 6 milioni di tonnellate all'anno di cui circa il 40% è costituito da bucce. Grazie al recupero di estratti ricchi di antiossidanti, esistono molte applicazioni commerciali per i derivati della buccia di melograno, tra cui si possono citare l'uso nel settore farmaceutico, in tinture e alimenti, in agenti terapeutici antitumorali e la sintesi di ossido di rame (Witt *et al.,*2022). Inoltre, è stata inclusa nella sperimentazione anche una pianta infestante, l'*Equisetum arvense* o codacavallina, in quanto, per le sue proprietà benefiche, è utilizzata a scopo fitoterapico per uso interno per il trattamento di infiammazioni del cavo orale, tonsillite, per la cura dell'acne, herpes labiale ed altre affezioni grazie alle sue note proprietà astringenti, diuretiche, antinfiammatorie, antibatteriche, antimicrobiche e antiossidanti.

2. Bibliografia

Aruna TE (2019) Production of value-added product from pineapple peels using solid state fermentation, Innov. Food Sci. Emerg. Technol. 57: 102193.

Aslam HKW, Raheem MIU, Ramzan R, Shakeel A, Shoaib M, Sakandar HA (2014) Utilization of mango waste material (peel, kernel) to enhance dietary fiber content and antioxidant properties of biscuit, J. Glob Innov. Agric. Soc. Sci. 2(2): 76-81.

Eurostat (2020) Agriculture, forestry and fisheries statistics- edizione 2020

FAOSTAT (2020) The Food and Agriculture Organization of the United Nations.

Hu X, Zeng J, Shen F, Xia X, Tian X, Wu Z (2022) Citrus pomace fermentation with autochthonous probiotics improves its nutrient composition and antioxidant activities, LWT. 157: 113076.

Maurya AK, Pandey RK, Rai D, Porwal P, Rai DC (2015) Waste product of fruits and vegetables processing as a source of dietary fibre: A review, Trends Biosci, 8(19): 5129-5140.

Mejri F, Selmi S, Martins A, Benkhoud H, Baati T, Chaabane H, Nijm L, Serralheiro MLM, Rauter AP, Hosni K (2018) Broad bean (*Vicia faba* L.) pods: a rich source of bioactive ingredients with antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic and health-promoting properties, Food Funct. 9(4): 2051–2069.

Witt EDP, Johann G, Triques CC, da Silva EA, da Silva C, Borsato D, da Silva Baumgartner TR (2022) Potential application of supercritical extract of pomegranate peel as bio-antioxidant for biodiesel, Environ. Prog. Sustain. 41(2), e13755.

3. Sviluppo della ricerca

La ricerca è stata sviluppata secondo i seguenti punti principali:

1) **Caratterizzazione di sottoprodotti alimentari,** quali pastazzo di agrumi, sansa di olive, residui della lavorazione di frutta e verdura; i sottoprodotti vengono valutati in sistema modello per alcuni caratteri di bioattività, ed in particolare la capacità antiossidante, antimicrobica, prebiotica.
2) **Valorizzazione dei sottoprodotti attraverso fermentazioni guidate** con ceppi selezionati di lieviti e batteri lattici appartenenti alla Collezione di Colture Microbiche Industriali del Dipartimento. Si realizzano fermentazioni allo stato solido valutando la capacità dei ceppi di incrementare la bioattività dei sottoprodotti o di produrre metaboliti ad azione antimicrobica.
3) **Formulazione di alimenti con i sottoprodotti:** prodotti lattiero-caseari e da forno vengono formulati con l'aggiunta dei sottoprodotti, tal quali o fermentati, e prodotti in scala da laboratorio. I prodotti così ottenuti sono analizzati durante la conservazione per valutare l'evoluzione del microbiota e delle caratteristiche qualitative e nutrizionali rispetto a prodotti di controllo di riferimento. Si realizzano inoltre dei challenge test con i microrganismi patogeni di riferimento per i diversi alimenti per verificare l'azione antimicrobica dei sottoprodotti quando impiegati come ingredienti alimentari.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

	Attività / Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	**Ricerca bibliografica**																		
A2)	**Caratterizzazione dei sottoprodotti alimentari**																		
	Attività antimicrobica e antifungina																		
	Attività antiossidante																		
	Attività prebiotica																		
	Caratteristiche tecnologiche																		
A3)	**Valorizzazione dei sottoprodotti attraverso fermentazioni guidate**																		
	Selezione dei microrganismi e dei ceppi																		
	Fermentazione allo stato solido																		
	Caratterizzazione funzionale delle matrici fermentati																		
A4)	**Formulazione di alimenti con i sottoprodotti**																		
	Formaggio fresco																		
	Latte fermentato																		
	Prodotti da forno																		
A5)	**Convegni, scrittura articoli e tesi di dottorato**																		

4. Principali risultati

Il presente progetto di ricerca si propone di valutare il possibile utilizzo di sottoprodotti del settore agro-alimentare, valorizzandone alcune caratteristiche interessanti sia in materia di componenti nutrizionali, sia in termini di funzionalità, ad esempio, nel miglioramento della sicurezza, qualità o nel prolungamento della shelf-life del prodotto al quale vengono addizionati come ingredienti. La strategia adottata per tale scopo prevede l'utilizzo di tali matrici tal quali o a seguito di fermentazioni guidate ad opera di microrganismi selezionati al fine di incrementarne la bioattività.

1. Caratterizzazione dei sottoprodotti

Le matrici prese in esame sono 2 diversi raccolti di *Equisetum arvense,* 2019 e 2021, (**eq_19** e **eq_21** rispettivamente), 2 campioni di pastazzo di agrumi di 2 diversi anni (2012 e 2021) (**citrus_12** e **citrus_21**), bucce di melograno edibile raccolte in Emilia-Romagna (**melo_ed_ER**) e nelle Marche (**melo_ed_MA**), bucce e arilli di melograno del tipo ornamentale raccolti nelle Marche (**melo_orn_MA**) e arilli ottenuti dalla spremitura del melograno (**arilli_MA**). Gli estratti metanolici sono stati valutati per: i) il contenuto fenolico (test di Folin-Ciocalteau – TPC); ii) l'attività antiossidante (test DPPH e $ABTS^{+}$); iii) l'attività antimicrobica nei confronti di 48 ceppi di generi e specie microbiche differenti includenti microrganismi patogeni, degradativi, probiotici e starter sia mediante la metodica dell'overlay assay, sia per la determinazione della MIC. Le matrici (non estratte) sono anche state valutate per la loro funzione prebiotica (prebiotic activity score assay) e per la possibile attività di protezione nei confronti di probiotici alle condizioni del tratto gastro-intestinale, e caratterizzate per la loro componente volatile mediante analisi SPME- GC/MS.

I campioni di bucce di melograno sono risultati essere quelli con i più alti valori di TPC compresi tra 3500 e 6300 GAE (mg/l), mentre gli arilli e i campioni di equiseto hanno mostrato contenuti inferiori (112 e 430 GAE(mg/l) rispettivamente). I valori di TPC sono in accordo con quelli dell'attività *scavenging*: infatti i campioni con il più alto contenuto di fenoli hanno presentato i minori valori di IC50. Per quanto riguarda l'attività antimicrobica, quasi tutti i ceppi sono risultati sensibili ai sottoprodotti anche se con differenze specie e ceppo- dipendenti. **Melo_ed_ER, melo_ed_MA** e **melo_orn_MA** sono risultati i più attivi nei confronti di patogeni, mentre **eq** e **citrus** nei confronti dei lieviti (Figura 1). È interessante notare come invece i batteri lattici sono risultati resistenti alla maggior parte delle sostanze testate. Nel complesso, tali risultati possono essere spiegati considerando i valori di TPC e l'analisi dei composti volatili di questi sottoprodotti che mostrano elevate quantità di terpeni, ad esempio D-limonene e (S)-D-carvone, ampiamente riconosciuti come agenti antimicrobici.

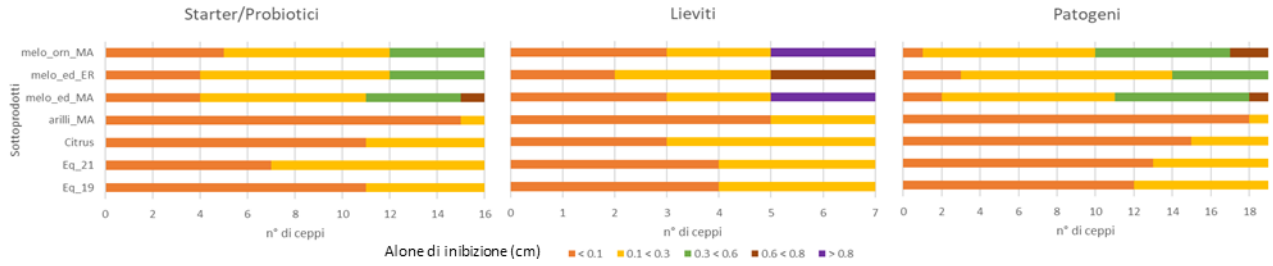

Figura 1. Valori medi di aloni di inibizione (cm) delle varie sostanze nei confronti dei ceppi microbici.

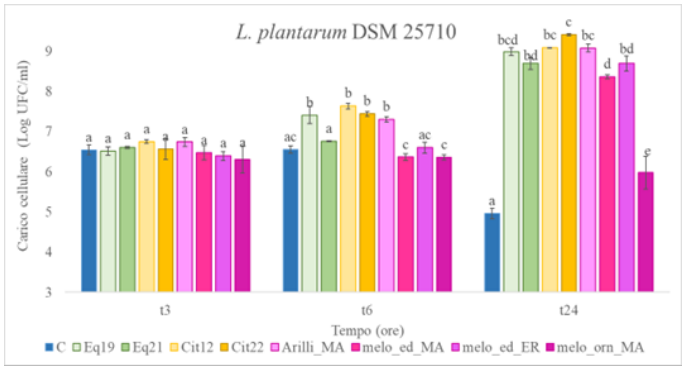

Figura 2. Attività prebiotica delle sostanze nei confronti della vitalità cellulare di *L. plantarum* in sistema simulante il fluido intestinale ($p<0,05$)

I sottoprodotti, quando testati per l'attività prebiotica nei confronti di *Lactiplantibacillus plantarum* DSM 25710 e *Bifidobacterium animalis* subsp *lactis* BB-12, sono risultati promettenti in quando in grado di proteggere e favorirne lo sviluppo in una simulazione del tratto gastro-intestinale. In particolare, tutti i sottoprodotti hanno promosso lo sviluppo del ceppo di *L. plantarum* di circa 3 cicli logaritmici ad eccezione del campione di **melo_orn_MA** che è risultato comunque significativamente diverso dal controllo che ha presentato una riduzione della vitalità cellulare dopo 24 ore (Figura 2). Una condizione simile è stata osservata anche per il ceppo di *Bifidobacterium animalis* subsp *lactis*.

2. *Fermentazione su stato solido di bucce di clementine da coltivazione biologica*

Una seconda fase della ricerca è stata quella di bio-trasformare le bucce di clementina biologiche, provenienti dalla produzione di succo di frutta, attraverso l'inoculo di diverse specie microbiche e in particolare 19 lieviti e 12 batteri lattici (LAB) appartenenti a generi e specie differenti appartenenti alla Collezione Microbica del DISTAL. I campioni sono stati fatti fermentare per 6 giorni e il processo è stato monitorato per la crescita microbica, l'acidificazione, il TPC, l'attività antiossidante ($ABTS^+$), il profilo in molecole volatili (SPME- GC/MS). Tutti i ceppi di LAB e la maggior parte dei lieviti (14 su 19) sono stati in grado di sopravvivere e crescere - anche con un aumento finale di oltre 2-3 Log UFC/g - su residui di clementina senza l'aggiunta di qualsiasi altro nutriente nonostante la matrice presenti delle caratteristiche avverse allo sviluppo dei microrganismi: pH basso (3.5-4.0), acidi organici, oli essenziali, carboidrati poco fermentescibili. Inoltre, la maggior parte dei ceppi durante la fermentazione ha aumentato il contenuto totale di fenoli, i quali sono ampiamente riconosciuti come antinfiammatori e antimicrobici. In generale, per i campioni fermentati con i diversi ceppi, è stato rilevato un accumulo di molecole volatili come alcoli e terpeni che presentano aromi positivi che possono essere sfruttati come agenti aromatizzanti.

3. *Uso di sottoprodotti per la produzione di un formaggio 'Primo Sale'*

Citrus_22 (2% p/p) e **Eq_21** (1% p/p) sono stati utilizzati come ingredienti per la produzione di formaggio "Primo Sale" addizionato di ceppi di LAB (*Lactiplantibacillus plantarum* B39.1.4A, *Pediococcus pentosaceus* B39.2.2A, *Enterococcus faecalis* B39.2.2B) (~7 Log UFC/g). La vitalità della flora microbica dei diversi formaggi e degli starter deliberatamente inoculati è stata monitorata durante una conservazione di 29 giorni a 4°C. I campioni sono stati analizzati anche per il contenuto fenolico e per l'attività antiossidante mediante test $ABTS^+$. Inoltre, è stato valutato l'impatto sul volatiloma durante la conservazione attraverso analisi SPME- GC/MS. Durante la conservazione si è rilevato come l'aggiunta dei sottoprodotti tal quale non abbia influito sulla vitalità degli starter; d'altra parte, si è osservata un'inibizione dello sviluppo di alcuni gruppi microbici come, ad esempio, le *Enterobacteriaceae* e *Pseudomonas* spp. (Figura 3). In particolare, per queste ultime si è rilevato un minor sviluppo già a seguito dell'aggiunta degli starter; tuttavia, la presenza di **eq** e **citrus** ha ulteriormente contribuito all'inibizione di oltre 3 cicli logaritmici rispetto al controllo con i LAB e di oltre 6 cicli rispetto al controllo senza gli starter.

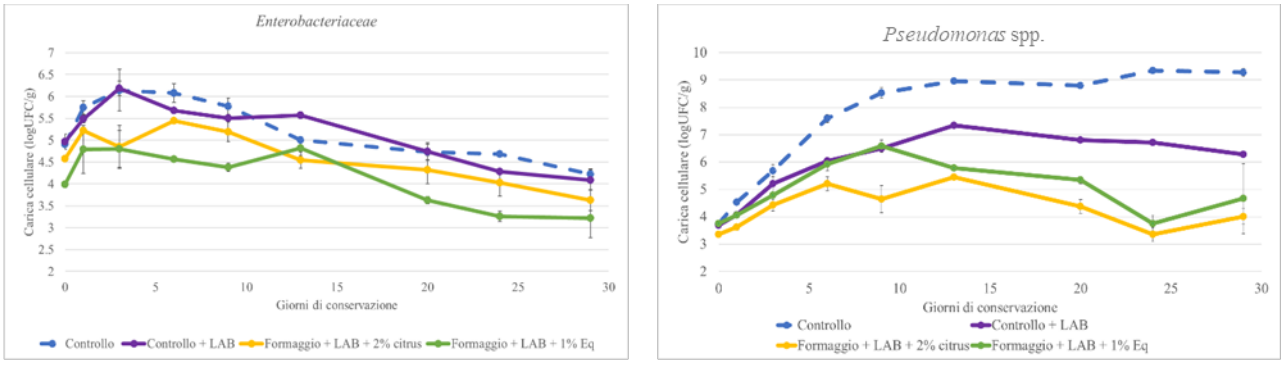

Figura 3. Cariche cellulari di *Enterobacteriaceae* e *Pseudomonas* spp. durante la conservazione del formaggio "Primo Sale" addizionato con Citrus, Eq, rispetto ai controlli.

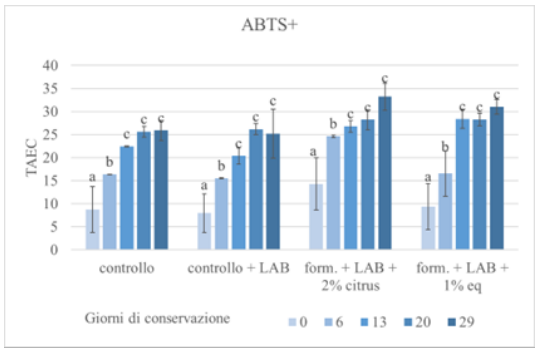

Figura 4. Attività antiossidante dei formaggi di controllo e addizionati con sottoprodotti durante il periodo di conservazione ($p<0,05$)

Per quanto riguarda la caratterizzazione del contenuto fenolico dei campioni, si è osservato come l'aggiunta di **citrus** abbia determinato un aumento di sostanze fenoliche di circa 50 ppm AGeq. rispetto al controllo. Inoltre, il contenuto fenolico di tutte le matrici è aumentato nel tempo raddoppiando alla fine della conservazione (da ~150 a 330 ppm AGeq.). Tale comportamento è stato osservato anche per l'attività antiossidante risultando significativamente differente intra campione per i diversi tempi di conservazione (Figura 4). Il maggiore incremento in contenuto fenolico è stato riscontrato per il campione contenente **citrus** che, da concentrazioni di 180 ppm AGeq., ha raggiunto un valore finale di circa 500 ppm AGeq. Inoltre, il formaggio con **citrus** ha presentato un'attività antiossidante maggiore rispetto al controllo e a tutti gli altri campioni; infine, nonostante il campione con **citrus** abbia mantenuto le migliori performance come attività antiossidante, anche il campione con **eq** ha presentato differenze significative rispetto al controllo. Per quanto riguarda l'analisi delle molecole volatili, il contenuto in composti appartenenti alle classi dei chetoni e degli acidi è aumentato durante la conservazione, mentre quello degli alcoli è diminuito dopo i primi giorni. È inoltre rilevante evidenziare come il campione con il **citrus** abbia presentato un contenuto di terpeni 100 volte maggiore rispetto agli altri campioni dovuto principalmente alla presenza di D-limonene, e come tale caratteristica abbia influenzato positivamente l'aroma del prodotto.

5. Elenco delle pubblicazioni prodotte nell'ambito dell'attività di dottorato

Contributi a convegni

Cellini B, Giordano E, Patrignani F, Malerba A, Fratebianchi De La Parra D, Armendariz Ferrandez N, Virto Resano R, Echeverria Goni I, Romani S, Dalla Rosa M, Lanciotti R, Rocculi P, Vannini L (2023) Biotechnological valorisation of by-products from clementine juice production into functional ingredients, Presentazione orale - Proc. of VIII international conference sustainable postharvest and food technologies - INOPTEP 2023 and XXXV scientific - professional conference processing and energy in agriculture - PTEP 2023 Subotica – Palić, 23 – 28 April 2023, pp.26.

Cellini B. (2022) Biotechnological valorisation of residues and by-products from agro-food industries. Poster - Proc. of XXVI Workshop on the developments in the Italian PhD research on food science technology and biotechnology, University of Turin, Asti, September 2022, pp. 220-221.

Cellini B, Gozzi G, Vannini L (2021) Valorisation of olive pomace and tomato by-products with selected yeasts strains. Poster - Proc. of 6th International Conference on Microbial Diversity 2021 - Advances in Microbial Diversity MD2021, San Casciano Val di Pesa, Firenze, Italy, SIMTREA, Società Italiana di Microbiologia Agro-Alimentare e Ambientale, 2021, pp. 55.

Vannini L, Gozzi G, Bruno V, **Cellini B** (2021) Valorization of agri-food by-products as functional ingredients for soft cheeses. Presentazione orale - Proc of 2nd International E-Conference on Nutrition and Food Science, 2021, pp. 21.

Gozzi G, **Cellini B**, Bruno V, Lanciotti R, Vannini L (2021) Valorisation of food by-products into functional "primo sale" cheese. Poster - Proc. 6th International ISEKI-Food Conference "Sustainable Development Goals in Food Systems: Challenges and Opportunities for the Future", 2021.

Cellini B. (2021) Biotechnological valorisation of residues and by-products from agro-food industries. Poster - Proc. of First Virtual (XXV) Workshop on the developments in the Italian PhD research on food science technology and biotechnology, University of Palermo, September 2021.

Studio e realizzazione di prodotti ittici innovativi attraverso l'applicazione di tecnologie di processo emergenti

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVI; Anno di frequenza: II
Tutor: Prof. Marco Dalla Rosa; Co-tutor: Prof. Pietro Rocculi; Prof.ssa Santina Romani

1. Stato dell'arte

Grazie alla crescente domanda da parte dei consumatori di alimenti minimamente lavorati e con una shelf-life estesa, sia la ricerca scientifica che quella industriale stanno studiando l'applicazione di processi innovativi per garantire prodotti alimentari sicuri e di alta qualità. Tra le varie categorie alimentari, i prodotti ittici sono stati oggetto di un significativo aumento della domanda, grazie alle loro interessanti proprietà nutrizionali. La FAO ha recentemente effettuato delle proiezioni preliminari per le possibili situazioni di pesca e acquacoltura entro il 2050. Sono state proposti 3 scenari sulla base delle aspettative di crescita del settore: scenario "*business-as-usual*", scenario a "crescita bassa" e scenario a "crescita alta". Lo scenario "*business-as-usual*" è considerato il più plausibile dalla FAO, mentre gli altri due sono stati proposti per mostrare l'intervallo delle possibilità previste. Secondo lo scenario più probabile, nel 2050 saranno pescati circa 98 milioni di tonnellate di prodotti ittici, di cui principalmente da cattura marina. Invece ci sarà una produzione di circa 140 milioni di tonnellate di prodotti ittici da acquacultura. Infine, si stima un consumo di circa 217 milioni di tonnellate di prodotti ittici da parte dell'uomo (SOFIA, 2022).

I prodotti ittici freschi sono altamente deperibili a causa della loro composizione biochimica. Per estenderne la shelf-life, potrebbero essere commercializzati prodotti ottenuti previo trattamento termico, che se da un lato fornisce un'inattivazione efficiente della carica microbica, dall'altro causa perdite significative di composti termolabili, e talvolta cambiamenti negativi nelle caratteristiche sensoriali, fisico-chimiche e nutrizionali degli alimenti. Al contrario, attraverso l'uso di trattamenti non termici emergenti, potrebbe risultare possibile migliorare la qualità igienica e sanitaria dei prodotti evitando le modificazioni promosse dall'utilizzo delle alte temperature, oltre a ridurre i costi energetici associati a questa operazione tecnologica (Ekonomou e Boziaris, 2021). In particolare, come riportato da diversi studi, attraverso l'utilizzo di tecnologie quali alte pressioni idrostatiche (HPP), ultrasuoni (US), luce ultravioletta (UV), luce pulsata (PL), irradiazione (IR), campi elettrici pulsati (PEF), plasma freddo (CP) etc., può essere possibile ottenere prodotti sicuri e di qualità superiore, se confrontati con quelli ottenuti con processi tradizionali (Ekonomou e Boziaris, 2021; Olatunde e Benjakul, 2018). La tecnologia HPP si basa sull'applicazione di alte pressioni su alimenti confezionati in imballaggi flessibili, sia solidi che liquidi, attraverso un mezzo fluido. L'applicazione dell'HPP ai prodotti ittici è principalmente finalizzata a prolungare la loro shelf-life, minimizzandone le alterazioni della qualità e del valore nutrizionale durante la conservazione (Romulo, 2021). La tecnologia ad ultrasuoni (US) è considerata un trattamento semplice, economico e con ridotti consumi energetici. Nel settore ittico, questa tecnologia può essere utilizzata come pre-trattamento per operazioni successive come l'estrazione di specifici composti bioattivi, il recupero di molti componenti dai sottoprodotti, ma anche per migliorare le *performances* di operazioni successive quali disidratazione, scongelamento, filtrazione ed essiccazione (Ekonomou e Boziaris, 2021). Il trattamento PEF si basa sull'uso di impulsi elettrici ad alto voltaggio e di breve durata su alimenti posti tra 2 elettrodi (Kotnik et al., 2019). Sono differenti le applicazioni del PEF nel settore dei prodotti ittici, ad esempio: migliorare la diffusione del sale nel muscolo del pesce e la capacità di reidratazione del pesce disidratato (Genovese et al., 2022); migliorare la capacità di trattenere l'acqua del pesce; intenerire la polpa di molluschi e accelerare il trasferimento di massa per migliorarne il processo di essiccazione (Cropotova et al., 2021). L'attività di dottorato ha inoltre riguardato alcune applicazioni basate sul trattamento con plasma freddo (CP) per la decontaminazione dei prodotti alimentari, mostrando un buon potenziale nella riduzione della carica microbica grazie alla formazione di particelle cariche, radicali liberi, fotoni, specie chimiche reattive e radiazioni ultraviolette generate durante il trattamento. Il CP è stato riconosciuto come una promettente tecnologia di sanificazione non termica per i prodotti ittici, grazie alla sua elevata efficacia di inibizione della crescita di diversi tipi di microrganismi, anche se a dosaggi troppo elevati può causare fenomeni indesiderati come la più rapida ossidazione di lipidi e proteine (Olatunde et al., 2021). Alla luce di una ridotta consistenza a livello di letteratura scientifica attualmente disponibile sul tema, appare di rilevante importanza l'esplorazione dell'effetto delle suddette tecnologie emergenti applicate ai prodotti ittici in quanto attraverso l'uso di trattamenti non termici, può essere possibile non solo per migliorare la qualità igienica e sanitaria dei prodotti, ma anche gli attributi di qualità, oltre a ridurre i costi energetici. Infine, nell'ottica dell'aumento della sostenibilità, questo approccio potrebbe risultare estremamente utile nel ridurre le perdite alimentari nonché l'impatto ambientale della produzione alimentare.

2. Bibliografia

Cropotova, J., Tappi, S., Genovese, J., Rocculi, P., Laghi, L., Dalla Rosa, M., & Rustad, T. (2021). Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting. Innovative Food Science & Emerging Technologies, 70, 102706.

Ekonomou, S. I., & Boziaris, I. S. (2021). Non-Thermal Methods for Ensuring the Microbiological Quality and Safety of Seafood. Applied Sciences, 11(2).

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Sustainability in action. Fao.

Genovese, J., Tappi, S., Tylewicz, U., D'Elia, F., De Aguiar Saldanha Pinheiro, A.C. and Rocculi, P. (2022), Dry-salted cod (Gadus morhua) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. J Sci Food Agric.

Kotnik, T., Rems, L., Tarek, M., & Miklavčič, D. (2019). Membrane Electroporation and Electropermeabilization: Mechanisms and Models. Annual Review of Biophysics, 48(1), 63–91.

Olatunde, O. O., & Benjakul, S. (2018). Nonthermal Processes for Shelf-Life Extension of Seafoods : A Revisit, 17, 892–904.

Olatunde, O. O., Shiekh, K. A., & Benjakul, S. (2021, May 1). Pros and cons of cold plasma technology as an alternative non-thermal processing technology in seafood industry. Trends in Food Science and Technology. Elsevier Ltd.

Romulo, A. (2021). The impact of high-pressure processing treatment on microbial inactivation of seafood - a review. Food Research, 5(2), 38–44.

3. Sviluppo della ricerca

In questo contesto, l'obiettivo di questo progetto di dottorato è quello di valutare l'efficacia di trattamenti non termici emergenti quali US, PEF, HPP e CP come operazioni unitarie, o in combinazione tra loro, per l'aumento della shelf-life e/o delle *performances* di processo per la preparazione di prodotti ittici freschi e/o processati al minimo, esplorando le potenzialità di innovazione di processo/prodotto di tali tecnologie, ed altre come l'affumicatura a freddo utilizzando un prototipo innovativo basato sull'utilizzo di azoto in forma gassosa e azoto in forma liquida. Lo scopo del progetto è quindi quello di ottenere prodotti alimentari innovativi, non solo in termini di stabilità, ma anche con nuove caratteristiche reologiche, cromatiche e strutturali. La comprensione dei fenomeni che interessano i prodotti studiati è effettuata principalmente attraverso la valutazione dei cambiamenti chimico-fisici e microstrutturali delle matrici, seguendo i diversi parametri di processo per ogni tecnologia utilizzata. In questa direzione, questo progetto di ricerca di dottorato può essere suddiviso nelle seguenti attività, secondo il diagramma di Gantt riportato nella tabella 1:

A1) Ricerca bibliografica.

A2) Studio della matrice (analisi preliminari).

A3) Valutazione dei parametri di processo US e PEF per ottenere il processo più efficace per sviluppare prodotti ittici innovativi.

A4) Valutazione dei parametri di processo CP e AFFUMICATURA per il controllo della carica microbica e l'impostazione del processo più efficace.

A5) Preparazione di poster scientifici, articoli e tesi.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Activity / Month		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1	***Ricerca bibliografica***																		
A2	***Studio della matrice (analisi preliminari)***																		
A3	***Valutazione dei parametri di processo per ottenere il più efficace processo per sviluppare prodotti ittici innovativi***																		
	1) PEF, US																		
	2) Analisi qualitative																		
	3) Studi di S*helf-life*																		
A4	***Valutazione dei parametri di processo per il controllo della carica microbica e impostazione del processo più efficace***																		
	1) CP; Affumicatura																		
	2) Analisi qualitative e microbiologiche																		
	3) Studi di S*helf-life*																		
A5	***Preparazione della tesi, di poster e articoli***																		

4. Principali risultati

Il presente progetto di ricerca si propone di valutare l'effetto di tecnologie emergenti non termiche sulle caratteristiche qualitative e microbiologiche di differenti specie ittiche. Di seguito sono riportate le ricerche condotte:

- Reidratazione di merluzzo salato a secco (*Gadus morhua*) assistita da campi elettrici pulsati;
- Valutazione dell'effetto dei campi elettrici pulsati sulle cinetiche di salagione di branzino fresco;
- Utilizzo di campi elettrici pulsati per la modulazione del trasferimento di massa durante la salagione di filetti di salmone;
- Effetto del trattamento con plasma freddo sulla shelf-life di filetti di orata;
- Ottimizzazione di un processo innovativo di crio-affumicatura di salmone;
- Studio della shelf-life di filetti di salmone crio-affumicati tramite un processo innovativo;
- Studio della shelf-life di filetti di branzino leggermente salati trattati con campi elettrici pulsati.

Come avanzamento dei risultati della ricerca, di seguito si riportano schematicamente i principali risultati degli studi di shelf-life su filetti di salmone crio-affumicati e di branzino leggermente salati e trattati con PEF.

Studio della shelf-life di filetti di salmone crio-affumicati tramite un processo innovativo

Il presente studio ha analizzato la shelf-life di filetti di salmone affumicato, utilizzando tre diverse tecniche di affumicatura: crio-affumicatura a 5^0C, affumicatura a freddo in azoto e affumicatura a freddo in aria. L'affumicatura dei filetti è stata condotta per 3 ore in tre condizioni differenti, così da suddividere i campioni in tre gruppi sperimentali, in base al trattamento subìto:

• AR (Aria): filetti affumicati a freddo, a 22°C, in aria;
• CH (Crio - *High temperature*): filetti affumicati a freddo, a 22°C, in azoto;
• CL (Crio - *Low temperature*): filetti crio-affumicati a 5°C, in azoto.

I filetti sono stati preparati utilizzando salmone proveniente dall'azienda Salmar in Norvegia, allevato in mare aperto e spedito in Italia in contenitori di polistirolo. Una volta che i pesci sono arrivati in Italia, sono stati sfilettati manualmente, tagliati in porzioni di 18 x 14 cm e peso medio di 600 g, confezionati sottovuoto in buste di plastica, surgelati a -40°C e conservati fino alle fasi di salatura e affumicatura.
Prima di essere sottoposti ad altre operazioni, i filetti sono stati fatti scongelare gradualmente in una cella frigorifera a 4°C per 24 ore. Successivamente, i filetti sono stati sottoposti a salatura a secco con una miscela di cloruro di sodio e saccarosio (rispettivamente 70% e 30%), confezionati sottovuoto in buste di plastica e conservati a 4°C per 24 ore. I filetti sono stati poi rimossi dall'imballaggio, lavati con acqua corrente e sottoposti ad affumicatura per 3 ore utilizzando trucioli di legno di faggio, secondo i gruppi sperimentali indicati precedentemente.
Le determinazioni analitiche dei filetti di salmone affumicato sono state effettuate a cadenza settimanale per un totale di 5 tempi di prelievo. Dal punto di vista fisico-chimico sono stati determinati: colore, contenuto di sale, pH, contenuto di umidità, a_w, texture e i contenuti di TBARS (sostanze reattive all'acido 2-tiobarbiturico), lipidi, fenoli e perossidi.
I primi risultati ottenuti sono stati associati al tempo 0 ed i prelievi successivi sono avvenuti a distanza di 10, 18, 23 e 32 giorni dalla data di inizio; più precisamente:

- Tempo 1: 10 giorni di conservazione
- Tempo 2: 18 giorni conservazione
- Tempo 3: 23 giorni di conservazione
- Tempo 4: 32 giorni di conservazione

Una volta scongelati, i filetti sono stati tagliati in modo da ottenere delle aliquote sufficienti per le varie analisi, coerentemente al numero di repliche previste. Per le determinazioni di TBARS, grassi e fenoli i campioni sono stati confezionati sottovuoto, congelati e analizzati successivamente.
Poiché i risultati delle analisi chimico-fisiche effettuate sui filetti possono variare in base alla parte anatomica da cui le aliquote vengono prelevate, per standardizzare le analisi sui filetti è stata utilizzata sempre la stessa area per ottenere i campioni per ogni determinazione. Per quanto riguarda le analisi microbiologiche dei filetti, i prelievi sono stati eseguiti con cadenza diversa da quella programmata per le analisi fisiche e chimiche, ovvero a 2, 5, 8, 15, 21, 32 e 45 giorni di conservazione dalla data di inizio (giorno 0).
I risultati delle analisi chimico-fisiche e microbiologiche sono stati associati ai tempi di prelievo programmati e hanno permesso di valutare l'effetto delle diverse tecniche di affumicatura sulle proprietà dei filetti di salmone affumicato durante la loro shelf-life.
L'innovativa tecnica di crio-affumicatura in azoto ha permesso di ottenere dei filetti di salmone affumicato con caratteristiche chimico-fisiche simili a quelle del salmone affumicato a freddo convenzionale, ma con alcune differenze rilevanti. Difatti, esaminando le peculiarità del salmone affumicato a 5°C in azoto, è stato possibile evincere le grandi potenzialità di questa tecnologia, soprattutto osservando il livello dei prodotti primari e secondari dell'ossidazione

lipidica presenti nei filetti. Nonostante l'utilizzo della crio-affumicatura eviti interruzioni lungo la catena del freddo, i filetti ottenuti attraverso di essa hanno presentato una minor durata di conservazione rispetto al salmone affumicato a freddo convenzionale, ottenuto senza alcuna modifica artificiale dell'atmosfera nell'affumicatore. La presenza di azoto gassoso al posto dell'aria comporta infatti un accumulo sensibilmente inferiore di sostanze fenoliche, con conseguenze negative sulla shelf-life microbica.

Studio della shelf-life di filetti di branzino leggermente salati trattati con campi elettrici pulsati

L'obiettivo di questo studio è stato quello di valutare l'effetto dell'applicazione dei campi elettrici pulsati sulla shelf-life di fletti di branzino sottoposti ad una leggera salagione e confezionati in atmosfera protetta (MAP). Durante questo lavoro sono state indagate le differenze tra i filetti salati trattati con PEF e quelli non trattati. La sperimentazione è stata eseguita su 160 filetti di branzino (origine Italia), forniti sfilettati dall'azienda Economia del Mare (Cesenatico) e tenuti sotto ghiaccio. I filetti dal peso di circa 80 g ciascuno sono stati suddivisi in 2 gruppi:

- A: Controllo, non trattato;
- B: Filetto trattato con PEF, con i seguenti settaggi: E= 0.6 Kv/cm; Frequenza degli impulsi: 100 Hz; Ampiezza dell'impulso: 10 us; Numero di impulsi: 1000; Tempo di trattamento: 10 s;

Ogni gruppo era composto da 80 filetti ciascuno. Il gruppo A è stato trattato con PEF. In seguito, tutti i 160 filetti sono stati inseriti in una salamoia al 5% di NaCl per 24h con un rapporto di pesce/acqua di 1:4. Infine, sono stati tutti confezionati in atmosfera controllata (20% CO_2 – 80% N_2) all'interno di confezioni in polipropilene e inseriti in cella frigorifera. Durante lo studio della shelf-life, per ogni giorno di campionamento sono stati prelevati 6 campioni (5 A e 5 B), in totale sono stati eseguite le analisi su 5 tempi di campionamento nei giorni 0, 1, 3, 6 e 8. Al fine di valutare la qualità dei gruppi sperimentali sono state condotte le seguenti determinazioni analitiche: variazione del peso; variazione dello spazio di testa; analisi del colore; analisi della texture; attività dell'acqua; variazione del contenuto in sostanza secca; variazione del contenuto di NaCl; analisi del pH; sostanze reattive all'acido tiobarbiturico (TBARS); analisi sensoriale; analisi microbiologiche.

Nel complesso, dai risultati ottenuti dalla ricerca, è possibile affermare che il trattamento PEF a intensità di 0.6 kV/cm, abbia permesso un incremento significativo della concentrazione di sale nei filetti, probabilmente attribuibile ad una distribuzione più omogenea di NaCl nel tessuto muscolare. Un altro effetto del PEF riguarda la riduzione del peso dei filetti durante la conservazione, che è risultata significativamente inferiore nei campioni trattati rispetto al controllo. Il fenomeno sembra essere legato alla capacità dei campi elettrici pulsati ad alto voltaggio di aumentare la capacità di ritenzione idrica (WHC) nei campioni trattati. Inoltre, nonostante sia riconosciuto come la tecnologia PEF possa innescare fenomeni ossidativi a carico della matrice lipidica, in questo studio non sono state osservate modificazioni, suggerendo quindi come il PEF non abbia negativamente influenzato la stabilità ossidativa dei filetti, non solo dopo il trattamento, ma anche durante la conservazione refrigerata.
In conclusione, poiché gli scostamenti di valore tra il controllo e il trattamento non sono risultati particolarmente significativi nella maggior parte dei casi, possiamo dedurre che il trattamento con campi elettrici pulsati ad alto voltaggio su filetti di branzino non abbia inciso né positivamente né negativamente sulla *shelf-life*.

5. Elenco delle pubblicazioni prodotte nell'ambito dell'attività di dottorato

D'Elia F., A.C. De Aguiar Saldanha Pinheiro; S. Tappi; P. Rocculi, Use of pulsed electric fields for modulating mass transfer during salting of salmon fillets, Poster presentation in Acquaculture Europe 2022, Rimini.

D'Elia F., Effect of cold plasma treatment on the shelf life of sea bream fillets; Oral presentation in Acquaculture Europe 2022, Rimini.

D'Elia F., De Aguiar Saldanha Pinheiro A. C., Di Gregorio C., Picone G., Tappi S., Capozzi F., Rocculi P., Innovative smoked salmon obtained by crio-smoking. In 6th International Conference on Foodomics 2020

Genovese, J., Tappi, S., Tylewicz, U., D'Elia, F., De Aguiar Saldanha Pinheiro, A.C. and Rocculi, P. (2022), Dry-salted cod (Gadus morhua) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. J Sci Food Agric.

Wine stability, implications of yeast mannoprotein additions prior bottling of wine

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1. State-of-the-Art

Yeast mannoproteins are highly glycosylated glycoproteins that contain about 80% of D-mannose associated with residues of D-glucose and N-acetylglucosamine, with 10-20% of proteins. They present a wide range of molecular weights that can typically vary from 5 to 400 kDa, but even up to 800 kDa. Their location is in the external layer of the yeast cell wall and are connected to a matrix of amorphous β-1,3 glucan by covalent bonds, making up to 35-40% of the cell wall. There are two moments in vinification when they are released: During alcoholic fermentation and after yeast autolysis by exogenous β-1,3-glucanase enzyme, being this last group similar but with less protein content (Rodrigues et al., 2012). Commercial preparations of yeast mannoprotein were first authorized for their addition in white wine to improve its tartaric and protein stability in the early 2000s, but then, its use quickly spread to red wines for other purposes than well-known chemical stabilization, starting to be attractive due to its influence on technological and organoleptic effect on these wines. Within the already known enological properties of mannoproteins in wine production, the following can be named: inhibition of tartrate salt crystallization, reduction of protein haze, stimulation of malolactic fermentation, wine enrichment during autolysis of lees, interaction with flor wines, yeast flocculation, and autolysis in sparkling wines, adsorption of toxic ochratoxin; interaction with aromatic compounds, color stabilization, reduction of astringency and increased body and mouthfeel sensations (Guadalupe and Ayestarán, 2008). **Table 1** lists some of these properties linked to a particular molecular weight, range, or method of extraction, together with the specific effect and the type of wine where it has been studied (Caridi 2006; Gawel et al. 2016; Guadalupe et al., 2010; Núñez et al., 2006).
This Ph.D. project aims to investigate the impact of mannoproteins on winemaking, especially when added just before the last filtration. Their physicochemical effects, especially on color and mouth sensations, crucial aspects of red wine quality (Merrell et al., 2018; Sacchi et al., 2005), are not fully understood. The study will explore the interaction between mannoproteins physicochemical characteristics and the wine matrix. The ultimate goal is to provide guidance on their selection and dosage before the final filtration, improving red wine quality.

Table 1. Enological properties of mannoproteins linked to a particular molecular weight.

Properties	Molecular weight (MW KDa)	Specific effect	Studied at
Inhibition of tartrate salt crystallization	30-50 kDa	Improve tartaric stability	WW
Interaction with flor wines	49 kDa	Velum formation and surface hydrophobicity	FW
Prevention of Haze	420 kDa	Decreasing the particle size of the haze	WW
	Enzymatic extracted 31,8 kDa	Heat-stability in the presence of them	WW
Improving foaming	Mild thermal extracted 10-21.5 kDa	Contribute to foam quality and stability	SW
Mouthfeel and taste improving	PS fraction of 13–93kDa*	Reduction of palate hotness and increase of viscosity at higher pH	WW
Tannin precipitation	high-MW ~110 kDa	Reduction of proanthocyanidins	RW
Color stability	high-MW ~110 kDa	Possible, stable color loss	RW

PS: polysaccharide; WW: White wine; FW: Flor wine; SW: Sparkling wine; RW: Red wine
*: Polysaccharide contains both grape and yeast polysaccharide

2. Selected References

Caridi, A. (2006) Enological functions of parietal yeast mannoproteins. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 89(3–4), pp. 417–422. doi: 10.1007/s10482-005-9050-x.

Gawel, R., Smith, P. A. and Waters, E. J. (2016) Influence of polysaccharides on the taste and mouthfeel of white wine. Australian Journal of Grape and Wine Research, 22(3), pp. 350–357. doi: 10.1111/ajgw.12222.

Guadalupe, Z. and Ayestarán, B. (2008) Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. Journal of Agricultural and Food Chemistry, 56(19), pp. 9022–9029. doi: 10.1021/jf801535k.

Guadalupe, Z., Martínez, L. and Ayestarán, B. (2010) Yeast mannoproteins in red winemaking: Effect on polysaccharide, polyphenolic, and color composition. American Journal of Enology and Viticulture, 61(2), pp. 191–200.

Merrell, C. P., Larsen, R. C. and Harbertson, J. F.(2018) Effects of berry maturity and wine alcohol on phenolic content during winemaking and aging. American Journal of Enology and Viticulture, 69(1), pp. 1–11. doi:

10.5344/ajev.2017.17035.

Núñez, Y. P., Carrascosa, A. V., González García, R., Polo, M. C., & Martínez-Rodríguez, A. (2006) Isolation and characterization of a thermally extracted yeast cell wall fraction potentially useful for improving the foaming properties of sparkling wines. Journal of Agricultural and Food Chemistry, 54(20), pp. 7898–7903. doi: 10.1021/jf0615496.

Rodrigues, A., Ricardo-da-Silva, J. M., Lucas, C., & Laureano, O. (2012) Effect of commercial mannoproteins on wine colour and tannins stability. Food Chemistry, 131(3), pp. 907–914. doi: 10.1016/j.foodchem.2011.09.075.

Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. American Journal of Enology and Viticulture, 53(3), pp. 197–206. DOI: 10.5344/ajev.2005.56.3.197

Levengood, J., & Boulton, R. (2004) The variation in the color due to copigmentation in young Cabernet Sauvignon wines. Red wine Color, Chapter 4, pp 35-52. doi: 10.1021/bk-2004-0886.ch004

3. Ph.D. Thesis Objectives and Milestones

Within the overall objective mentioned above, this Ph.D. thesis project is organized into the following main activities according to the Gantt diagram given in **Table 2**:

A1) **Lab training and Literature review** about the latest researches associated with wine stability in relation to mannoproteins and evaluation of methods of determination mannoproteins and wines stability status;

A2) **Commercial MP Characterization** specifically selected for addition before bottling;

A3) **Study the effect of normalized dose of different MPs in two wine qualities** of Cabernet Sauvignon wine in a winery pilot scale through the mannose concentration of each mannoprotein as previously determined in A2;

A4) **Study the effect of different doses of selected MP in two wine qualities and two bottling aging time** in Cabernet Sauvignon wine using the selected mannoprotein in A3;

A5) **Data analysis, writing, and Editing** of the Ph.D. thesis, scientific papers, and oral and/or poster communications.

Table 2. Gantt diagram for this Ph.D. thesis project

Activity / Months		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
A1)	***Lab training and literature review***																
	1) Literature review																
	2) Basic Experimental Design and Laboratory Training																
A2)	***Commercial MP Characterization***																
	1) Molecular weight distribution																
	2) Total concentration of polysaccharides, monosaccharides, and proteins																
	3) Technological characteristics																
A3)	***Study the effect of normalized dose of different MPs in two wine qualities***																
	1) Physicochemical analysis of wines																
	2) Sensorial analysis of wines																
	3) selection of one mannoprotein to continue the studies																
A4)	***Study the effect of different doses of selected MP in two wine qualities and two bottling aging time***																
	1) Physicochemical analysis of wines																
	2) Sensorial analysis of wines																
A5)	***Thesis and Papers***																

4. The research progress and principal results

At the experimental winery of the Innovation and Research Center of Concha y Toro (Chile), Chilean Cabernet Sauvignon from the central-south zone was used for the experiments. Two commercial qualities were selected, "Blend" and "Premium", which differ markedly from each other in both physicochemical and sensory terms. The first experiment involved applying a standardized dosage of 5 different commercial mannoprotein, previously analyzed enzymatically during the first years of the doctoral study, to the two wines in triplicate. The doses were standardized to the mannose concentration of a typical commercial dose for this purpose and added prior to bottling.

Figure 1 presents the results of the principal component analysis (PCA) of the sensory variables that were significant for both qualities of wine, namely warmth, sweet, and smoothness. The plot shows that all the commercial mannoproteins tested were separated from the control at normalized dose of mannose. Among the commercial mannoproteins, MP5 was highlighted as the most different and was the only one that exhibited significant differences in all three sensory parameters compared to the control.

In parallel, the molecular weight distribution, total concentration of polysaccharides, monosaccharides, and proteins were determinated to the same mannoproteins. The results, presented in **Figure 2 A-F**, shows that certain mannoproteins had a mixture of medium and high molecular weights, while others contained only medium molecular weights. It was also found an important concentration of low molecular weights <5 kDa for some of the mannoproteins that were associated with oligosaccharides. The above finding could explain the different results obtained in sensory analysis, considering that a standardized dose of mannose was applied for both qualities of wine.

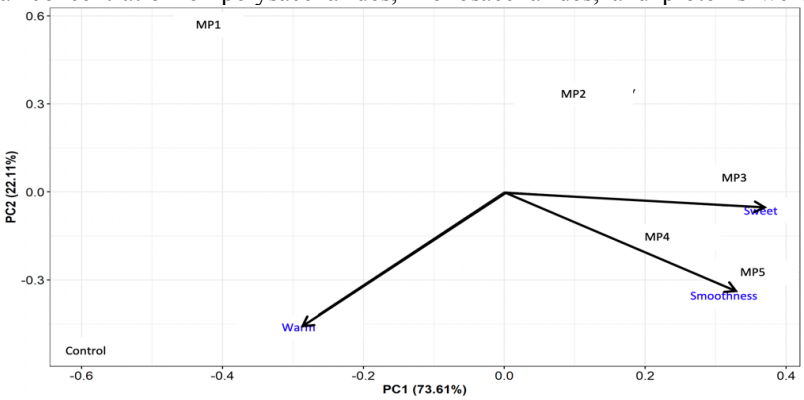

Figure 1. PCA plot of RATA (Rate All that Apply) significant sensorial variables ($p<0,05$) according to HSD Tukey post-hoc test.

Another important finding was the elevated concentration of monosaccharides structurally associated with arabic gum in mannoprotein MP3, an not allowed additive in winemaking exported to China, a crucial market for Chilean producers. Based on the available evidence, it was decided to proceed the experiments with the mannoprotein MP5. This was based on its high concentration of polysaccharides and mannose, moderate molecular weight as a polysaccharide, and absence of arabic gum. Furthermore, MP5 was the only mannoprotein with significant differences compared to the control in all three significant RATA sensory variables, and its medium molecular weight would not represent the possibility of color instability, according to bibliography.

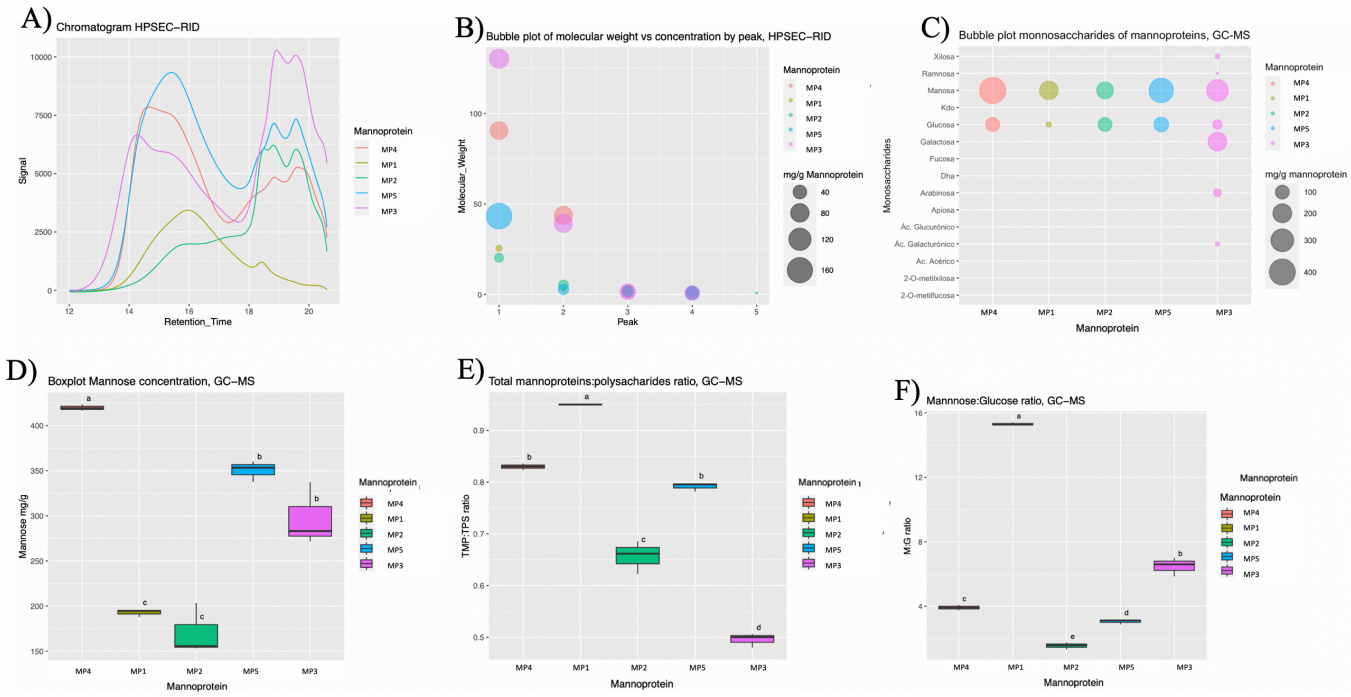

Figure 2. Polysaccharide and monosaccharide analysis of commercial mannoproteins by HPSEC-RID: **A)** Chromatogram, **B)** molecular weight distribution, and GS-MS**: C)** monosaccharides concentration, **D)** mannose concentration, **E)** Total mannoprotein to polysaccharides ratio, **F)** mannose to glucose ratio.

A factorial experiment was conducted using two red wines added before bottling with the selected commercial mannoprotein, at three different dosages in triplicate (3 , 13.5 and 30 g/HL), along with a control. The measurements were taken at two aging times (3 and 6 months). The results of the MANOVA analysis presented in **Table 3** and the PCA analysis shown in **Figure 8a-d** indicate that the physico-chemical analyses conducted at 3 months of aging reveal a significant increase in hue for both with increasing doses of MP5, when compared to the control. Blend wine quality distances itself from the control at any dosage, while Premium wine does it only from 13.5 g/HL onwards. it was observed that there was a very different and opposite evolution of color for both wine qualities, specifically in terms of % of total color (WC) due to monomeric anthocyanins (MAC%) and % of total color due to bisulfite stable anthocyanins (BSC%), but also in CIE L*a*b* color space L* parameter, indicating

Table 3. MANOVA summary of physicochemical analysis at 3 months of bottle aging

Type of analysis	Analysis	Blend wine				Premium wine			
		0g/HL	3g/HL	13.5g/HL	30g/HL	0g/HL	3g/HL	13.5g/HL	30g/HL
Levengood, J., & Boulton, R. (2004)	WC	7.39 ± 0.02 c	6.94 ± 0.12 a	7.07 ± 0.01 b	7.01 ± 0.01 ab	5.31 ± 0.03 a	5.34 ± 0.19 a	5.38 ± 0.02 a	5.40 ± 0.02 a
	CC	0.65 ± 0.03 ab	0.72 ± 0.16 b	0.45 ± 0.17 a	0.57 ± 0.06 ab	0.62 ± 0.02 b	0.56 ± 0.17 ab	0.33 ± 0.03 a	0.45 ± 0.17 ab
	MAC	2.29 ± 0.02 b	1.52 ± 0.11 a	1.35 ± 0.18 a	1.4 ± 0.05 a	1.49 ± 0.02 a	1.59 ± 0.07 a	1.81 ± 0.04 b	1.99 ± 0.15 c
	BSC	4.44 ± 0.01 a	4.97 ± 0.05 b	5.00 ± 0.01 b	5.04 ± 0.05 b	3.19 ± 0.01 b	3.19 ± 0.05 b	3.26 ± 0.02 c	2.94 ± 0.01 a
% of WC	CC%	8.87% ± 0.45% ab	6.47% ± 2.2% a	10.2% ± 2.45% b	8.13% ± 0.86% ab	11.7% ± 0.35% b	10.37% ± 2.95% ab	6.17% ± 0.5% a	8.33% ± 3.12% ab
	MAC%	31% ± 0.3% b	21.87% ± 1.81% a	19.03% ± 2.56% a	19.97% ± 0.65% a	28.1% ± 0.17% a	29.87% ± 0.31% a	33.5% ± 0.7% b	37.07% ± 2.95% c
	BSC%	60.13% ± 0.15% a	71.67% ± 0.57% c	70.73% ± 0.21% b	71.9% ± 0.7% c	60.2% ± 0.46% b	59.8% ± 3.13% b	60.37% ± 0.32% b	54.63% ± 0.21% a
Spectophotometric indexes	420nm	4.79 ± 0.02 a	4.77 ± 0.09 a	4.83 ± 0.02 a	4.78 ± 0.01 a	4.26 ± 0.02 a	4.36 ± 0.01 a	4.27 ± 0.19 a	4.37 ± 0.02 a
	520nm	6.97 ± 0.03 b	6.64 ± 0.14 a	6.74 ± 0.02 a	6.75 ± 0.01 a	4.99 ± 0.03 a	5.13 ± 0.01 a	4.99 ± 0.23 a	5.09 ± 0.01 a
	620nm	2.11 ± 0.01 b	1.94 ± 0.04 a	1.97 ± 0.02 a	1.99 ± 0 a	1.38b ± 0.01 c	1.40 ± 0 c	1.35 ± 0.05 ab	1.30 ± 0.01 ab
	CI	13.87 ± 0 b	13.35 ± 0 a	13.54 ± 0 a	13.52 ± 0 a	10.63 ± 0 a	10.89 ± 0 a	10.61 ± 0 a	10.77 ± 0 a
	Hue	0.69 ± 0.06 a	0.72 ± 0.27 d	0.72 ± 0.05 c	0.71 ± 0.03 b	0.85 ± 0.06 a	0.85 ± 0.03 a	0.86 ± 0.46 b	0.86 ± 0.03 b
	TPI	16.68 ± 0.23 ab	17.79 ± 0.57 c	17.4 ± 0.42 bc	16.41 ± 0.45 a	16.89 ± 0.27 a	17.43 ± 0.59 a	21.77 ± 0.06 b	21.86 ± 0.06 b
CIE L*a*b* color space	L*	63.59 ± 0.15 a	64.85 ± 0.05 c	64.8 ± 0.06 c	64.26 ± 0.19 b	72.68 ± 0.13 c	71.92 ± 0.33 b	67.97 ± 0.08 a	68.12 ± 0.07 a
	a*	32.22 ± 0.04 b	31.11 ± 0.08 a	31.16 ± 0.03 a	31.04 ± 0.34 a	23.93 ± 0.09 a	25.3 ± 1.23 b	23.48 ± 0.03 a	23.83 ± 0.07 a
	b*	5.42 ± 0.05 a	6.26 ± 0.04 b	6.36 ± 0.06 b	6.5 ± 0.55 b	10.62 ± 0.08 a	10.71 ± 0.48 a	10.43 ± 0.02 a	10.59 ± 0.02 a

Different letters in the same line indicate statistically significant differences ($p < 0.05$) according to the Duncan post-hoc test.

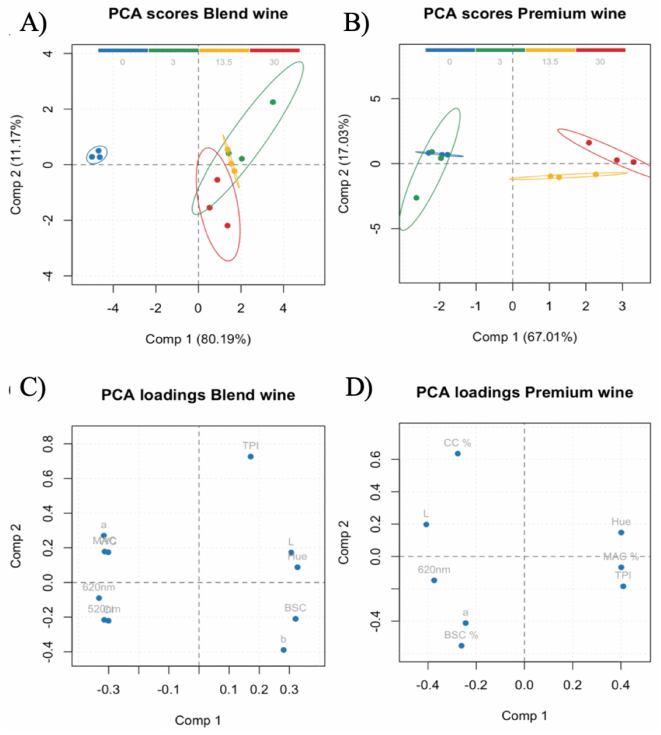

Figure 8. PCA analysis using MANOVA significant variables ($p<0.05$) for each wine quality. A-D) score and loading plots for Blend and Premium quality wines. Doses: 0 (blue), 3 (green), 13.5

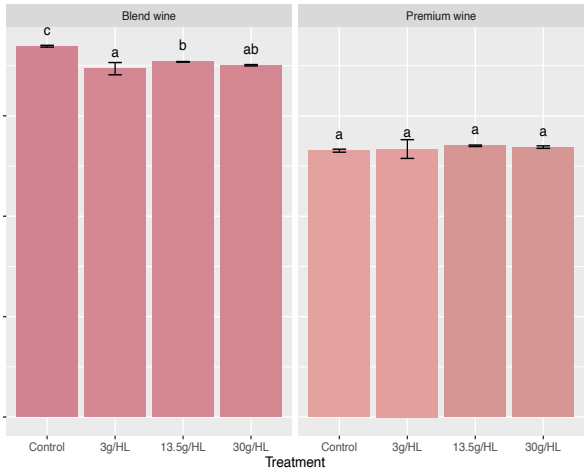

Figure 9. Total color according to Boulton for each dosage and wine quality. Different letters on the same line indicate statistically significant differences ($p < 0.05$) according to Duncan's post hoc test. The different observed colors of each bar are obtained through L*, a* and b* parameters.

that different polymerization and precipitation processes took place depending on the wine matrix and the dose of mannoprotein prior to bottling. In this sense, **Figure 9** illustrates how the dosage has a greater impact on WC of blend wines, particularly at doses exceeding 3 g/HL, while having no significant effect on the total color of premium wines. However, in the case of the last, the perceived darkness of color, may exhibit a slight increase at higher doses, due to a marginal but significant reduction in L*. The results are being contrasted by determining the total concentration of anthocyanins and phenols, as well as polysaccharides, monosaccharides, and sensorial analysis and volatile compounds for the first three months. factors. Analysis of 6 months of bottle aging will be carried, as stated in the experiment, to better understand which and why a given dosage is best suited to each wine matrix in terms of physicochemical and sensory characteristics, being able to extract the effect of dosage alone, the effect of wine quality and bottle aging, as well as the interactions between these two.

Sustainability of technology and quality control of olive oil

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1. State of the art

Nowadays, one of the main worldwide challenges is the achievement of the 17 sustainable development goals, known as SDGs in the framework of UN Agenda 2030. Among these, it is important to mention the SDG 12, namely "responsible consumption and production", and specifically the target 12.3, which focuses on the halve per capita food waste at the retail and consumer level and reduce food losses along the food production and supply chains; and target 12.4, that focuses on the management of chemicals and all wastes to significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment.
In addition, Europe is trying to become the first continent with zero impact in 2050 and to do this the European Green Deal provides a roadmap promoting an efficient use of resources by moving to a clean, circular economy and to restore biodiversity and cut pollution.
In the Mediterranean basin, olive oil represents one of the main food products, since almost the 90% of the world production comes from this area, concentrating mainly on European countries like Spain, Italy, and Greece, but also on others, such as Tunisia and Morocco. In the European Union, virgin olive oils (VOOs) can be classified in three commercial categories depending on their quality degree: extra virgin (EV), virgin (V) and lampante (L) (Reg. EU n. 2022/2104). The different quality level of each commercial category corresponds to a different value and, subsequently, to various price. In the context of olive oil production and quality control, it is important to consider that most of the official analytical methods to assess the quality and genuineness of VOOs consist of time-consuming and complex procedures, often with the use of toxic chemicals and solvents which are dangerous for human health and the environment (Valli et al., 2016). For these reasons, there is a strong and growing demand for rapid, easy-to use and environmentally friendly analytical procedures. This includes procedures that do not require solvents at all, such as the determination of volatile compounds by gas-chromatographic techniques with headspace-solid phase microextraction (HS-SPME-GC), ion mobility spectrometry (HS-GC-IMS) or HS-Flash-GC (Quintanilla-Casas et al., 2020). It is well known that volatile compounds have a crucial role to determinate VOOs quality, since they are directly responsible for the olfactory notes, and the application of methods for their determination could be used as support for sensory analysis in the classification of VOO based on the quality grade (Barbieri et al., 2020; Quintanilla-Casas et al., 2020).
In the context of rapid, innovative, and sustainable techniques for the assessment of VOOs quality and genuineness, the current investigations are also focused on the adoption of optical techniques (Valli et al., 2016). In particular, NIR, MIR, Raman and FT-IR spectroscopic methods can be considered useful for the rapid determination of food composition and molecular structure, also in the case of olive oils.
Agronomic factors, such as olive farming-systems, fertilization, irrigation, pests and diseases influence olive oil composition (Malheiro et al., 2014). Consequently, the adoption of sustainable agronomical practices can affect olive oil quality. In addition, olive oil production, as agro-industrial activity, in the Mediterranean area has a strong environmental impact, since it generates up to 30 million tons of waste per year, in which olive pomace is one of the principal by-products (Chandra and Sathiavelu, 2009). Olive pomace is the main residue from the mechanical extraction of the olive oil from the olive fruits and it is composed of skin, pulp and stone pieces, water, and oil. The major problem related to olive pomace is that it contains organic compounds with phytotoxic properties, that are dangerous for the environment. Although olive mill wastes represent an important environmental issue, they also contain high added value molecules, such as phenolic compounds (Dermeche et al., 2013), widely recognised for their beneficial properties (e.g. antioxidant activity). For this reason, this by-product can be considered a natural and economic source of phenolic compounds and their valorisation as functional ingredients in pharmaceutical, cosmetic and food industries (Nunes et al., 2016) represents a promising sustainable strategy, especially with a view to circular economy.

2. Bibliography

Barbieri S, Cevoli C, Bendini A, Quintanilla-Casas B, García-González DL, Gallina Toschi T (2020) Flash Gas Chromatography in tandem with chemometrics: a rapid screening tool for quality grades of virgin olive oils, Foods 9: 862.

Chandra M, Sathiavelu S (2009) Waste management in the olive oil industry in the Mediterranean region by composting, Clean Technol Environ Policy 11: 293–298.

Dermeche S, Nadour M, Larroche C, Moulti-Mati F, Michaud P. (2013) Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem 48: 1532–1552.

European Commission. Reg. (EU) n. 2022/2104 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards marketing standards for olive oil, and repealing Commission Regulation (EEC) No 2568/91 and Commission Implementing Regulation (EU) No 29/2012.

Nunes MA, Pimentel FB, Costa ASG, Alves RC, Oliveira MBPP (2016) Olive by-products for functional and food applications: challenging opportunities to face environmental constraints, IFSET 35: 139–148.

Malheiro R, Casal S, Baptista P, Pereira JA (2014) How agronomic factors affects olive oil composition and quality. In De Leonardis (Ed.) Virgin Olive Oil: Production, Composition, Uses and Benefits for Man, Nova Science Publishers: 118-141.

Quintanilla-Casas B, Bustamante J, Guardiola F, García-González DL, Barbieri S, Bendini A, Gallina Toschi T, Vichi S, Tres A (2020) Virgin olive oil volatile fingerprint and chemometrics: Towards an instrumental screening tool to grade the sensory quality, LWT - Food Sci Technol 121: 108936.

Valli E, Bendini A, Berardinelli A, Ragni L, Riccò B, Grossi M, Gallina Toschi T (2016) Rapid and innovative instrumental approaches for quality and authenticity of olive oils, Eur J Lipid Sci Technol 118: 1601–1619.

3. Research development

This PhD research project has been developed according to the following activities:

1) Bibliographic research.
2) Olive pomace valorisation: development of sustainable methods for the extraction of phenolic compounds from olive pomace, as well as characterization and shelf-life evaluation of the phenolic extracts.
3) Rapid and sustainable analytical methods for quality and authenticity of virgin olive oils: development and application of easy-to use, innovative and sustainable analytical approaches to evaluate the quality and genuineness of virgin olive oils.
4) Comparative study of virgin olive oils produced in experimental fields using sustainable and non-sustainable agricultural practices.
5) Spectroscopic analysis of virgin olive oils and development of chemometric models to predict the commercial category to support the sensory analysis (panel test).
6) Writing of the PhD thesis, scientific papers, oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***Olive pomace valorisation***																		
	1) Development of sustainable extractions																		
	2) Characterization and shelf-life study																		
A3)	***Rapid and sustainable analytical methods***																		
	1) Methods development																		
	2) Methods application																		
A4)	***Comparative study of virgin olive oils***																		
A5)	***Spectroscopic analysis***																		
A6)	***Thesis and paper preparation***																		

4. Research main results

This PhD project is focused on sustainability aspects in relation to technology and quality control of olive oils. The research has started from the application of rapid instrumental methods to evaluate the quality and genuineness of VOOs, based on the study of the volatile fraction, since it shows a relevant potential to support the sensory analysis (panel test) for the determination of the commercial category of VOOs, thus pre-classifying the samples. In this context, Flash-GC and HS-GC-IMS analyses were performed on an olive oil set composed of 120 samples, collected in order to have a relevant and balanced variety in the commercial categories of VOOs (among extra virgin, virgin and lampante) determined by sensory analysis (panel test). At now the data elaboration has regarded a set composed of 52 samples, classified into the commercial category using previously developed prediction approaches based on a PLS-DA models, both for Flash-GC (Barbieri et al., 2020) and HS-GC-IMS (Valli et al., 2020) results. The results show comparable effectiveness between the two techniques, and they are satisfactory in terms of percentage of correctly classified samples for the different commercial categories (see **Table 2)**, confirming the robustness of the developed models.

Table 2. Flash-GC and HS-GC-IMS outcomes, in terms of samples correctly classified, compared to the sensory assessment, by the prediction models.

	Flash-GC		*HS-GC-IMS*	
COMMERCIAL CATEGORY	**SAMPLES CORRECTLY CLASSIFIED**	**%**	**SAMPLES CORRECTLY CLASSIFIED**	**%**
EVOO	16/17	94.12	15/17	88.24
VOO	17/19	89.47	17/19	84.21
LOO	13/16	81.25	15/16	93.75
TOTAL	**31/52**	**88.46**	**47/52**	**90.38**

In addition, regarding the HS-GC-IMS, the whole sample set was analysed also by using improved analytical conditions with respect to the published ones (Valli et al., 2020) and the data elaboration is now ongoing.
Finally, the same samples set was analysed by spectroscopic methods (NIR, FT-IR, Raman), during a 3-months visiting period at Queen's University Belfast from November 2022 to February 2023. Firstly, a preliminary classification based on the three commercial categories was performed applying PLS-DA models on the results obtained from each technique, giving not completely satisfactory results, in terms of samples correctly classified. Then, a focus on the rancid defect is under investigation, since it is directly related to the oxidation status of olive oil, and subsequently to its quality. Also, a "data fusion" between the results of the different analytical techniques will be considered to obtain more robust predictive models.
Moreover, the experimental activities aimed to the technological valorisation of olive pomace by obtaining sustainable extracts rich in phenolic compounds, potentially usable in different industrial sectors, such as pharmaceutical, food and cosmetic, are now ending. This research is carried out in the framework of the Prima project SUSTAINOLIVE "Novel approaches to promote the SUSTAInability of OLIVE cultivation in the Mediterranean" (Grant Agreement no. 813904), 2019 – 2023. After the set-up of a sustainable method for the extraction of phenolic compounds without the use of toxic solvent, a mechanical approach (using a lab scale screw-press) was applied on the olive pomace by adding a mixture of water and food grade ethanol (80:20 % v/v) and two types of samples were obtained: one more liquid drained from the lower part of the mill (named *SI*) and one drier from the frontal part (named *SF*). On the samples, including olive pomace as it is (named *TQ*), a study of the phenolic fraction was carried out through the application of the Folin-Ciocalteu method, and by UHPLC-DAD, UHPLC-MS/MS analyses. About the UHPLC-MS/MS analytical approach, data elaboration is still ongoing to reach a complete phenolic profile.

Table 3. Average concentrations and relative standard deviations of the extracts from the sample TQ, SI, SF (see the description of these samples in the text above). In the first column the total concentrations of the sum of unknown compounds (Unk), hydroxytyrosol (HTyr), and tyrosol (Tyr), obtained after hydrolysis of the extracts, are reported. In the second column, the concentration in total reducing molecules contents obtained by Folin-Ciocalteu method are reported. Statistical analysis: ANOVA, HSD Tukey, p <0,05.

Sample	Concentration (g Tyr + HTyr + Unk/kg olive pomace)	SD	Concentration (g gallic acid/kg olive pomace)	SD
TQ	1.12^{b}	0.25	3.29^{b}	0.38
SI	1.54^{a}	0.14	7.10^{a}	0.42
SF	0.64^{b}	0.02	3.09^{b}	0.39

The results show that the extract obtained from the olive pomace drained from the central part of the lab-scale mill, *SI*, is the richest in the concentration of both total reducing molecules and the detected simple phenolic molecules after hydrolysis (**Table 3**). For this reason, it has been selected as the more suitable sample to obtain stable hydroalcoholic phenolic extracts. The UHPLC-MS/MS data elaboration is now ongoing, in order to obtain a complete phenolic profile of the considered extracts. Subsequently, the best technological conditions to obtain this extract were set-up: the procedure included filtration of the olive pomace, evaporation, and addition of food grade ethanol. On the selected extract, the phenolic compounds characterization and the assessment of its stability during a shelf-life study were performed, including both sensory (descriptive analysis) and instrumental (Folin-Ciocalteu method, UHPLC-DAD, UHPLC-MS/MS) evaluation. In particular, the shelf-life study was performed on a monthly basis and during three months (T0, T1 and T2, see **Table 4**), and carried out on the selected extract stored at room temperature and dark conditions.

Table 4. Average concentrations and relative standard deviations of the extracts from the sample T1, T2, T3 (see the description of these samples in the text above). In the first column the total concentrations of the sum of unknown compounds (Unk), hydroxytyrosol (HTyr), and tyrosol (Tyr), obtained after hydrolysis of the extracts, are reported. In the second column, the concentration in total reducing molecules contents obtained by Folin-Ciocalteu method are reported. Statistical analysis: ANOVA, HSD Tukey, p <0,05.

Sample	Concentration (mg Tyr + HTyr + Unk/mL extract)	SD	Concentration (mg gallic acid/mL extract)	SD
T0	0.23^{b}	0.00	0.55^{c}	0.01
T1	0.25^{a}	0.00	0.63^{a}	0.02
T2	$0.24^{a,b}$	0.00	0.60^{b}	0.01

The increase in the concentration of total reducing molecules observed at T1 with respect to T0 can be ascribable to a degradation of the sugar fraction of the extracts, since the difference in the related concentration of the phenolic molecules after hydrolysis, despite being significant, was very low in absolute value (**Table 4**).
On the other side, the sensory analysis was carried out by 8 panelists trained for olive oil assessment, through an olfactory evaluation, and they were asked to exclude the perception of ethanol. During the sensory assessment, relevant defects and other negative attributes were not perceived. The attributes mainly perceived were positive and related mostly to specific notes resembling vanilla, caramel, red fruits, and olive fruits.
Moreover, in the frame of the Prima project SUSTAINOLIVE, a comparative study is currently ongoing between VOOs obtained during the last olive season in experimental fields in which sustainable agricultural practices are adopted and others in which they are not followed. The olive oil samples have been provided by different countries (Italy, Greece, Tunisia, Spain, Portugal, and Morocco) in which farms, involved in the project, were previously selected. Other variables, such as olive fruit variety, location, olives maturity, technology conditions of milling and storage, will be taken into account. The main aim was to investigate the effect of sustainable agricultural solutions in the olive oil quality and composition by assessing free acidity, peroxide value, specific extinctions in UV, fatty acids profile, total phenolic compounds, as well as sensory analysis. The data elaboration is still ongoing.
Finally, the writing of the PhD thesis and of three scientific papers, based on the results obtained from the experimental activities that will be submitted in the next few weeks to scientific journals, is currently ongoing.

5. Publications produced during the PhD activities

Lazzarini C, Valli E, Casadei E, Grigoletto I, Ragni L, Bendini A, Gallina Toschi T (2021) A sustainable approach for the valorisation of a tomato by-product: green techniques for lycopene extraction, Book of Abstracts of the 6th International ISEKI-Food Conference, "Sustainable Development Goals in Food Systems: Challenges and Opportunities for the Future", pp. 142.

Grigoletto I, García Salas P, Valli E, Bendini A, Pasini F, Sánchez Villasclaras S, García Ruiz R, Gallina Toschi T (2022) Study of the phenolic fraction for the valorization of olive pomace as a functional ingredient, J Am Oil Chem Soc, Supplement: Book of Abstracts of 2022 AOCS Annual Meeting & Expo, May 1–4, 2022, 99, S1: pp. 107.

Grigoletto I, García Salas P, Valli E, Bendini A, Pasini F, Sánchez Villasclaras S, García Ruiz R, Gallina Toschi T (2022) Valorizzazione della sansa: metodi sostenibili per estrarre i composti fenolici, Book of Abstract of Congress SISSG 2022 "Edible Oils and Fats: Innovation and Sustainability in Production and Control", pp. 52.

Panni F, Valli E, Grigoletto I, Casadei E, Cevoli C, Bendini A, Focante F, Gallina Toschi T, Savino AF, Carpino S (2022) HS-GC-IMS e SPME-GC-FID: Metodi di screening e "targeted" per la classificazione degli oli vergini di oliva a supporto del Panel test, mediante studio della frazione volatile, Book of Abstract of Congress SISSG 2022 "Edible Oils and Fats: Innovation and Sustainability in Production and Control", pp. 32-33.

Grigoletto I, Casadei E, Panni F, Valli E, Cevoli C, Bendini A, Gallina Toschi T, Birse N, Vanhaecke L (2022) High throughput and field deployable instrumental screening methods to guarantee olive oil authenticity, Book of abstracts of the 10th International symposium on recent advances in food analysis (RAFA 2022), pp. 191.

Grigoletto I (2022) Sustainability of technology, quality control and consumption of olive oil, Proceedings of the 26th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology Asti (Italy), pp. 262.

DOTTORANDI ISCRITTI AL III ANNO (XXXVI CICLO)

Biotechnological valorization of residues and by-products from agro-food industries

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Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Water-Food-Energy-Sustainable Agriculture Nexus; Ciclo di dottorato: XXXVI; Anno di frequenza: III
Tutor: Prof.ssa Lucia Vannini

1. Stato dell'arte

Ad oggi, secondo Eurostat (Agriculture, foresty and fisheries statistics, 2020), nella sola Europa vengono generati oltre 22,4 milioni di tonnellate di rifiuti dalla produzione di alimenti e bevande. Questo è dovuto al fatto che gli scarti e sottoprodotti di lavorazione di diversi alimenti possono rappresentare anche oltre il 50% della materia prima. Tra quelli più importanti vi sono i semi, le bucce, i baccelli, la pula, la sansa, il mallo, le bucce, i gambi. Ad oggi le maggiori destinazioni di uso possono essere il riutilizzo per la mangimistica o per la produzione di energia, oltre all'estrazione di alcuni prodotti ad alto valore aggiunto; tuttavia, la maggior parte dei sottoprodotti è inutilizzata e scartata (Maurya *et al*., 2015), nonostante siano ricchi di sostanze che possono essere recuperate o utilizzate, come zuccheri semplici e complessi, lipidi, proteine, micronutrienti, oli essenziali, fibre alimentari (Mejri *et al.*, 2018). Negli ultimi decenni l'attenzione alla dieta e alla salute è diventata il focus della vita di tutti i giorni, e il consumo di alimenti a basso contenuto di grassi, con minor apporto calorico e ricchi di fibre e antiossidanti ha attirato l'attenzione di numerosi consumatori in quanto contribuiscono in modo significativo alla riduzione del colesterolo e alla prevenzione di malattie cardiovascolari e la stipsi (Aslam *et al.,* 2014). Nonostante la loro ricchezza in fattori nutrizionali, essi presentano delle problematiche legate alla poca biodisponibilità delle diverse componenti. Per questo motivo, l'utilizzo di fermentazioni di precisione con ceppi che presentano delle caratteristiche metaboliche specifiche in funzione di quelle chimico-fisiche e compositive dei sottoprodotti, quali attività amilasica, proteolitica, lipolitica o capacità di produrre fenoli (Aruna, 2019), può essere sfruttata come strategia per migliorare le proprietà del sottoprodotto e, conseguentemente, del prodotto finito (Hu *et al.,* 2022). Inoltre, l'utilizzo in alimenti convenzionali di questi sottoprodotti, sia tal quali che dopo fermentazione, può migliorarne le proprietà funzionali, ad esempio favorendo un aumento del contenuto in fibre, dell'attività antiossidante o prebiotica, oltre che prolungarne la shelf-life. Infatti, l'industria alimentare è alla continua ricerca di nuove strategie, anche attraverso la sostituzione dei conservanti tradizionali ad esempio con sostanze naturali, che vadano a soddisfare il desiderio del consumatore di prodotti freschi e "naturali", mantenendo sempre gli standard di sicurezza, qualità e stabilità dell'alimento.
In quest'ottica sono stati scelti 2 diversi di sottoprodotti - quelli dell'industria agrumaria (buccia e semi di arancio e clementine) e quelli del melograno (bucce e arilli). Per quanto riguarda gli agrumi, questi sono colture frutticole ampiamente coltivate con 14,49 milioni di tonnellate prodotte nel mondo nel 2019 (FAOSTAT 2020). A differenza di altri frutti, la porzione commestibile degli agrumi è bassa rispetto a quella non commestibile: infatti, quest'ultima risulta essere circa il 50-60% e comprende principalmente semi, buccia e residui dell'estrazione del succo. Per quanto riguarda la produzione mondiale di melograno, questa risulta essere di circa 6 milioni di tonnellate all'anno di cui circa il 40% è costituito da bucce. Grazie al recupero di estratti ricchi di antiossidanti, esistono molte applicazioni commerciali per i derivati della buccia di melograno, tra cui si possono citare l'uso nel settore farmaceutico, in tinture e alimenti, in agenti terapeutici antitumorali e la sintesi di ossido di rame (Witt *et al.,*2022). Inoltre, è stata inclusa nella sperimentazione anche una pianta infestante, l'*Equisetum arvense* o codacavallina, in quanto, per le sue proprietà benefiche, è utilizzata a scopo fitoterapico per uso interno per il trattamento di infiammazioni del cavo orale, tonsillite, per la cura dell'acne, herpes labiale ed altre affezioni grazie alle sue note proprietà astringenti, diuretiche, antinfiammatorie, antibatteriche, antimicrobiche e antiossidanti.

2. Bibliografia

Aruna TE (2019) Production of value-added product from pineapple peels using solid state fermentation, Innov. Food Sci. Emerg. Technol. 57: 102193.

Aslam HKW, Raheem MIU, Ramzan R, Shakeel A, Shoaib M, Sakandar HA (2014) Utilization of mango waste material (peel, kernel) to enhance dietary fiber content and antioxidant properties of biscuit, J. Glob Innov. Agric. Soc. Sci. 2(2): 76-81.

Eurostat (2020) Agriculture, forestry and fisheries statistics- edizione 2020

FAOSTAT (2020) The Food and Agriculture Organization of the United Nations.

Hu X, Zeng J, Shen F, Xia X, Tian X, Wu Z (2022) Citrus pomace fermentation with autochthonous probiotics improves its nutrient composition and antioxidant activities, LWT. 157: 113076.

Maurya AK, Pandey RK, Rai D, Porwal P, Rai DC (2015) Waste product of fruits and vegetables processing as a source of dietary fibre: A review, Trends Biosci, 8(19): 5129-5140.

Mejri F, Selmi S, Martins A, Benkhoud H, Baati T, Chaabane H, Nijm L, Serralheiro MLM, Rauter AP, Hosni K (2018) Broad bean (*Vicia faba* L.) pods: a rich source of bioactive ingredients with antimicrobial, antioxidant, enzyme inhibitory, anti-diabetic and health-promoting properties, Food Funct. 9(4): 2051–2069.

Witt EDP, Johann G, Triques CC, da Silva EA, da Silva C, Borsato D, da Silva Baumgartner TR (2022) Potential application of supercritical extract of pomegranate peel as bio-antioxidant for biodiesel, Environ. Prog. Sustain. 41(2), e13755.

3. Sviluppo della ricerca

La ricerca è stata sviluppata secondo i seguenti punti principali:

1) **Caratterizzazione di sottoprodotti alimentari,** quali pastazzo di agrumi, sansa di olive, residui della lavorazione di frutta e verdura; i sottoprodotti vengono valutati in sistema modello per alcuni caratteri di bioattività, ed in particolare la capacità antiossidante, antimicrobica, prebiotica.
2) **Valorizzazione dei sottoprodotti attraverso fermentazioni guidate** con ceppi selezionati di lieviti e batteri lattici appartenenti alla Collezione di Colture Microbiche Industriali del Dipartimento. Si realizzano fermentazioni allo stato solido valutando la capacità dei ceppi di incrementare la bioattività dei sottoprodotti o di produrre metaboliti ad azione antimicrobica.
3) **Formulazione di alimenti con i sottoprodotti:** prodotti lattiero-caseari e da forno vengono formulati con l'aggiunta dei sottoprodotti, tal quali o fermentati, e prodotti in scala da laboratorio. I prodotti così ottenuti sono analizzati durante la conservazione per valutare l'evoluzione del microbiota e delle caratteristiche qualitative e nutrizionali rispetto a prodotti di controllo di riferimento. Si realizzano inoltre dei challenge test con i microrganismi patogeni di riferimento per i diversi alimenti per verificare l'azione antimicrobica dei sottoprodotti quando impiegati come ingredienti alimentari.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

	Attività / Mese	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	**Ricerca bibliografica**																		
A2)	**Caratterizzazione dei sottoprodotti alimentari**																		
	Attività antimicrobica e antifungina																		
	Attività antiossidante																		
	Attività prebiotica																		
	Caratteristiche tecnologiche																		
A3)	**Valorizzazione dei sottoprodotti attraverso fermentazioni guidate**																		
	Selezione dei microrganismi e dei ceppi																		
	Fermentazione allo stato solido																		
	Caratterizzazione funzionale delle matrici fermentati																		
A4)	**Formulazione di alimenti con i sottoprodotti**																		
	Formaggio fresco																		
	Latte fermentato																		
	Prodotti da forno																		
A5)	**Convegni, scrittura articoli e tesi di dottorato**																		

4. Principali risultati

Il presente progetto di ricerca si propone di valutare il possibile utilizzo di sottoprodotti del settore agro-alimentare, valorizzandone alcune caratteristiche interessanti sia in materia di componenti nutrizionali, sia in termini di funzionalità, ad esempio, nel miglioramento della sicurezza, qualità o nel prolungamento della shelf-life del prodotto al quale vengono addizionati come ingredienti. La strategia adottata per tale scopo prevede l'utilizzo di tali matrici tal quali o a seguito di fermentazioni guidate ad opera di microrganismi selezionati al fine di incrementarne la bioattività.

1. Caratterizzazione dei sottoprodotti

Le matrici prese in esame sono 2 diversi raccolti di *Equisetum arvense,* 2019 e 2021, (**eq_19** e **eq_21** rispettivamente), 2 campioni di pastazzo di agrumi di 2 diversi anni (2012 e 2021) (**citrus_12** e **citrus_21**), bucce di melograno edibile raccolte in Emilia-Romagna (**melo_ed_ER**) e nelle Marche (**melo_ed_MA**), bucce e arilli di melograno del tipo ornamentale raccolti nelle Marche (**melo_orn_MA**) e arilli ottenuti dalla spremitura del melograno (**arilli_MA**). Gli estratti metanolici sono stati valutati per: i) il contenuto fenolico (test di Folin-Ciocalteau – TPC); ii) l'attività antiossidante (test DPPH e $ABTS^{+}$); iii) l'attività antimicrobica nei confronti di 48 ceppi di generi e specie microbiche differenti includenti microrganismi patogeni, degradativi, probiotici e starter sia mediante la metodica dell'overlay assay, sia per la determinazione della MIC. Le matrici (non estratte) sono anche state valutate per la loro funzione prebiotica (prebiotic activity score assay) e per la possibile attività di protezione nei confronti di probiotici alle condizioni del tratto gastro-intestinale, e caratterizzate per la loro componente volatile mediante analisi SPME- GC/MS.

I campioni di bucce di melograno sono risultati essere quelli con i più alti valori di TPC compresi tra 3500 e 6300 GAE (mg/l), mentre gli arilli e i campioni di equiseto hanno mostrato contenuti inferiori (112 e 430 GAE(mg/l) rispettivamente). I valori di TPC sono in accordo con quelli dell'attività *scavenging*: infatti i campioni con il più alto contenuto di fenoli hanno presentato i minori valori di IC50. Per quanto riguarda l'attività antimicrobica, quasi tutti i ceppi sono risultati sensibili ai sottoprodotti anche se con differenze specie e ceppo- dipendenti. **Melo_ed_ER, melo_ed_MA** e **melo_orn_MA** sono risultati i più attivi nei confronti di patogeni, mentre **eq** e **citrus** nei confronti dei lieviti (Figura 1). È interessante notare come invece i batteri lattici sono risultati resistenti alla maggior parte delle sostanze testate. Nel complesso, tali risultati possono essere spiegati considerando i valori di TPC e l'analisi dei composti volatili di questi sottoprodotti che mostrano elevate quantità di terpeni, ad esempio D-limonene e (S)-D-carvone, ampiamente riconosciuti come agenti antimicrobici.

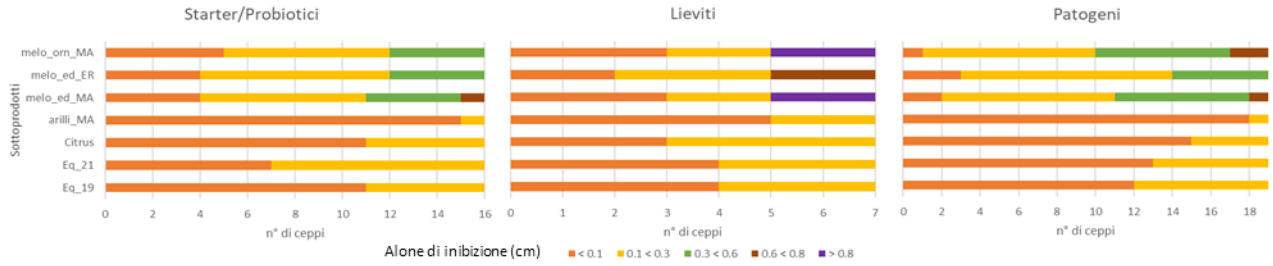

Figura 1. Valori medi di aloni di inibizione (cm) delle varie sostanze nei confronti dei ceppi microbici.

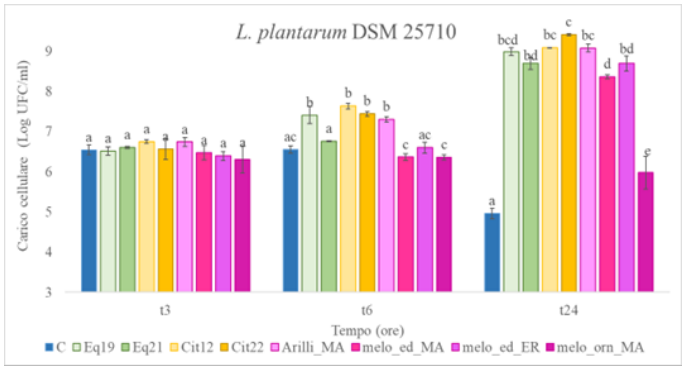

Figura 2. Attività prebiotica delle sostanze nei confronti della vitalità cellulare di *L. plantarum* in sistema simulante il fluido intestinale ($p<0,05$)

I sottoprodotti, quando testati per l'attività prebiotica nei confronti di *Lactiplantibacillus plantarum* DSM 25710 e *Bifidobacterium animalis* subsp *lactis* BB-12, sono risultati promettenti in quando in grado di proteggere e favorirne lo sviluppo in una simulazione del tratto gastro-intestinale. In particolare, tutti i sottoprodotti hanno promosso lo sviluppo del ceppo di *L. plantarum* di circa 3 cicli logaritmici ad eccezione del campione di **melo_orn_MA** che è risultato comunque significativamente diverso dal controllo che ha presentato una riduzione della vitalità cellulare dopo 24 ore (Figura 2). Una condizione simile è stata osservata anche per il ceppo di *Bifidobacterium animalis* subsp *lactis*.

2. *Fermentazione su stato solido di bucce di clementine da coltivazione biologica*

Una seconda fase della ricerca è stata quella di bio-trasformare le bucce di clementina biologiche, provenienti dalla produzione di succo di frutta, attraverso l'inoculo di diverse specie microbiche e in particolare 19 lieviti e 12 batteri lattici (LAB) appartenenti a generi e specie differenti appartenenti alla Collezione Microbica del DISTAL. I campioni sono stati fatti fermentare per 6 giorni e il processo è stato monitorato per la crescita microbica, l'acidificazione, il TPC, l'attività antiossidante ($ABTS^+$), il profilo in molecole volatili (SPME- GC/MS). Tutti i ceppi di LAB e la maggior parte dei lieviti (14 su 19) sono stati in grado di sopravvivere e crescere - anche con un aumento finale di oltre 2-3 Log UFC/g - su residui di clementina senza l'aggiunta di qualsiasi altro nutriente nonostante la matrice presenti delle caratteristiche avverse allo sviluppo dei microrganismi: pH basso (3.5-4.0), acidi organici, oli essenziali, carboidrati poco fermentescibili. Inoltre, la maggior parte dei ceppi durante la fermentazione ha aumentato il contenuto totale di fenoli, i quali sono ampiamente riconosciuti come antinfiammatori e antimicrobici. In generale, per i campioni fermentati con i diversi ceppi, è stato rilevato un accumulo di molecole volatili come alcoli e terpeni che presentano aromi positivi che possono essere sfruttati come agenti aromatizzanti.

3. *Uso di sottoprodotti per la produzione di un formaggio 'Primo Sale'*

Citrus_22 (2% p/p) e **Eq_21** (1% p/p) sono stati utilizzati come ingredienti per la produzione di formaggio "Primo Sale" addizionato di ceppi di LAB (*Lactiplantibacillus plantarum* B39.1.4A, *Pediococcus pentosaceus* B39.2.2A, *Enterococcus faecalis* B39.2.2B) (~7 Log UFC/g). La vitalità della flora microbica dei diversi formaggi e degli starter deliberatamente inoculati è stata monitorata durante una conservazione di 29 giorni a 4°C. I campioni sono stati analizzati anche per il contenuto fenolico e per l'attività antiossidante mediante test $ABTS^+$. Inoltre, è stato valutato l'impatto sul volatiloma durante la conservazione attraverso analisi SPME- GC/MS. Durante la conservazione si è rilevato come l'aggiunta dei sottoprodotti tal quale non abbia influito sulla vitalità degli starter; d'altra parte, si è osservata un'inibizione dello sviluppo di alcuni gruppi microbici come, ad esempio, le *Enterobacteriaceae* e *Pseudomonas* spp. (Figura 3). In particolare, per queste ultime si è rilevato un minor sviluppo già a seguito dell'aggiunta degli starter; tuttavia, la presenza di **eq** e **citrus** ha ulteriormente contribuito all'inibizione di oltre 3 cicli logaritmici rispetto al controllo con i LAB e di oltre 6 cicli rispetto al controllo senza gli starter.

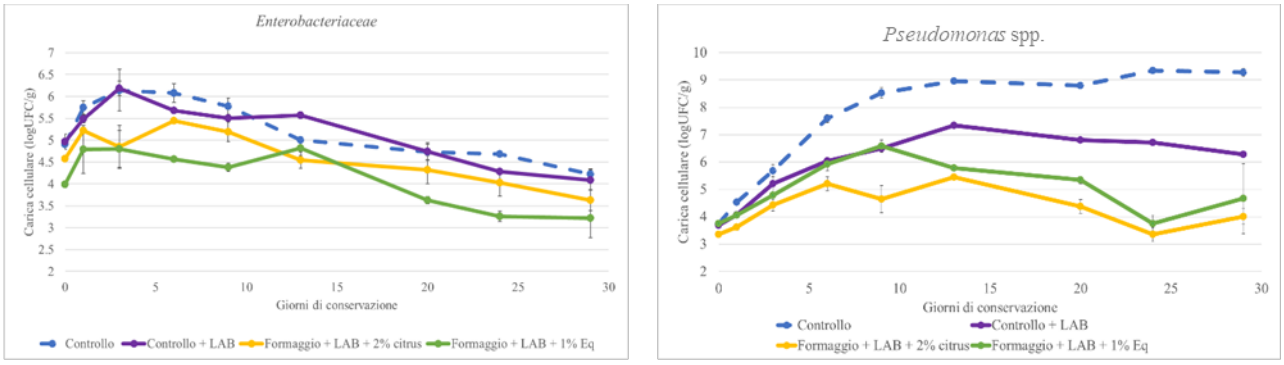

Figura 3. Cariche cellulari di *Enterobacteriaceae* e *Pseudomonas* spp. durante la conservazione del formaggio "Primo Sale" addizionato con Citrus, Eq, rispetto ai controlli.

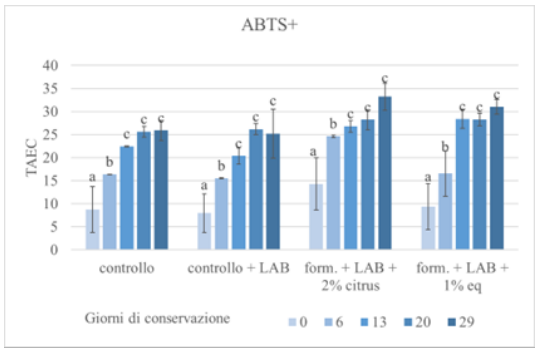

Figura 4. Attività antiossidante dei formaggi di controllo e addizionati con sottoprodotti durante il periodo di conservazione ($p<0,05$)

Per quanto riguarda la caratterizzazione del contenuto fenolico dei campioni, si è osservato come l'aggiunta di **citrus** abbia determinato un aumento di sostanze fenoliche di circa 50 ppm AGeq. rispetto al controllo. Inoltre, il contenuto fenolico di tutte le matrici è aumentato nel tempo raddoppiando alla fine della conservazione (da ~150 a 330 ppm AGeq.). Tale comportamento è stato osservato anche per l'attività antiossidante risultando significativamente differente intra campione per i diversi tempi di conservazione (Figura 4). Il maggiore incremento in contenuto fenolico è stato riscontrato per il campione contenente **citrus** che, da concentrazioni di 180 ppm AGeq., ha raggiunto un valore finale di circa 500 ppm AGeq. Inoltre, il formaggio con **citrus** ha presentato un'attività antiossidante maggiore rispetto al controllo e a tutti gli altri campioni; infine, nonostante il campione con **citrus** abbia mantenuto le migliori performance come attività antiossidante, anche il campione con **eq** ha presentato differenze significative rispetto al controllo. Per quanto riguarda l'analisi delle molecole volatili, il contenuto in composti appartenenti alle classi dei chetoni e degli acidi è aumentato durante la conservazione, mentre quello degli alcoli è diminuito dopo i primi giorni. È inoltre rilevante evidenziare come il campione con il **citrus** abbia presentato un contenuto di terpeni 100 volte maggiore rispetto agli altri campioni dovuto principalmente alla presenza di D-limonene, e come tale caratteristica abbia influenzato positivamente l'aroma del prodotto.

5. Elenco delle pubblicazioni prodotte nell'ambito dell'attività di dottorato

Contributi a convegni

Cellini B, Giordano E, Patrignani F, Malerba A, Fratebianchi De La Parra D, Armendariz Ferrandez N, Virto Resano R, Echeverria Goni I, Romani S, Dalla Rosa M, Lanciotti R, Rocculi P, Vannini L (2023) Biotechnological valorisation of by-products from clementine juice production into functional ingredients, Presentazione orale - Proc. of VIII international conference sustainable postharvest and food technologies - INOPTEP 2023 and XXXV scientific - professional conference processing and energy in agriculture - PTEP 2023 Subotica – Palić, 23 – 28 April 2023, pp.26.

Cellini B. (2022) Biotechnological valorisation of residues and by-products from agro-food industries. Poster - Proc. of XXVI Workshop on the developments in the Italian PhD research on food science technology and biotechnology, University of Turin, Asti, September 2022, pp. 220-221.

Cellini B, Gozzi G, Vannini L (2021) Valorisation of olive pomace and tomato by-products with selected yeasts strains. Poster - Proc. of 6th International Conference on Microbial Diversity 2021 - Advances in Microbial Diversity MD2021, San Casciano Val di Pesa, Firenze, Italy, SIMTREA, Società Italiana di Microbiologia Agro-Alimentare e Ambientale, 2021, pp. 55.

Vannini L, Gozzi G, Bruno V, **Cellini B** (2021) Valorization of agri-food by-products as functional ingredients for soft cheeses. Presentazione orale - Proc of 2nd International E-Conference on Nutrition and Food Science, 2021, pp. 21.

Gozzi G, **Cellini B**, Bruno V, Lanciotti R, Vannini L (2021) Valorisation of food by-products into functional "primo sale" cheese. Poster - Proc. 6th International ISEKI-Food Conference "Sustainable Development Goals in Food Systems: Challenges and Opportunities for the Future", 2021.

Cellini B. (2021) Biotechnological valorisation of residues and by-products from agro-food industries. Poster - Proc. of First Virtual (XXV) Workshop on the developments in the Italian PhD research on food science technology and biotechnology, University of Palermo, September 2021.

Studio e realizzazione di prodotti ittici innovativi attraverso l'applicazione di tecnologie di processo emergenti

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVI; Anno di frequenza: II
Tutor: Prof. Marco Dalla Rosa; Co-tutor: Prof. Pietro Rocculi; Prof.ssa Santina Romani

1. Stato dell'arte

Grazie alla crescente domanda da parte dei consumatori di alimenti minimamente lavorati e con una shelf-life estesa, sia la ricerca scientifica che quella industriale stanno studiando l'applicazione di processi innovativi per garantire prodotti alimentari sicuri e di alta qualità. Tra le varie categorie alimentari, i prodotti ittici sono stati oggetto di un significativo aumento della domanda, grazie alle loro interessanti proprietà nutrizionali. La FAO ha recentemente effettuato delle proiezioni preliminari per le possibili situazioni di pesca e acquacoltura entro il 2050. Sono state proposti 3 scenari sulla base delle aspettative di crescita del settore: scenario "*business-as-usual*", scenario a "crescita bassa" e scenario a "crescita alta". Lo scenario "*business-as-usual*" è considerato il più plausibile dalla FAO, mentre gli altri due sono stati proposti per mostrare l'intervallo delle possibilità previste. Secondo lo scenario più probabile, nel 2050 saranno pescati circa 98 milioni di tonnellate di prodotti ittici, di cui principalmente da cattura marina. Invece ci sarà una produzione di circa 140 milioni di tonnellate di prodotti ittici da acquacultura. Infine, si stima un consumo di circa 217 milioni di tonnellate di prodotti ittici da parte dell'uomo (SOFIA, 2022).

I prodotti ittici freschi sono altamente deperibili a causa della loro composizione biochimica. Per estenderne la shelf-life, potrebbero essere commercializzati prodotti ottenuti previo trattamento termico, che se da un lato fornisce un'inattivazione efficiente della carica microbica, dall'altro causa perdite significative di composti termolabili, e talvolta cambiamenti negativi nelle caratteristiche sensoriali, fisico-chimiche e nutrizionali degli alimenti. Al contrario, attraverso l'uso di trattamenti non termici emergenti, potrebbe risultare possibile migliorare la qualità igienica e sanitaria dei prodotti evitando le modificazioni promosse dall'utilizzo delle alte temperature, oltre a ridurre i costi energetici associati a questa operazione tecnologica (Ekonomou e Boziaris, 2021). In particolare, come riportato da diversi studi, attraverso l'utilizzo di tecnologie quali alte pressioni idrostatiche (HPP), ultrasuoni (US), luce ultravioletta (UV), luce pulsata (PL), irradiazione (IR), campi elettrici pulsati (PEF), plasma freddo (CP) etc., può essere possibile ottenere prodotti sicuri e di qualità superiore, se confrontati con quelli ottenuti con processi tradizionali (Ekonomou e Boziaris, 2021; Olatunde e Benjakul, 2018). La tecnologia HPP si basa sull'applicazione di alte pressioni su alimenti confezionati in imballaggi flessibili, sia solidi che liquidi, attraverso un mezzo fluido. L'applicazione dell'HPP ai prodotti ittici è principalmente finalizzata a prolungare la loro shelf-life, minimizzandone le alterazioni della qualità e del valore nutrizionale durante la conservazione (Romulo, 2021). La tecnologia ad ultrasuoni (US) è considerata un trattamento semplice, economico e con ridotti consumi energetici. Nel settore ittico, questa tecnologia può essere utilizzata come pre-trattamento per operazioni successive come l'estrazione di specifici composti bioattivi, il recupero di molti componenti dai sottoprodotti, ma anche per migliorare le *performances* di operazioni successive quali disidratazione, scongelamento, filtrazione ed essiccazione (Ekonomou e Boziaris, 2021). Il trattamento PEF si basa sull'uso di impulsi elettrici ad alto voltaggio e di breve durata su alimenti posti tra 2 elettrodi (Kotnik et al., 2019). Sono differenti le applicazioni del PEF nel settore dei prodotti ittici, ad esempio: migliorare la diffusione del sale nel muscolo del pesce e la capacità di reidratazione del pesce disidratato (Genovese et al., 2022); migliorare la capacità di trattenere l'acqua del pesce; intenerire la polpa di molluschi e accelerare il trasferimento di massa per migliorarne il processo di essiccazione (Cropotova et al., 2021). L'attività di dottorato ha inoltre riguardato alcune applicazioni basate sul trattamento con plasma freddo (CP) per la decontaminazione dei prodotti alimentari, mostrando un buon potenziale nella riduzione della carica microbica grazie alla formazione di particelle cariche, radicali liberi, fotoni, specie chimiche reattive e radiazioni ultraviolette generate durante il trattamento. Il CP è stato riconosciuto come una promettente tecnologia di sanificazione non termica per i prodotti ittici, grazie alla sua elevata efficacia di inibizione della crescita di diversi tipi di microrganismi, anche se a dosaggi troppo elevati può causare fenomeni indesiderati come la più rapida ossidazione di lipidi e proteine (Olatunde et al., 2021). Alla luce di una ridotta consistenza a livello di letteratura scientifica attualmente disponibile sul tema, appare di rilevante importanza l'esplorazione dell'effetto delle suddette tecnologie emergenti applicate ai prodotti ittici in quanto attraverso l'uso di trattamenti non termici, può essere possibile non solo per migliorare la qualità igienica e sanitaria dei prodotti, ma anche gli attributi di qualità, oltre a ridurre i costi energetici. Infine, nell'ottica dell'aumento della sostenibilità, questo approccio potrebbe risultare estremamente utile nel ridurre le perdite alimentari nonché l'impatto ambientale della produzione alimentare.

2. Bibliografia

Cropotova, J., Tappi, S., Genovese, J., Rocculi, P., Laghi, L., Dalla Rosa, M., & Rustad, T. (2021). Study of the influence of pulsed electric field pre-treatment on quality parameters of sea bass during brine salting. Innovative Food Science & Emerging Technologies, 70, 102706.

Ekonomou, S. I., & Boziaris, I. S. (2021). Non-Thermal Methods for Ensuring the Microbiological Quality and Safety of Seafood. Applied Sciences, 11(2).

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Sustainability in action. Fao.

Genovese, J., Tappi, S., Tylewicz, U., D'Elia, F., De Aguiar Saldanha Pinheiro, A.C. and Rocculi, P. (2022), Dry-salted cod (Gadus morhua) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. J Sci Food Agric.

Kotnik, T., Rems, L., Tarek, M., & Miklavčič, D. (2019). Membrane Electroporation and Electropermeabilization: Mechanisms and Models. Annual Review of Biophysics, 48(1), 63–91.

Olatunde, O. O., & Benjakul, S. (2018). Nonthermal Processes for Shelf-Life Extension of Seafoods : A Revisit, 17, 892–904.

Olatunde, O. O., Shiekh, K. A., & Benjakul, S. (2021, May 1). Pros and cons of cold plasma technology as an alternative non-thermal processing technology in seafood industry. Trends in Food Science and Technology. Elsevier Ltd.

Romulo, A. (2021). The impact of high-pressure processing treatment on microbial inactivation of seafood - a review. Food Research, 5(2), 38–44.

3. Sviluppo della ricerca

In questo contesto, l'obiettivo di questo progetto di dottorato è quello di valutare l'efficacia di trattamenti non termici emergenti quali US, PEF, HPP e CP come operazioni unitarie, o in combinazione tra loro, per l'aumento della shelf-life e/o delle *performances* di processo per la preparazione di prodotti ittici freschi e/o processati al minimo, esplorando le potenzialità di innovazione di processo/prodotto di tali tecnologie, ed altre come l'affumicatura a freddo utilizzando un prototipo innovativo basato sull'utilizzo di azoto in forma gassosa e azoto in forma liquida. Lo scopo del progetto è quindi quello di ottenere prodotti alimentari innovativi, non solo in termini di stabilità, ma anche con nuove caratteristiche reologiche, cromatiche e strutturali. La comprensione dei fenomeni che interessano i prodotti studiati è effettuata principalmente attraverso la valutazione dei cambiamenti chimico-fisici e microstrutturali delle matrici, seguendo i diversi parametri di processo per ogni tecnologia utilizzata. In questa direzione, questo progetto di ricerca di dottorato può essere suddiviso nelle seguenti attività, secondo il diagramma di Gantt riportato nella tabella 1:

A1) Ricerca bibliografica.

A2) Studio della matrice (analisi preliminari).

A3) Valutazione dei parametri di processo US e PEF per ottenere il processo più efficace per sviluppare prodotti ittici innovativi.

A4) Valutazione dei parametri di processo CP e AFFUMICATURA per il controllo della carica microbica e l'impostazione del processo più efficace.

A5) Preparazione di poster scientifici, articoli e tesi.

Tabella 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Activity / Month		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1	***Ricerca bibliografica***																		
A2	***Studio della matrice (analisi preliminari)***																		
A3	***Valutazione dei parametri di processo per ottenere il più efficace processo per sviluppare prodotti ittici innovativi***																		
	1) PEF, US																		
	2) Analisi qualitative																		
	3) Studi di S*helf-life*																		
A4	***Valutazione dei parametri di processo per il controllo della carica microbica e impostazione del processo più efficace***																		
	1) CP; Affumicatura																		
	2) Analisi qualitative e microbiologiche																		
	3) Studi di S*helf-life*																		
A5	***Preparazione della tesi, di poster e articoli***																		

4. Principali risultati

Il presente progetto di ricerca si propone di valutare l'effetto di tecnologie emergenti non termiche sulle caratteristiche qualitative e microbiologiche di differenti specie ittiche. Di seguito sono riportate le ricerche condotte:

- Reidratazione di merluzzo salato a secco (*Gadus morhua*) assistita da campi elettrici pulsati;
- Valutazione dell'effetto dei campi elettrici pulsati sulle cinetiche di salagione di branzino fresco;
- Utilizzo di campi elettrici pulsati per la modulazione del trasferimento di massa durante la salagione di filetti di salmone;
- Effetto del trattamento con plasma freddo sulla shelf-life di filetti di orata;
- Ottimizzazione di un processo innovativo di crio-affumicatura di salmone;
- Studio della shelf-life di filetti di salmone crio-affumicati tramite un processo innovativo;
- Studio della shelf-life di filetti di branzino leggermente salati trattati con campi elettrici pulsati.

Come avanzamento dei risultati della ricerca, di seguito si riportano schematicamente i principali risultati degli studi di shelf-life su filetti di salmone crio-affumicati e di branzino leggermente salati e trattati con PEF.

Studio della shelf-life di filetti di salmone crio-affumicati tramite un processo innovativo

Il presente studio ha analizzato la shelf-life di filetti di salmone affumicato, utilizzando tre diverse tecniche di affumicatura: crio-affumicatura a 5^0C, affumicatura a freddo in azoto e affumicatura a freddo in aria. L'affumicatura dei filetti è stata condotta per 3 ore in tre condizioni differenti, così da suddividere i campioni in tre gruppi sperimentali, in base al trattamento subìto:

• AR (Aria): filetti affumicati a freddo, a 22°C, in aria;
• CH (Crio - *High temperature*): filetti affumicati a freddo, a 22°C, in azoto;
• CL (Crio - *Low temperature*): filetti crio-affumicati a 5°C, in azoto.

I filetti sono stati preparati utilizzando salmone proveniente dall'azienda Salmar in Norvegia, allevato in mare aperto e spedito in Italia in contenitori di polistirolo. Una volta che i pesci sono arrivati in Italia, sono stati sfilettati manualmente, tagliati in porzioni di 18 x 14 cm e peso medio di 600 g, confezionati sottovuoto in buste di plastica, surgelati a -40°C e conservati fino alle fasi di salatura e affumicatura.
Prima di essere sottoposti ad altre operazioni, i filetti sono stati fatti scongelare gradualmente in una cella frigorifera a 4°C per 24 ore. Successivamente, i filetti sono stati sottoposti a salatura a secco con una miscela di cloruro di sodio e saccarosio (rispettivamente 70% e 30%), confezionati sottovuoto in buste di plastica e conservati a 4°C per 24 ore. I filetti sono stati poi rimossi dall'imballaggio, lavati con acqua corrente e sottoposti ad affumicatura per 3 ore utilizzando trucioli di legno di faggio, secondo i gruppi sperimentali indicati precedentemente.
Le determinazioni analitiche dei filetti di salmone affumicato sono state effettuate a cadenza settimanale per un totale di 5 tempi di prelievo. Dal punto di vista fisico-chimico sono stati determinati: colore, contenuto di sale, pH, contenuto di umidità, a_w, texture e i contenuti di TBARS (sostanze reattive all'acido 2-tiobarbiturico), lipidi, fenoli e perossidi.
I primi risultati ottenuti sono stati associati al tempo 0 ed i prelievi successivi sono avvenuti a distanza di 10, 18, 23 e 32 giorni dalla data di inizio; più precisamente:

- Tempo 1: 10 giorni di conservazione
- Tempo 2: 18 giorni conservazione
- Tempo 3: 23 giorni di conservazione
- Tempo 4: 32 giorni di conservazione

Una volta scongelati, i filetti sono stati tagliati in modo da ottenere delle aliquote sufficienti per le varie analisi, coerentemente al numero di repliche previste. Per le determinazioni di TBARS, grassi e fenoli i campioni sono stati confezionati sottovuoto, congelati e analizzati successivamente.
Poiché i risultati delle analisi chimico-fisiche effettuate sui filetti possono variare in base alla parte anatomica da cui le aliquote vengono prelevate, per standardizzare le analisi sui filetti è stata utilizzata sempre la stessa area per ottenere i campioni per ogni determinazione. Per quanto riguarda le analisi microbiologiche dei filetti, i prelievi sono stati eseguiti con cadenza diversa da quella programmata per le analisi fisiche e chimiche, ovvero a 2, 5, 8, 15, 21, 32 e 45 giorni di conservazione dalla data di inizio (giorno 0).
I risultati delle analisi chimico-fisiche e microbiologiche sono stati associati ai tempi di prelievo programmati e hanno permesso di valutare l'effetto delle diverse tecniche di affumicatura sulle proprietà dei filetti di salmone affumicato durante la loro shelf-life.
L'innovativa tecnica di crio-affumicatura in azoto ha permesso di ottenere dei filetti di salmone affumicato con caratteristiche chimico-fisiche simili a quelle del salmone affumicato a freddo convenzionale, ma con alcune differenze rilevanti. Difatti, esaminando le peculiarità del salmone affumicato a 5°C in azoto, è stato possibile evincere le grandi potenzialità di questa tecnologia, soprattutto osservando il livello dei prodotti primari e secondari dell'ossidazione

lipidica presenti nei filetti. Nonostante l'utilizzo della crio-affumicatura eviti interruzioni lungo la catena del freddo, i filetti ottenuti attraverso di essa hanno presentato una minor durata di conservazione rispetto al salmone affumicato a freddo convenzionale, ottenuto senza alcuna modifica artificiale dell'atmosfera nell'affumicatore. La presenza di azoto gassoso al posto dell'aria comporta infatti un accumulo sensibilmente inferiore di sostanze fenoliche, con conseguenze negative sulla shelf-life microbica.

Studio della shelf-life di filetti di branzino leggermente salati trattati con campi elettrici pulsati

L'obiettivo di questo studio è stato quello di valutare l'effetto dell'applicazione dei campi elettrici pulsati sulla shelf-life di fletti di branzino sottoposti ad una leggera salagione e confezionati in atmosfera protetta (MAP). Durante questo lavoro sono state indagate le differenze tra i filetti salati trattati con PEF e quelli non trattati. La sperimentazione è stata eseguita su 160 filetti di branzino (origine Italia), forniti sfilettati dall'azienda Economia del Mare (Cesenatico) e tenuti sotto ghiaccio. I filetti dal peso di circa 80 g ciascuno sono stati suddivisi in 2 gruppi:

- A: Controllo, non trattato;
- B: Filetto trattato con PEF, con i seguenti settaggi: E= 0.6 Kv/cm; Frequenza degli impulsi: 100 Hz; Ampiezza dell'impulso: 10 us; Numero di impulsi: 1000; Tempo di trattamento: 10 s;

Ogni gruppo era composto da 80 filetti ciascuno. Il gruppo A è stato trattato con PEF. In seguito, tutti i 160 filetti sono stati inseriti in una salamoia al 5% di NaCl per 24h con un rapporto di pesce/acqua di 1:4. Infine, sono stati tutti confezionati in atmosfera controllata (20% CO_2 – 80% N_2) all'interno di confezioni in polipropilene e inseriti in cella frigorifera. Durante lo studio della shelf-life, per ogni giorno di campionamento sono stati prelevati 6 campioni (5 A e 5 B), in totale sono stati eseguite le analisi su 5 tempi di campionamento nei giorni 0, 1, 3, 6 e 8. Al fine di valutare la qualità dei gruppi sperimentali sono state condotte le seguenti determinazioni analitiche: variazione del peso; variazione dello spazio di testa; analisi del colore; analisi della texture; attività dell'acqua; variazione del contenuto in sostanza secca; variazione del contenuto di NaCl; analisi del pH; sostanze reattive all'acido tiobarbiturico (TBARS); analisi sensoriale; analisi microbiologiche.

Nel complesso, dai risultati ottenuti dalla ricerca, è possibile affermare che il trattamento PEF a intensità di 0.6 kV/cm, abbia permesso un incremento significativo della concentrazione di sale nei filetti, probabilmente attribuibile ad una distribuzione più omogenea di NaCl nel tessuto muscolare. Un altro effetto del PEF riguarda la riduzione del peso dei filetti durante la conservazione, che è risultata significativamente inferiore nei campioni trattati rispetto al controllo. Il fenomeno sembra essere legato alla capacità dei campi elettrici pulsati ad alto voltaggio di aumentare la capacità di ritenzione idrica (WHC) nei campioni trattati. Inoltre, nonostante sia riconosciuto come la tecnologia PEF possa innescare fenomeni ossidativi a carico della matrice lipidica, in questo studio non sono state osservate modificazioni, suggerendo quindi come il PEF non abbia negativamente influenzato la stabilità ossidativa dei filetti, non solo dopo il trattamento, ma anche durante la conservazione refrigerata.
In conclusione, poiché gli scostamenti di valore tra il controllo e il trattamento non sono risultati particolarmente significativi nella maggior parte dei casi, possiamo dedurre che il trattamento con campi elettrici pulsati ad alto voltaggio su filetti di branzino non abbia inciso né positivamente né negativamente sulla *shelf-life*.

5. Elenco delle pubblicazioni prodotte nell'ambito dell'attività di dottorato

D'Elia F., A.C. De Aguiar Saldanha Pinheiro; S. Tappi; P. Rocculi, Use of pulsed electric fields for modulating mass transfer during salting of salmon fillets, Poster presentation in Acquaculture Europe 2022, Rimini.

D'Elia F., Effect of cold plasma treatment on the shelf life of sea bream fillets; Oral presentation in Acquaculture Europe 2022, Rimini.

D'Elia F., De Aguiar Saldanha Pinheiro A. C., Di Gregorio C., Picone G., Tappi S., Capozzi F., Rocculi P., Innovative smoked salmon obtained by crio-smoking. In 6th International Conference on Foodomics 2020

Genovese, J., Tappi, S., Tylewicz, U., D'Elia, F., De Aguiar Saldanha Pinheiro, A.C. and Rocculi, P. (2022), Dry-salted cod (Gadus morhua) rehydration assisted by pulsed electric fields: modelling of mass transfer kinetics. J Sci Food Agric.

Wine stability, implications of yeast mannoprotein additions prior bottling of wine

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1. State-of-the-Art

Yeast mannoproteins are highly glycosylated glycoproteins that contain about 80% of D-mannose associated with residues of D-glucose and N-acetylglucosamine, with 10-20% of proteins. They present a wide range of molecular weights that can typically vary from 5 to 400 kDa, but even up to 800 kDa. Their location is in the external layer of the yeast cell wall and are connected to a matrix of amorphous β-1,3 glucan by covalent bonds, making up to 35-40% of the cell wall. There are two moments in vinification when they are released: During alcoholic fermentation and after yeast autolysis by exogenous β-1,3-glucanase enzyme, being this last group similar but with less protein content (Rodrigues et al., 2012). Commercial preparations of yeast mannoprotein were first authorized for their addition in white wine to improve its tartaric and protein stability in the early 2000s, but then, its use quickly spread to red wines for other purposes than well-known chemical stabilization, starting to be attractive due to its influence on technological and organoleptic effect on these wines. Within the already known enological properties of mannoproteins in wine production, the following can be named: inhibition of tartrate salt crystallization, reduction of protein haze, stimulation of malolactic fermentation, wine enrichment during autolysis of lees, interaction with flor wines, yeast flocculation, and autolysis in sparkling wines, adsorption of toxic ochratoxin; interaction with aromatic compounds, color stabilization, reduction of astringency and increased body and mouthfeel sensations (Guadalupe and Ayestarán, 2008). **Table 1** lists some of these properties linked to a particular molecular weight, range, or method of extraction, together with the specific effect and the type of wine where it has been studied (Caridi 2006; Gawel et al. 2016; Guadalupe et al., 2010; Núñez et al., 2006).
This Ph.D. project aims to investigate the impact of mannoproteins on winemaking, especially when added just before the last filtration. Their physicochemical effects, especially on color and mouth sensations, crucial aspects of red wine quality (Merrell et al., 2018; Sacchi et al., 2005), are not fully understood. The study will explore the interaction between mannoproteins physicochemical characteristics and the wine matrix. The ultimate goal is to provide guidance on their selection and dosage before the final filtration, improving red wine quality.

Table 1. Enological properties of mannoproteins linked to a particular molecular weight.

Properties	Molecular weight (MW KDa)	Specific effect	Studied at
Inhibition of tartrate salt crystallization	30-50 kDa	Improve tartaric stability	WW
Interaction with flor wines	49 kDa	Velum formation and surface hydrophobicity	FW
Prevention of Haze	420 kDa	Decreasing the particle size of the haze	WW
	Enzymatic extracted 31,8 kDa	Heat-stability in the presence of them	WW
Improving foaming	Mild thermal extracted 10-21.5 kDa	Contribute to foam quality and stability	SW
Mouthfeel and taste improving	PS fraction of 13–93kDa*	Reduction of palate hotness and increase of viscosity at higher pH	WW
Tannin precipitation	high-MW ~110 kDa	Reduction of proanthocyanidins	RW
Color stability	high-MW ~110 kDa	Possible, stable color loss	RW

PS: polysaccharide; WW: White wine; FW: Flor wine; SW: Sparkling wine; RW: Red wine
*: Polysaccharide contains both grape and yeast polysaccharide

2. Selected References

Caridi, A. (2006) Enological functions of parietal yeast mannoproteins. Antonie van Leeuwenhoek, International Journal of General and Molecular Microbiology, 89(3–4), pp. 417–422. doi: 10.1007/s10482-005-9050-x.

Gawel, R., Smith, P. A. and Waters, E. J. (2016) Influence of polysaccharides on the taste and mouthfeel of white wine. Australian Journal of Grape and Wine Research, 22(3), pp. 350–357. doi: 10.1111/ajgw.12222.

Guadalupe, Z. and Ayestarán, B. (2008) Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. Journal of Agricultural and Food Chemistry, 56(19), pp. 9022–9029. doi: 10.1021/jf801535k.

Guadalupe, Z., Martínez, L. and Ayestarán, B. (2010) Yeast mannoproteins in red winemaking: Effect on polysaccharide, polyphenolic, and color composition. American Journal of Enology and Viticulture, 61(2), pp. 191–200.

Merrell, C. P., Larsen, R. C. and Harbertson, J. F.(2018) Effects of berry maturity and wine alcohol on phenolic content during winemaking and aging. American Journal of Enology and Viticulture, 69(1), pp. 1–11. doi:

10.5344/ajev.2017.17035.

Núñez, Y. P., Carrascosa, A. V., González García, R., Polo, M. C., & Martínez-Rodríguez, A. (2006) Isolation and characterization of a thermally extracted yeast cell wall fraction potentially useful for improving the foaming properties of sparkling wines. Journal of Agricultural and Food Chemistry, 54(20), pp. 7898–7903. doi: 10.1021/jf0615496.

Rodrigues, A., Ricardo-da-Silva, J. M., Lucas, C., & Laureano, O. (2012) Effect of commercial mannoproteins on wine colour and tannins stability. Food Chemistry, 131(3), pp. 907–914. doi: 10.1016/j.foodchem.2011.09.075.

Sacchi, K. L., Bisson, L. F. and Adams, D. O. (2005) A review of the effect of winemaking techniques on phenolic extraction in red wines. American Journal of Enology and Viticulture, 53(3), pp. 197–206. DOI: 10.5344/ajev.2005.56.3.197

Levengood, J., & Boulton, R. (2004) The variation in the color due to copigmentation in young Cabernet Sauvignon wines. Red wine Color, Chapter 4, pp 35-52. doi: 10.1021/bk-2004-0886.ch004

3. Ph.D. Thesis Objectives and Milestones

Within the overall objective mentioned above, this Ph.D. thesis project is organized into the following main activities according to the Gantt diagram given in **Table 2**:

A1) **Lab training and Literature review** about the latest researches associated with wine stability in relation to mannoproteins and evaluation of methods of determination mannoproteins and wines stability status;

A2) **Commercial MP Characterization** specifically selected for addition before bottling;

A3) **Study the effect of normalized dose of different MPs in two wine qualities** of Cabernet Sauvignon wine in a winery pilot scale through the mannose concentration of each mannoprotein as previously determined in A2;

A4) **Study the effect of different doses of selected MP in two wine qualities and two bottling aging time** in Cabernet Sauvignon wine using the selected mannoprotein in A3;

A5) **Data analysis, writing, and Editing** of the Ph.D. thesis, scientific papers, and oral and/or poster communications.

Table 2. Gantt diagram for this Ph.D. thesis project

Activity / Months		2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32
A1)	***Lab training and literature review***																
	1) Literature review																
	2) Basic Experimental Design and Laboratory Training																
A2)	***Commercial MP Characterization***																
	1) Molecular weight distribution																
	2) Total concentration of polysaccharides, monosaccharides, and proteins																
	3) Technological characteristics																
A3)	***Study the effect of normalized dose of different MPs in two wine qualities***																
	1) Physicochemical analysis of wines																
	2) Sensorial analysis of wines																
	3) selection of one mannoprotein to continue the studies																
A4)	***Study the effect of different doses of selected MP in two wine qualities and two bottling aging time***																
	1) Physicochemical analysis of wines																
	2) Sensorial analysis of wines																
A5)	***Thesis and Papers***																

4. The research progress and principal results

At the experimental winery of the Innovation and Research Center of Concha y Toro (Chile), Chilean Cabernet Sauvignon from the central-south zone was used for the experiments. Two commercial qualities were selected, "Blend" and "Premium", which differ markedly from each other in both physicochemical and sensory terms. The first experiment involved applying a standardized dosage of 5 different commercial mannoprotein, previously analyzed enzymatically during the first years of the doctoral study, to the two wines in triplicate. The doses were standardized to the mannose concentration of a typical commercial dose for this purpose and added prior to bottling.

Figure 1 presents the results of the principal component analysis (PCA) of the sensory variables that were significant for both qualities of wine, namely warmth, sweet, and smoothness. The plot shows that all the commercial mannoproteins tested were separated from the control at normalized dose of mannose. Among the commercial mannoproteins, MP5 was highlighted as the most different and was the only one that exhibited significant differences in all three sensory parameters compared to the control.

In parallel, the molecular weight distribution, total concentration of polysaccharides, monosaccharides, and proteins were determinated to the same mannoproteins. The results, presented in **Figure 2 A-F**, shows that certain mannoproteins had a mixture of medium and high molecular weights, while others contained only medium molecular weights. It was also found an important concentration of low molecular weights <5 kDa for some of the mannoproteins that were associated with oligosaccharides. The above finding could explain the different results obtained in sensory analysis, considering that a standardized dose of mannose was applied for both qualities of wine.

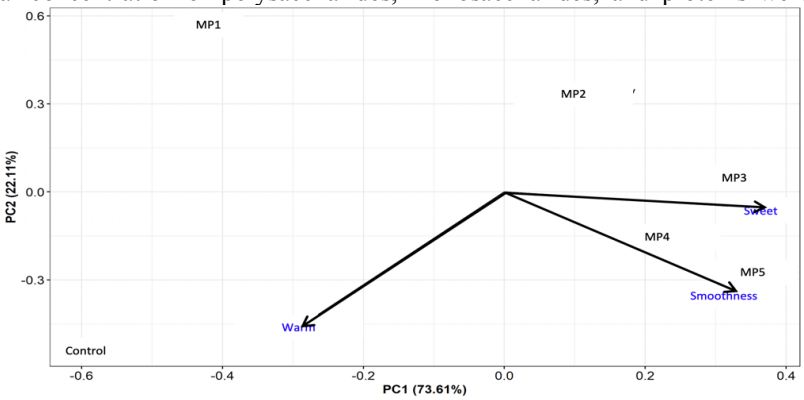

Figure 1. PCA plot of RATA (Rate All that Apply) significant sensorial variables ($p<0,05$) according to HSD Tukey post-hoc test.

Another important finding was the elevated concentration of monosaccharides structurally associated with arabic gum in mannoprotein MP3, an not allowed additive in winemaking exported to China, a crucial market for Chilean producers. Based on the available evidence, it was decided to proceed the experiments with the mannoprotein MP5. This was based on its high concentration of polysaccharides and mannose, moderate molecular weight as a polysaccharide, and absence of arabic gum. Furthermore, MP5 was the only mannoprotein with significant differences compared to the control in all three significant RATA sensory variables, and its medium molecular weight would not represent the possibility of color instability, according to bibliography.

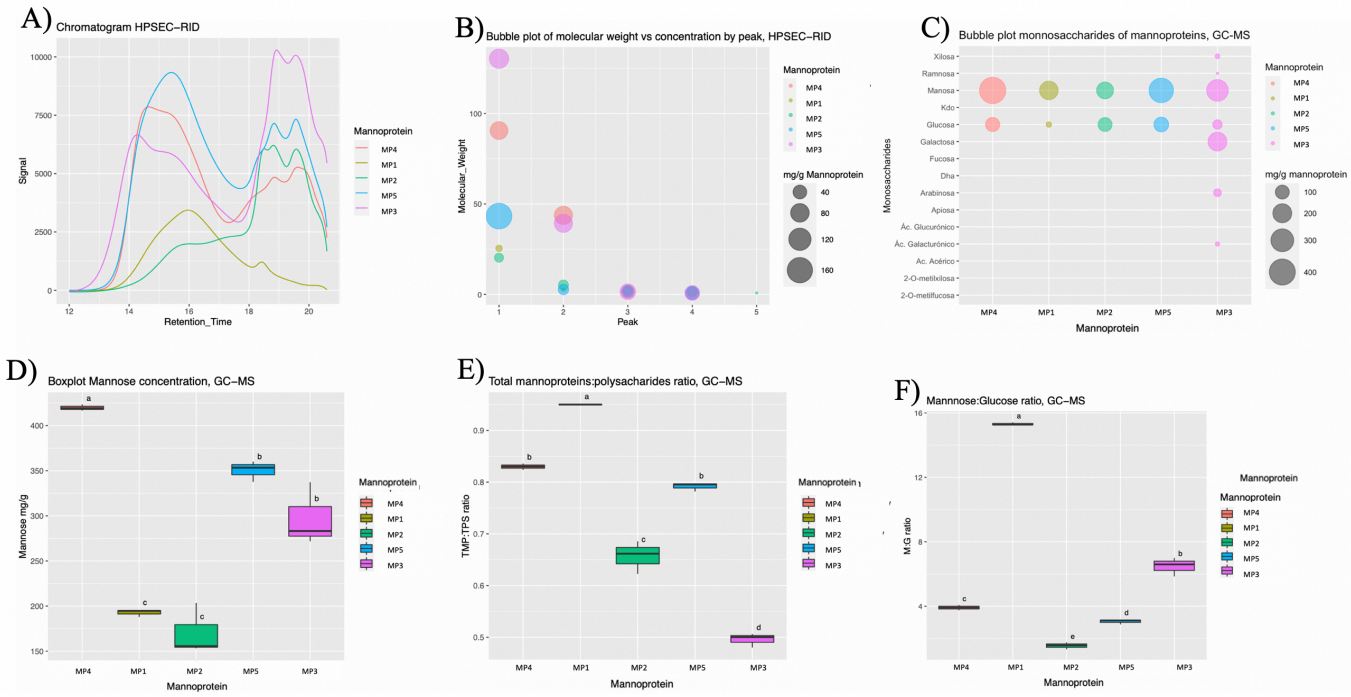

Figure 2. Polysaccharide and monosaccharide analysis of commercial mannoproteins by HPSEC-RID: **A)** Chromatogram, **B)** molecular weight distribution, and GS-MS**: C)** monosaccharides concentration, **D)** mannose concentration, **E)** Total mannoprotein to polysaccharides ratio, **F)** mannose to glucose ratio.

A factorial experiment was conducted using two red wines added before bottling with the selected commercial mannoprotein, at three different dosages in triplicate (3 , 13.5 and 30 g/HL), along with a control. The measurements were taken at two aging times (3 and 6 months). The results of the MANOVA analysis presented in **Table 3** and the PCA analysis shown in **Figure 8a-d** indicate that the physico-chemical analyses conducted at 3 months of aging reveal a significant increase in hue for both with increasing doses of MP5, when compared to the control. Blend wine quality distances itself from the control at any dosage, while Premium wine does it only from 13.5 g/HL onwards. it was observed that there was a very different and opposite evolution of color for both wine qualities, specifically in terms of % of total color (WC) due to monomeric anthocyanins (MAC%) and % of total color due to bisulfite stable anthocyanins (BSC%), but also in CIE L*a*b* color space L* parameter, indicating

Table 3. MANOVA summary of physicochemical analysis at 3 months of bottle aging

Type of analysis	Analysis	Blend wine				Premium wine			
		0g/HL	3g/HL	13.5g/HL	30g/HL	0g/HL	3g/HL	13.5g/HL	30g/HL
Levengood, J., & Boulton, R. (2004)	WC	7.39 ± 0.02 c	6.94 ± 0.12 a	7.07 ± 0.01 b	7.01 ± 0.01 ab	5.31 ± 0.03 a	5.34 ± 0.19 a	5.38 ± 0.02 a	5.40 ± 0.02 a
	CC	0.65 ± 0.03 ab	0.72 ± 0.16 b	0.45 ± 0.17 a	0.57 ± 0.06 ab	0.62 ± 0.02 b	0.56 ± 0.17 ab	0.33 ± 0.03 a	0.45 ± 0.17 ab
	MAC	2.29 ± 0.02 b	1.52 ± 0.11 a	1.35 ± 0.18 a	1.4 ± 0.05 a	1.49 ± 0.02 a	1.59 ± 0.07 a	1.81 ± 0.04 b	1.99 ± 0.15 c
	BSC	4.44 ± 0.01 a	4.97 ± 0.05 b	5.00 ± 0.01 b	5.04 ± 0.05 b	3.19 ± 0.01 b	3.19 ± 0.05 b	3.26 ± 0.02 c	2.94 ± 0.01 a
% of WC	CC%	8.87% ± 0.45% ab	6.47% ± 2.2% a	10.2% ± 2.45% b	8.13% ± 0.86% ab	11.7% ± 0.35% b	10.37% ± 2.95% ab	6.17% ± 0.5% a	8.33% ± 3.12% ab
	MAC%	31% ± 0.3% b	21.87% ± 1.81% a	19.03% ± 2.56% a	19.97% ± 0.65% a	28.1% ± 0.17% a	29.87% ± 0.31% a	33.5% ± 0.7% b	37.07% ± 2.95% c
	BSC%	60.13% ± 0.15% a	71.67% ± 0.57% c	70.73% ± 0.21% b	71.9% ± 0.7% c	60.2% ± 0.46% b	59.8% ± 3.13% b	60.37% ± 0.32% b	54.63% ± 0.21% a
Spectophotometric indexes	420nm	4.79 ± 0.02 a	4.77 ± 0.09 a	4.83 ± 0.02 a	4.78 ± 0.01 a	4.26 ± 0.02 a	4.36 ± 0.01 a	4.27 ± 0.19 a	4.37 ± 0.02 a
	520nm	6.97 ± 0.03 b	6.64 ± 0.14 a	6.74 ± 0.02 a	6.75 ± 0.01 a	4.99 ± 0.03 a	5.13 ± 0.01 a	4.99 ± 0.23 a	5.09 ± 0.01 a
	620nm	2.11 ± 0.01 b	1.94 ± 0.04 a	1.97 ± 0.02 a	1.99 ± 0 a	1.38b ± 0.01 c	1.40 ± 0 c	1.35 ± 0.05 ab	1.30 ± 0.01 ab
	CI	13.87 ± 0 b	13.35 ± 0 a	13.54 ± 0 a	13.52 ± 0 a	10.63 ± 0 a	10.89 ± 0 a	10.61 ± 0 a	10.77 ± 0 a
	Hue	0.69 ± 0.06 a	0.72 ± 0.27 d	0.72 ± 0.05 c	0.71 ± 0.03 b	0.85 ± 0.06 a	0.85 ± 0.03 a	0.86 ± 0.46 b	0.86 ± 0.03 b
	TPI	16.68 ± 0.23 ab	17.79 ± 0.57 c	17.4 ± 0.42 bc	16.41 ± 0.45 a	16.89 ± 0.27 a	17.43 ± 0.59 a	21.77 ± 0.06 b	21.86 ± 0.06 b
CIE L*a*b* color space	L*	63.59 ± 0.15 a	64.85 ± 0.05 c	64.8 ± 0.06 c	64.26 ± 0.19 b	72.68 ± 0.13 c	71.92 ± 0.33 b	67.97 ± 0.08 a	68.12 ± 0.07 a
	a*	32.22 ± 0.04 b	31.11 ± 0.08 a	31.16 ± 0.03 a	31.04 ± 0.34 a	23.93 ± 0.09 a	25.3 ± 1.23 b	23.48 ± 0.03 a	23.83 ± 0.07 a
	b*	5.42 ± 0.05 a	6.26 ± 0.04 b	6.36 ± 0.06 b	6.5 ± 0.55 b	10.62 ± 0.08 a	10.71 ± 0.48 a	10.43 ± 0.02 a	10.59 ± 0.02 a

Different letters in the same line indicate statistically significant differences ($p < 0.05$) according to the Duncan post-hoc test.

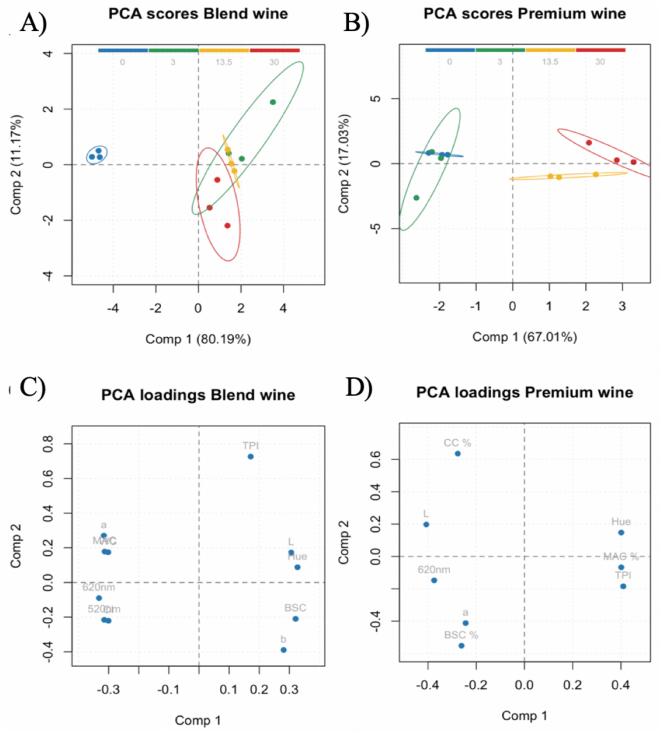

Figure 8. PCA analysis using MANOVA significant variables ($p<0.05$) for each wine quality. A-D) score and loading plots for Blend and Premium quality wines. Doses: 0 (blue), 3 (green), 13.5

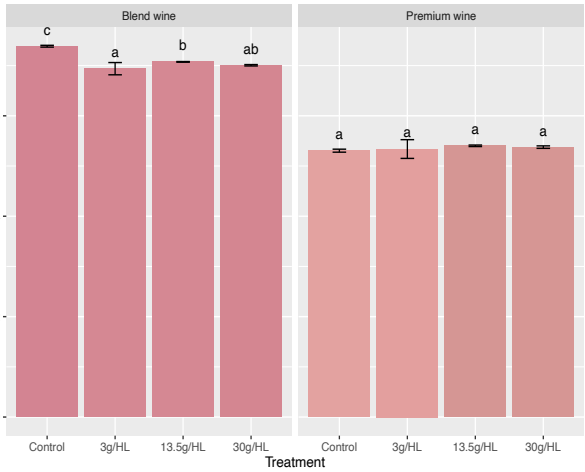

Figure 9. Total color according to Boulton for each dosage and wine quality. Different letters on the same line indicate statistically significant differences ($p < 0.05$) according to Duncan's post hoc test. The different observed colors of each bar are obtained through L*, a* and b* parameters.

that different polymerization and precipitation processes took place depending on the wine matrix and the dose of mannoprotein prior to bottling. In this sense, **Figure 9** illustrates how the dosage has a greater impact on WC of blend wines, particularly at doses exceeding 3 g/HL, while having no significant effect on the total color of premium wines. However, in the case of the last, the perceived darkness of color, may exhibit a slight increase at higher doses, due to a marginal but significant reduction in L*. The results are being contrasted by determining the total concentration of anthocyanins and phenols, as well as polysaccharides, monosaccharides, and sensorial analysis and volatile compounds for the first three months. factors. Analysis of 6 months of bottle aging will be carried, as stated in the experiment, to better understand which and why a given dosage is best suited to each wine matrix in terms of physicochemical and sensory characteristics, being able to extract the effect of dosage alone, the effect of wine quality and bottle aging, as well as the interactions between these two.

Sustainability of technology and quality control of olive oil

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1. State of the art

Nowadays, one of the main worldwide challenges is the achievement of the 17 sustainable development goals, known as SDGs in the framework of UN Agenda 2030. Among these, it is important to mention the SDG 12, namely "responsible consumption and production", and specifically the target 12.3, which focuses on the halve per capita food waste at the retail and consumer level and reduce food losses along the food production and supply chains; and target 12.4, that focuses on the management of chemicals and all wastes to significantly reduce their release to air, water and soil in order to minimize their adverse impacts on human health and the environment.
In addition, Europe is trying to become the first continent with zero impact in 2050 and to do this the European Green Deal provides a roadmap promoting an efficient use of resources by moving to a clean, circular economy and to restore biodiversity and cut pollution.
In the Mediterranean basin, olive oil represents one of the main food products, since almost the 90% of the world production comes from this area, concentrating mainly on European countries like Spain, Italy, and Greece, but also on others, such as Tunisia and Morocco. In the European Union, virgin olive oils (VOOs) can be classified in three commercial categories depending on their quality degree: extra virgin (EV), virgin (V) and lampante (L) (Reg. EU n. 2022/2104). The different quality level of each commercial category corresponds to a different value and, subsequently, to various price. In the context of olive oil production and quality control, it is important to consider that most of the official analytical methods to assess the quality and genuineness of VOOs consist of time-consuming and complex procedures, often with the use of toxic chemicals and solvents which are dangerous for human health and the environment (Valli et al., 2016). For these reasons, there is a strong and growing demand for rapid, easy-to use and environmentally friendly analytical procedures. This includes procedures that do not require solvents at all, such as the determination of volatile compounds by gas-chromatographic techniques with headspace-solid phase microextraction (HS-SPME-GC), ion mobility spectrometry (HS-GC-IMS) or HS-Flash-GC (Quintanilla-Casas et al., 2020). It is well known that volatile compounds have a crucial role to determinate VOOs quality, since they are directly responsible for the olfactory notes, and the application of methods for their determination could be used as support for sensory analysis in the classification of VOO based on the quality grade (Barbieri et al., 2020; Quintanilla-Casas et al., 2020).
In the context of rapid, innovative, and sustainable techniques for the assessment of VOOs quality and genuineness, the current investigations are also focused on the adoption of optical techniques (Valli et al., 2016). In particular, NIR, MIR, Raman and FT-IR spectroscopic methods can be considered useful for the rapid determination of food composition and molecular structure, also in the case of olive oils.
Agronomic factors, such as olive farming-systems, fertilization, irrigation, pests and diseases influence olive oil composition (Malheiro et al., 2014). Consequently, the adoption of sustainable agronomical practices can affect olive oil quality. In addition, olive oil production, as agro-industrial activity, in the Mediterranean area has a strong environmental impact, since it generates up to 30 million tons of waste per year, in which olive pomace is one of the principal by-products (Chandra and Sathiavelu, 2009). Olive pomace is the main residue from the mechanical extraction of the olive oil from the olive fruits and it is composed of skin, pulp and stone pieces, water, and oil. The major problem related to olive pomace is that it contains organic compounds with phytotoxic properties, that are dangerous for the environment. Although olive mill wastes represent an important environmental issue, they also contain high added value molecules, such as phenolic compounds (Dermeche et al., 2013), widely recognised for their beneficial properties (e.g. antioxidant activity). For this reason, this by-product can be considered a natural and economic source of phenolic compounds and their valorisation as functional ingredients in pharmaceutical, cosmetic and food industries (Nunes et al., 2016) represents a promising sustainable strategy, especially with a view to circular economy.

2. Bibliography

Barbieri S, Cevoli C, Bendini A, Quintanilla-Casas B, García-González DL, Gallina Toschi T (2020) Flash Gas Chromatography in tandem with chemometrics: a rapid screening tool for quality grades of virgin olive oils, Foods 9: 862.

Chandra M, Sathiavelu S (2009) Waste management in the olive oil industry in the Mediterranean region by composting, Clean Technol Environ Policy 11: 293–298.

Dermeche S, Nadour M, Larroche C, Moulti-Mati F, Michaud P. (2013) Olive mill wastes: Biochemical characterizations and valorization strategies, Process Biochem 48: 1532–1552.

European Commission. Reg. (EU) n. 2022/2104 supplementing Regulation (EU) No 1308/2013 of the European Parliament and of the Council as regards marketing standards for olive oil, and repealing Commission Regulation (EEC) No 2568/91 and Commission Implementing Regulation (EU) No 29/2012.

Nunes MA, Pimentel FB, Costa ASG, Alves RC, Oliveira MBPP (2016) Olive by-products for functional and food applications: challenging opportunities to face environmental constraints, IFSET 35: 139–148.

Malheiro R, Casal S, Baptista P, Pereira JA (2014) How agronomic factors affects olive oil composition and quality. In De Leonardis (Ed.) Virgin Olive Oil: Production, Composition, Uses and Benefits for Man, Nova Science Publishers: 118-141.

Quintanilla-Casas B, Bustamante J, Guardiola F, García-González DL, Barbieri S, Bendini A, Gallina Toschi T, Vichi S, Tres A (2020) Virgin olive oil volatile fingerprint and chemometrics: Towards an instrumental screening tool to grade the sensory quality, LWT - Food Sci Technol 121: 108936.

Valli E, Bendini A, Berardinelli A, Ragni L, Riccò B, Grossi M, Gallina Toschi T (2016) Rapid and innovative instrumental approaches for quality and authenticity of olive oils, Eur J Lipid Sci Technol 118: 1601–1619.

3. Research development

This PhD research project has been developed according to the following activities:

1) Bibliographic research.
2) Olive pomace valorisation: development of sustainable methods for the extraction of phenolic compounds from olive pomace, as well as characterization and shelf-life evaluation of the phenolic extracts.
3) Rapid and sustainable analytical methods for quality and authenticity of virgin olive oils: development and application of easy-to use, innovative and sustainable analytical approaches to evaluate the quality and genuineness of virgin olive oils.
4) Comparative study of virgin olive oils produced in experimental fields using sustainable and non-sustainable agricultural practices.
5) Spectroscopic analysis of virgin olive oils and development of chemometric models to predict the commercial category to support the sensory analysis (panel test).
6) Writing of the PhD thesis, scientific papers, oral and/or poster communications.

Table 1. Gantt diagram for this PhD thesis project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***																		
A2)	***Olive pomace valorisation***																		
	1) Development of sustainable extractions																		
	2) Characterization and shelf-life study																		
A3)	***Rapid and sustainable analytical methods***																		
	1) Methods development																		
	2) Methods application																		
A4)	***Comparative study of virgin olive oils***																		
A5)	***Spectroscopic analysis***																		
A6)	***Thesis and paper preparation***																		

4. Research main results

This PhD project is focused on sustainability aspects in relation to technology and quality control of olive oils. The research has started from the application of rapid instrumental methods to evaluate the quality and genuineness of VOOs, based on the study of the volatile fraction, since it shows a relevant potential to support the sensory analysis (panel test) for the determination of the commercial category of VOOs, thus pre-classifying the samples. In this context, Flash-GC and HS-GC-IMS analyses were performed on an olive oil set composed of 120 samples, collected in order to have a relevant and balanced variety in the commercial categories of VOOs (among extra virgin, virgin and lampante) determined by sensory analysis (panel test). At now the data elaboration has regarded a set composed of 52 samples, classified into the commercial category using previously developed prediction approaches based on a PLS-DA models, both for Flash-GC (Barbieri et al., 2020) and HS-GC-IMS (Valli et al., 2020) results. The results show comparable effectiveness between the two techniques, and they are satisfactory in terms of percentage of correctly classified samples for the different commercial categories (see **Table 2)**, confirming the robustness of the developed models.

Table 2. Flash-GC and HS-GC-IMS outcomes, in terms of samples correctly classified, compared to the sensory assessment, by the prediction models.

	Flash-GC		*HS-GC-IMS*	
COMMERCIAL CATEGORY	**SAMPLES CORRECTLY CLASSIFIED**	**%**	**SAMPLES CORRECTLY CLASSIFIED**	**%**
EVOO	16/17	94.12	15/17	88.24
VOO	17/19	89.47	17/19	84.21
LOO	13/16	81.25	15/16	93.75
TOTAL	**31/52**	**88.46**	**47/52**	**90.38**

In addition, regarding the HS-GC-IMS, the whole sample set was analysed also by using improved analytical conditions with respect to the published ones (Valli et al., 2020) and the data elaboration is now ongoing.
Finally, the same samples set was analysed by spectroscopic methods (NIR, FT-IR, Raman), during a 3-months visiting period at Queen's University Belfast from November 2022 to February 2023. Firstly, a preliminary classification based on the three commercial categories was performed applying PLS-DA models on the results obtained from each technique, giving not completely satisfactory results, in terms of samples correctly classified. Then, a focus on the rancid defect is under investigation, since it is directly related to the oxidation status of olive oil, and subsequently to its quality. Also, a "data fusion" between the results of the different analytical techniques will be considered to obtain more robust predictive models.
Moreover, the experimental activities aimed to the technological valorisation of olive pomace by obtaining sustainable extracts rich in phenolic compounds, potentially usable in different industrial sectors, such as pharmaceutical, food and cosmetic, are now ending. This research is carried out in the framework of the Prima project SUSTAINOLIVE "Novel approaches to promote the SUSTAInability of OLIVE cultivation in the Mediterranean" (Grant Agreement no. 813904), 2019 – 2023. After the set-up of a sustainable method for the extraction of phenolic compounds without the use of toxic solvent, a mechanical approach (using a lab scale screw-press) was applied on the olive pomace by adding a mixture of water and food grade ethanol (80:20 % v/v) and two types of samples were obtained: one more liquid drained from the lower part of the mill (named *SI*) and one drier from the frontal part (named *SF*). On the samples, including olive pomace as it is (named *TQ*), a study of the phenolic fraction was carried out through the application of the Folin-Ciocalteu method, and by UHPLC-DAD, UHPLC-MS/MS analyses. About the UHPLC-MS/MS analytical approach, data elaboration is still ongoing to reach a complete phenolic profile.

Table 3. Average concentrations and relative standard deviations of the extracts from the sample TQ, SI, SF (see the description of these samples in the text above). In the first column the total concentrations of the sum of unknown compounds (Unk), hydroxytyrosol (HTyr), and tyrosol (Tyr), obtained after hydrolysis of the extracts, are reported. In the second column, the concentration in total reducing molecules contents obtained by Folin-Ciocalteu method are reported. Statistical analysis: ANOVA, HSD Tukey, p <0,05.

Sample	Concentration (g Tyr + HTyr + Unk/kg olive pomace)	SD	Concentration (g gallic acid/kg olive pomace)	SD
TQ	1.12^{b}	0.25	3.29^{b}	0.38
SI	1.54^{a}	0.14	7.10^{a}	0.42
SF	0.64^{b}	0.02	3.09^{b}	0.39

The results show that the extract obtained from the olive pomace drained from the central part of the lab-scale mill, *SI*, is the richest in the concentration of both total reducing molecules and the detected simple phenolic molecules after hydrolysis (**Table 3**). For this reason, it has been selected as the more suitable sample to obtain stable hydroalcoholic phenolic extracts. The UHPLC-MS/MS data elaboration is now ongoing, in order to obtain a complete phenolic profile of the considered extracts. Subsequently, the best technological conditions to obtain this extract were set-up: the procedure included filtration of the olive pomace, evaporation, and addition of food grade ethanol. On the selected extract, the phenolic compounds characterization and the assessment of its stability during a shelf-life study were performed, including both sensory (descriptive analysis) and instrumental (Folin-Ciocalteu method, UHPLC-DAD, UHPLC-MS/MS) evaluation. In particular, the shelf-life study was performed on a monthly basis and during three months (T0, T1 and T2, see **Table 4**), and carried out on the selected extract stored at room temperature and dark conditions.

Table 4. Average concentrations and relative standard deviations of the extracts from the sample T1, T2, T3 (see the description of these samples in the text above). In the first column the total concentrations of the sum of unknown compounds (Unk), hydroxytyrosol (HTyr), and tyrosol (Tyr), obtained after hydrolysis of the extracts, are reported. In the second column, the concentration in total reducing molecules contents obtained by Folin-Ciocalteu method are reported. Statistical analysis: ANOVA, HSD Tukey, p <0,05.

Sample	Concentration (mg Tyr + HTyr + Unk/mL extract)	SD	Concentration (mg gallic acid/mL extract)	SD
T0	0.23^{b}	0.00	0.55^{c}	0.01
T1	0.25^{a}	0.00	0.63^{a}	0.02
T2	$0.24^{a,b}$	0.00	0.60^{b}	0.01

The increase in the concentration of total reducing molecules observed at T1 with respect to T0 can be ascribable to a degradation of the sugar fraction of the extracts, since the difference in the related concentration of the phenolic molecules after hydrolysis, despite being significant, was very low in absolute value (**Table 4**).
On the other side, the sensory analysis was carried out by 8 panelists trained for olive oil assessment, through an olfactory evaluation, and they were asked to exclude the perception of ethanol. During the sensory assessment, relevant defects and other negative attributes were not perceived. The attributes mainly perceived were positive and related mostly to specific notes resembling vanilla, caramel, red fruits, and olive fruits.
Moreover, in the frame of the Prima project SUSTAINOLIVE, a comparative study is currently ongoing between VOOs obtained during the last olive season in experimental fields in which sustainable agricultural practices are adopted and others in which they are not followed. The olive oil samples have been provided by different countries (Italy, Greece, Tunisia, Spain, Portugal, and Morocco) in which farms, involved in the project, were previously selected. Other variables, such as olive fruit variety, location, olives maturity, technology conditions of milling and storage, will be taken into account. The main aim was to investigate the effect of sustainable agricultural solutions in the olive oil quality and composition by assessing free acidity, peroxide value, specific extinctions in UV, fatty acids profile, total phenolic compounds, as well as sensory analysis. The data elaboration is still ongoing.
Finally, the writing of the PhD thesis and of three scientific papers, based on the results obtained from the experimental activities that will be submitted in the next few weeks to scientific journals, is currently ongoing.

5. Publications produced during the PhD activities

Lazzarini C, Valli E, Casadei E, Grigoletto I, Ragni L, Bendini A, Gallina Toschi T (2021) A sustainable approach for the valorisation of a tomato by-product: green techniques for lycopene extraction, Book of Abstracts of the 6th International ISEKI-Food Conference, "Sustainable Development Goals in Food Systems: Challenges and Opportunities for the Future", pp. 142.

Grigoletto I, García Salas P, Valli E, Bendini A, Pasini F, Sánchez Villasclaras S, García Ruiz R, Gallina Toschi T (2022) Study of the phenolic fraction for the valorization of olive pomace as a functional ingredient, J Am Oil Chem Soc, Supplement: Book of Abstracts of 2022 AOCS Annual Meeting & Expo, May 1–4, 2022, 99, S1: pp. 107.

Grigoletto I, García Salas P, Valli E, Bendini A, Pasini F, Sánchez Villasclaras S, García Ruiz R, Gallina Toschi T (2022) Valorizzazione della sansa: metodi sostenibili per estrarre i composti fenolici, Book of Abstract of Congress SISSG 2022 "Edible Oils and Fats: Innovation and Sustainability in Production and Control", pp. 52.

Panni F, Valli E, Grigoletto I, Casadei E, Cevoli C, Bendini A, Focante F, Gallina Toschi T, Savino AF, Carpino S (2022) HS-GC-IMS e SPME-GC-FID: Metodi di screening e "targeted" per la classificazione degli oli vergini di oliva a supporto del Panel test, mediante studio della frazione volatile, Book of Abstract of Congress SISSG 2022 "Edible Oils and Fats: Innovation and Sustainability in Production and Control", pp. 32-33.

Grigoletto I, Casadei E, Panni F, Valli E, Cevoli C, Bendini A, Gallina Toschi T, Birse N, Vanhaecke L (2022) High throughput and field deployable instrumental screening methods to guarantee olive oil authenticity, Book of abstracts of the 10th International symposium on recent advances in food analysis (RAFA 2022), pp. 191.

Grigoletto I (2022) Sustainability of technology, quality control and consumption of olive oil, Proceedings of the 26th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology Asti (Italy), pp. 262.

Metabolomics to investigate the effects of treatments on food and of food consumption on health

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Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVI; Anno di frequenza: II
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1. State-of-the-art

Metabolomics is the field of research that aims to identify and quantify the complete collection of small metabolites of a biological system, its so-called metabolome. When the system is a food, metabolomics offers a systematic approach to determine its components and, consequently, the chemical and biochemical changes caused by technological transformations or by the action of microorganisms. These changes define the final characteristics of products, such as nutritional quality, safety and sensory characteristics. Therefore, metabolomic approaches can be a versatile way to obtain information on the consequences of any food trait or transformation on consumer acceptability by monitoring changes in the food as a whole. Furthermore, as the whole set of metabolites in food can directly influence the health status of humans at the time of consumption, a comprehensive metabolomic observation of food intake could include the metabolite profile of body fluids (Trimigno, et al., 2020).

To investigate the metabolome of a food and of human biofluids simultaneously, proton high-resolution nuclear magnetic resonance (^{1}H-NMR) is one of the election analytical platforms for metabolomics investigation. Examples of its application can be traced in the work by Yang et al. (2020), who investigated the taste difference of different Chinese sauce-stewed beef by the quantification of the taste-active metabolites using ^{1}H-NMR. Trimigno et al. (2020) compared the metabolic effects of nutritionally healthy New Nordic Diet with an Average Danish Diet by monitoring the alteration of the human urine metabolome using ^{1}H-NMR spectroscopy. Therefore, ^{1}H-NMR has been proven as a valuable tool to acquire information about changes in food quality with different treatments as well as connections between food composition and health.

Fermentation is considered the most ancient food preservation technology and is perfectly tailored to be studied through metabolomics approaches, as it is a metabolic process that consumes and produces a range of metabolites, through the biological activity of microorganisms. It has been used in a range of foodstuffs. As an example, *Lactobacillus sakei* is often used to ferment dry-fermented sausages (Hugas, et al., 1993). Because it can outperform undesired microorganisms, including pathogens (Chaillou, et al., 2013). Besides, it is able to use a variety of substrates to obtain energy for survival whenever its main source of energy, hexose, and pentose sugars, is depleted (Cocconcelli & Fontana, 2010). To further optimize the use of this species in the fermentation processing of meat sausage, we can rely on metabolomic approaches to gain insight into these mechanisms and characteristics.

Regarding food processing, non-thermal technologies are gaining traction, especially in developed countries, because they imply milder treatment conditions than heat exchange, granting therefore higher a quality, with equal results in terms of food safety. Among them, high hydrostatic pressure (HHP) has been proven to be an effective preservation technique for various seafood, by reducing the growth of undesirable spoilage microorganisms (Ekonomou & Boziaris, 2021). Metabolomics can give valuable pieces of information also in his context, because many consequences of microbial growth on the sensory characteristics related to freshness and flavors can be conveniently followed by observing the evolution of the metabolome of fish flesh (Lou, et al., 2020). As examples, the concentrations of adenosine triphosphate (ATP) and its breakdown products, named adenosine-5-diphosphate (ADP), adenosine-5-monophosphate (AMP), inosine-5-monophosphate (IMP), inosine and hypoxanthine, are used as indices of freshness in various seafood. Moreover, some water-soluble, low-weight molecules are considered taste-active compounds that contribute to the specific flavors of seafood, classified as umami, sweet, sour, and bitter (Nishimura & Kato, 1988). As the effects of HHP on seafood are diverse and depend not only on process parameters but also on seafood species (Puértolas & Lavilla, 2020), more research is needed to better understand the effects of HHP on specific seafood metabolomes.

A research field where the extension of metabolomics observation to the human body upon nutrition is particularly appealing is the application of probiotics. Probiotic bacteria have been applied to the production of functional foods for a healthy diet by exploiting their beneficial effects on the immune system and health. Very interestingly, the mechanisms underlying their effects on health is largely unknown. This is true for many applications, one which described by Román *et al.* (2019), who investigated by a double-blind, placebo-controlled, randomized clinical trial the effects of a multistrain probiotic on subjects affected by cirrhosis.

2. Bibliography

Chaillou S, Lucquin I, Najjari A, et al. (2013) Population Genetics of *Lactobacillus sakei* Reveals Three Lineages with

Distinct Evolutionary Histories. Plos One, 8(9): e73253.
Cocconcelli PS, Fontana CA. (2010) Starter Cultures for Meat Fermentation: Wiley ‐ Blackwell, pp. 199 ‐ 218.
Ekonomou SI, Boziaris IS. (2021) Non-Thermal Methods for Ensuring the Microbiological Quality and Safety of Seafood. Appl. Sci. 11 (2): 833.
Hugas M, Garriga M, Aymerich T, et al. (1993) Biochemical characterization of lactobacilli from dry fermented sausages. International Journal of Food Microbiology, 18(2): 107-113.
Lou XW, Zhai DD, Yang HS. (2020) Changes of metabolite profiles of fish models inoculated with Shewanella baltica during spoilage. Food Control, 123: 107697.
Nishimura T, and Kato H. (1988). Taste of free amino acids and peptides. Food Reviews International, 4(2).
Puértolas E, and María L. (2020) HPP in seafood products: impact on quality and applications. In Present and future of high pressure processing. Elsevier, pp 201-220.
Román E, Nieto JC, Gely C, et al. Effect of a Multistrain Probiotic on Cognitive Function and Risk of Falls in Patients With Cirrhosis: A Randomized Trial. Hepatol Commun. 2019;3(5):632-645.
Trimigno A, Khakimov B, Savorani F, et al. (2020). Human urine 1H NMR metabolomics reveals alterations of protein and carbohydrate metabolism when comparing habitual Average Danish diet vs. healthy New Nordic diet. Nutrition, 79, 110867.
Yang Y, Wang Y, Pan D, et al. The application of 1H NMR to explore the taste difference caused by taste-active metabolites of different Chinese sauce-stewed beef. Food Science & Nutrition. 2020 Sep;8(9):4868-76.

3. Objectives

To complete the project of the doctoral thesis, the relevant academic activities may be carried out according to the Gantt diagram in Table 1:

A1) Literature review of latest research related to investigating the effects of food processes on the physiological response and metabolism of food-related microorganisms.
A2) Set up and application of specific NMR SOP (Standard operation procedures).
A3) Metabolomics-oriented experiments focusing on the effects of treatments on food composition and quality.
A4) Metabolomics-oriented experiments focusing on the relationship between food composition and health.
A5) Writing and publishing the doctoral thesis, posters, scientific articles, and oral presentations.

Table 1. Diagramma di Gantt dell'attività di ricerca del dottorato

Activity	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Literature review and Experimental design***																		
	1) preliminary studies in metabolomics																		
	2) experiment design																		
A2)	***Set up and apply specific NMR SOP***																		
A3)	***Experiments focusing on the metabolites of food under treatments***																		
	1) read papers to select the most appropriate foods to investigate																		
	2) assessments of the changes of metabolites with treatments																		
A4)	***Experiments focusing on the metabolites and health***																		
	1) read papers to select the most appropriate diet to investigate																		
	2) assessments of the relationship between food composition and health																		
A5)	***To write and publish the doctoral thesis***																		

4. Research progress and principal results

In the first year, I focused on researching and testing concerning the metabolome of fermented food. In order to set up and apply specific SOPs (standard operating procedures), pre-tests were performed on the metabolome of soy sauce and tempeh by ^{1}H-NMR spectroscopy on their water extracts. These efforts gave me confidence in the complete workflow required to analyze ^{1}H-NMR data, through the development of in-house scripts based on the R computational language and the application of the ^{1}H-NMR specific software, such as MNOVA (Mestrelab Research, Spain) and Chenomx (Chenomx. Inc., Canada). Subsequently, metabolomic-oriented experiments were carried out about the adaptation of *Latilactobacillus sakei* to different carbon sources. The resulting paper, "Insights into the metabolome diversity of Latilactobacillus sakei", describes the cultivation of five strains of different origins in defined media containing two concentrations of glucose or ribose. The media were analyzed by ^{1}H-NMR spectroscopy to monitor amino acid depletion and the accumulation of organic acids and aromatic compounds.

Table 2. The microbiological shelf life (day) of packaged rose shrimp, striped prawn, and grey mullet untreated or after treatment with HHP at 400,500 or 600 MPa.

	striped prawn	rose shrimp	grey mullet
control	9	7	9
400 MPa	21	14	21
500 MPa	35	28	35
600 MPa	35	35	35

In the second year, another metabolomics-oriented experiment was performed focusing on the effect of high hydrostatic

pressure (HHP) on the metabolome of different seafood. This experiment considered rose shrimp (*Parapenaeus longirostris*), striped shrimp (*Penaeus kerathurus*), and grey mullet (*Mugil cephalus*). Three pressure levels (400, 500 and 600 MPa) were applied for 10 min. Metabolomic by ^{1}H-NMR and microbiological analyses were performed on the considered seafood during chill storage, until the microbial load reached 6 log cfu/g, a value witnessing the end of the microbiological shelf life.

As listed in Table 2, the application of 600 MPa allowed to extend the microbiological shelf life of the considered seafood up to 35 days. Besides, any treated sample showed lower viable counts of total mesophilic bacteria, *Lactobacillus spp.*, *Pseudomonas spp.*, *E. coli*, total Coliforms, sulfite-reducing anaerobic bacteria (AB), and/or positive coagulase staphylococci throughout the storage compared to the untreated counterpart (data not shown).

^{1}H-NMR spectra allowed us to quantify the main nucleotides typically constituting seafood, as well as their breakdown derivatives, accumulated during storage. Based on the different nucleotide compositions detected by ^{1}H-NMR in three seafood products (data not shown), this made possible to calculate different nucleotide breakdown freshness indices for rose shrimp, striped prawn and grey mullet, namely Ki value, K value, and H value. Overall, as shown in Figure 1, it is obvious that the HHP treatment delayed the degradation of nucleotides and effectively slowed down the deterioration of freshness. In further detail, from the changes in the nucleotide concentration of each treatment during storage (data not shown), we speculated that HHP treatment delay the conversion of IMP to inosine and/ or inosine to hypoxanthine.

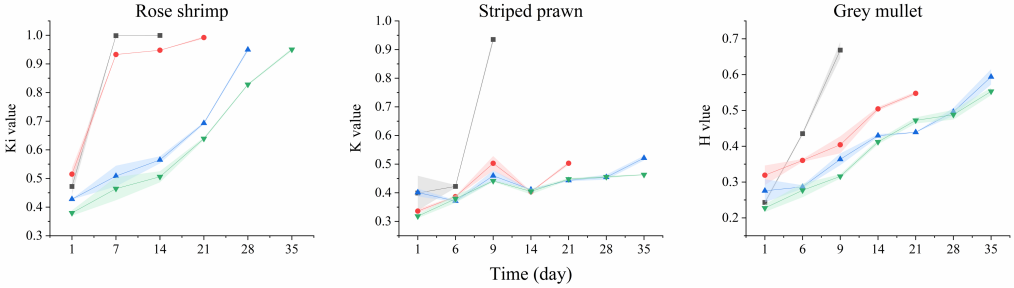

Figure. 1 Changes of nucleotide degradation-related indices for rose shrimp, striped prawn, and grey mullet untreated (black squares) and in samples treated with HHP at 400 (red circles), 500 (blue upward triangles), and 600 MPa (purple downward triangles). Ki value is the ratio of the total amount of inosine and hypoxanthine to that of IMP, inosine, and hypoxanthine. K value is the ratio of the total amount of inosine and hypoxanthine to that of all nucleotides. H value is the ratio of hypoxanthine to the total amount of IMP, inosine, and hypoxanthine.

It is intriguing to notice that a single ^{1}H-NMR spectrum made possible not only to calculate the above-mentioned indices of freshness but also to quantify some small molecules that can be directly tasted by consumers. To express the concentration of taste active molecules in terms of their contribution to sensorial characteristics, their taste active value (TAV) was calculated as the ratio of the compound concentration to its taste recognition threshold. Generally, the compounds with TAV greater than 1 were considered active compounds in food taste analysis (Figure 2).

As seen in Figure 2, different taste active molecules contributed to the taste of rose shrimp, striped prawn, and grey mullet. In rose shrimp, on day 1 glutamate and IMP showed a concentration above the threshold for umami taste. Lysine, glycine, and alanine could contribute significant sweet taste impacts to rose shrimp. In striped prawn, on day 1 lactate, IMP, and arginine showed a concentration above the threshold for sour, umami, and bitter tastes, respectively, while glycine, lysine, and alanine exceeded the threshold for sweet taste. In grey mullet, on day 1 histidine and lactate exceeded their threshold for bitter and sour tastes, respectively.

Another interesting observation was that both HHP treatment and storage had an impact on the TAV of taste-active compounds. This is particularly evident with the TAV of acetate, which tended to reach levels above 1 in the three untreated seafood after 7 or 9 days of storage. While in 500 and 600 MPa treated seafood samples, the concentrations of acetate were present at lower than the threshold value after 7 or 9 days. A similar result was observed for succinate concentrations of rose shrimp and striped prawn. Lower concentrations of acetate and succinate in treated samples compared with this in untreated samples suggest that HHP can reduce the metabolism production of acetate and succinate, without preventing it completely.

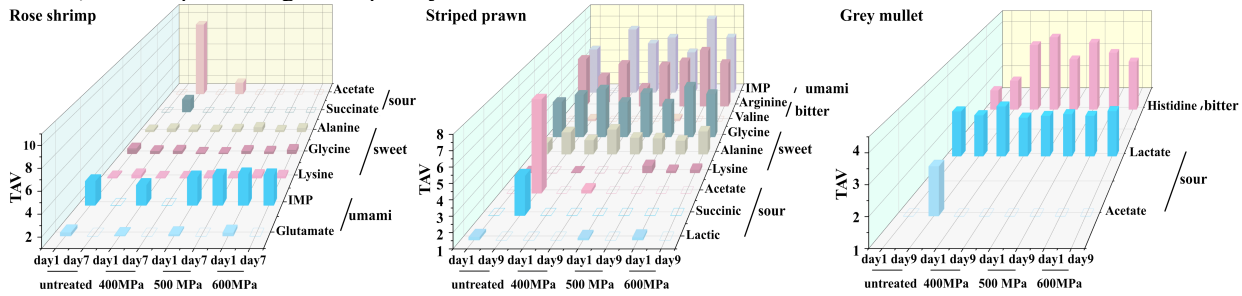

Figure. 2 Taste-active molecules with TAVs greater than 1 in untreated and treated (400, 500, 600 MPa) rose shrimp, striped prawn, and grey mullet on day 1 and day 7 or 9. No fill rectangle represents TAV less than 1.

In the third year, a further metabolomics-oriented experiment targeted the potential mechanisms underlying the effect of a multistrain probiotic on cirrhotic patients. A multistrain probiotic or placebo was randomized to 32 patients with

cirrhosis and cognitive dysfunction or falls. All patients were treated for 12 weeks and assessed at baseline and at 12 weeks (end of treatment) for clinical and analytical data. The metabolome of serum at baseline and at 12 weeks was analyzed by ^{1}H-NMR spectroscopy.

^{1}H-NMR spectra representative of serum was shown in Figure 3. The untargeted observation of the serum metabolome by ^{1}H-NMR enabled the identification of 54 metabolites. Among these metabolites, the main change after the use of probiotics is in the amount of glutamine and glutamate.

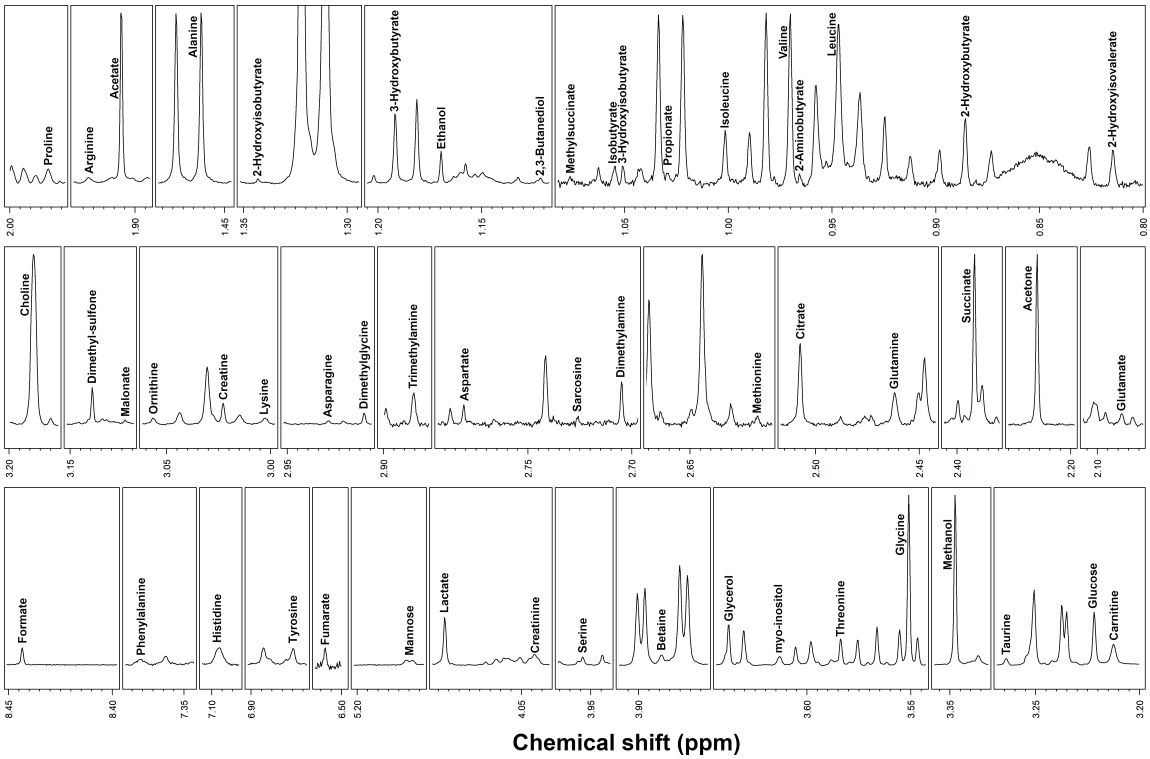

Figure. 3 Example of the spectra obtained by 1H-NMR metabolomic analysis of cirrhotic patients' serum.

Pairwise comparisons between values at baseline and at 12 weeks (Figure 4) showed an increase in blood glutamine (0.002, FDR p=0.007) and a decrease in glutamate (p=0.03, FDR p=0.03) in the probiotic group, resulting in an increase in the glutamine/glutamate ratio (p=0.009, FDR p=0.01). In contrast, the placebo group showed an increase in glutamate concentration (p=0.01, FDR p=0.02) and a decrease in glutamine/glutamate ratio (p=0.02, FDR p=0.03). No statistically significant changes were observed in any of the other metabolites identified. Our results suggest that multispecies probiotics can influence glutamine/glutamate metabolism and increase the ability to detoxify ammonia.

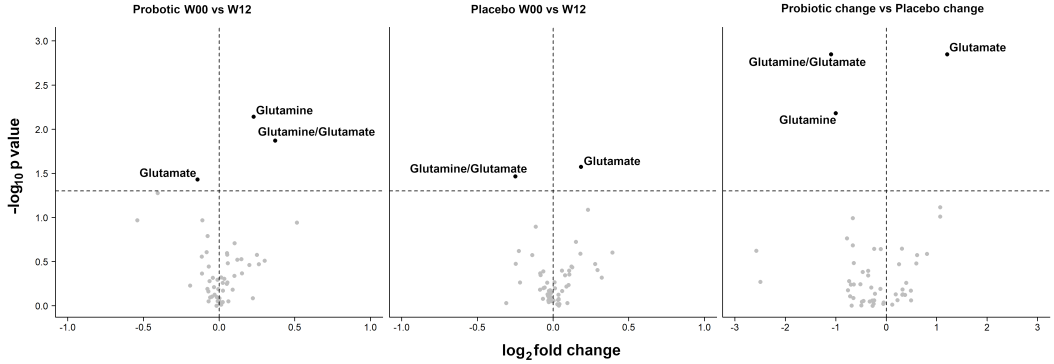

Figure. 4 Volcano plots showing the change between baseline and 12 weeks in all metabolites identified in the probiotic group and in the placebo group.

5. Publication produced during the PhD activities

1. Barbieri F, Laghi L, Montanari C, Lan Q, Levante A, Gardini F, Tabanelli G. (2022) Insights into the Metabolomic Diversity of *Latilactobacillus sakei*. Foods, 11(3), 477.
2. Lan Q, Tappi S, Braschi G, Picone G, Rocculi P, Laghi L. (2022) Effect of High Hydrostatic Pressure on the Metabolite Profile of Striped Prawn (Melicertus kerathurus) during Chilled Storage. Foods, 11(22), 3677.
3. Laghi L, Román E, Lan Q, Nieto JC, Canalda-Baltrons A, Poca M, Sánchez-Rodríguez MB, Clària J, Alvarado E, Cuyàs B, Sánchez E, Vidal S, Guarner C, Escorsell À, Manichanh C, Soriano G. (2023) A multistrain probiotic increases the serum glutamine/glutamate ratio in patients with cirrhosis: a metabolomic analysis. Hepatol Commun. Apr 4;7(4):e0072.