ALMA MATER STUDIORUM
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Dottorato in Scienze e Tecnologie Agrarie, Ambientali e Alimentari

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ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA
PHD PROGRAMME
AGRICULTURAL, ENVIRONMENTAL AND FOOD SCIENCE AND TECHNOLOGY

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DOTTORANDI ISCRITTI AL I ANNO (XXXIX CICLO)

Risk assessment dell'effetto degli xenobiotici sul microbiota intestinale umano

Davide Addazii (e-mail: davide.addazii2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Andrea Gianotti; Co-tutor: Lorenzo Nissen

1. Curriculum

Laurea magistrale in Biosicurezza e Qualità degli Alimenti (LM-7) conseguita a Febbraio 2023 presso l'Università di Pisa con la votazione di 110/110 *cum Laude*. Titolo tesi: Valutazione quantitativa della produzione di biofilm di *Listeria monocytogenes* isolati da prodotti a base di carne suina e dal loro ambiente di produzione.
Tirocinio curriculare da Settembre 2022 a Gennaio 2023 presso il laboratorio di microbiologia degli alimenti dell'Istituto Zooprofilattico Sperimentale del Lazio e Toscana (IZSLT) sede di Roma.
Eletto rappresentate degli studenti e membro della commissione paritetica, presso l'Università di Pisa, Giugno 2021.
Iscritto al corso di laurea magistrale in Biosicurezza e Qualità degli Alimenti presso l'Università di Pisa, Settembre 2020/2021
Laureato frequentatore da Gennaio 2020 a Marzo 2020 presso il laboratorio di microbiologia alimentare, dell'Università degli Studi di Teramo-Facoltà di Bioscienze.
Laurea triennale in Scienze e Tecnologie Alimentari (L-26) conseguita a Dicembre 2019 presso l'Università degli Studi di Teramo. Titolo della tesi: Attività antimicrobica di estratti di insetti edibili su *Listeria monocytogenes* e *Pseudomonas fluorescens*
Erasmus+ da settembre 2016 a Giugno 2017 presso Universidad Miguel Hernandez de Elche-Politecnico (Spagna).
Iscritto al corso di laurea triennale in Scienze e Tecnologie Alimentari presso l'Università degli Studi di Teramo, Settembre 2014/2015.

2. Stato dell'arte

Gli esseri umani ingeriscono una moltitudine di molecole estranee all'organismo (xenobiotici), tra cui componenti della dieta, sostanze chimiche ambientali e farmaci. Il termine "xenobiotico" è utilizzato per descrivere le sostanze chimiche estranee alla vita umana, come farmaci, pesticidi, cosmetici, aromi, fragranze, additivi alimentari, sostanze chimiche industriali ed inquinanti ambientali (Idle Jeffrey R.; Gonzalez Frank J. 2007).
Gli xenobiotici ingeriti possono influenzare direttamente il microbiota influendo potenzialmente sulla salute dell'ospite mediante soppressione o potenziamento selettivo di specifiche specie batteriche all'interno della complessa comunità microbica (Suez J. *et al.*, 2014; Chassaing B. *et al.,* 2017). Poiché il microbiota è noto per il suo coinvolgimento nel metabolismo di vari farmaci e inquinanti (Spanogiannopoulos P. *et al.,* 2016), le variazioni nella composizione della comunità batterica tra gli individui possono portare a risposte diverse a specifici composti xenobiotici. Lo studio pre-clinico di tali risposte è da sempre realizzato prevalentemente su modelli animali che oggi stanno perdendo importanza sia per scarsa trasferibilità dei risultati all'umano che, in tempi più recenti, per una maggiore sensibilità verso l'eticità della sperimentazione animale.
Questa considerazione sottolinea l'importanza di condurre una valutazione del rischio (risk assesment) utilizzando modelli *in vitro*, strumenti eccellenti per lo screening di numerose sostanze specialmente quando i loro effetti dipendono dalle interazioni con il microbiota intestinale. Un modello ampiamente utilizzato è il *Multi-Unit in vitro Colon Model* (MICODE), un nuovo modello intestinale sviluppato presso il DISTAL (Università di Bologna), che simula il colon umano o animale con grande versatilità. Tale modello, che può essere preceduto da una simulazione di digestione gastro-duodenale, si è dimostrato utile nella valutazione degli effetti della formulazione e dei processi sull'ecosistema intestinale attraverso la valutazione della dinamica dei cambiamenti della popolazione intestinale (Nissen *et al.,* 2021; Nissen *et al.,* 2022). Fornendo così informazioni cruciali sulla biodiversità microbica e sull'analisi delle vie metaboliche in grado di spiegare alcuni meccanismi alla base delle caratteristiche prebiotiche di alimenti o ingredienti. MICODE offre la possibilità di simulare diverse condizioni *in vitro*, sia in modalità continua che in batch rendendolo un modello estremamente versatile.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di studiare *in vitro* gli effetti sulla microflora intestinale derivanti dall'ingestione degli xenobiotici tramite il modello intestinale MICODE e validarne l'utilizzo nell'ambito del risk assesment. Tra i fattori xenobiotici, saranno considerati quelli veicolati all'uomo attraverso la dieta e in particolare: i) contaminanti alimentari, ii) additivi alimentari, e iii) microrganismi patogeni e loro metaboliti tossici. A tale scopo saranno considerati gli effetti perturbativi di tali fattori sia sulle popolazioni microbiche che sui loro metaboliti al fine di

ipotizzare meccanismi alla base degli effetti tossici mediati dal microbiota intestinale. Il progetto mira ad ottenere i seguenti risultati:
- Studiare un protocollo sperimentale per adattare il modello intestinale MICODE alla valutazione del rischio derivante da fattori xenobiotici di origine dietetica;
- Individuare target microbici e metabolici o loro indicatori in grado di descrivere gli effetti benefici e dannosi degli xenobiotici considerati
- Individuare alcuni casi studio per realizzare un modello di risk assesment dell'esposizione basato su marcatori microbiologici e metabolici;
- Stabilire delle linee guida per l'utilizzo del modello intestinale MICODE come strumento preclinico nel risk assesment di xenobiotici di origine alimentare.

Il progetto di tesi di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt riportato in tabella 1:
A1) Ricerca bibliografica degli xenobiotici come i contaminanti alimentari, additivi alimentari e microrganismi patogeni;
A2) Messa a punto del modello *in vitro* per lo studio dell'ecologia microbica del tratto intestinale e delle metodiche per la determinazione del microbiota e dei suoi metaboliti;
A3) Selezione degli xenobiotici oggetto dello studio sulla base di uno screening delle loro caratteristiche;
A4) Valutazione degli effetti sulla microflora intestinale sul modello in vitro;
A5) Scrittura e pubblicazione di poster, articoli scientifici e della tesi di dottorato.

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 modello in vitro***		■	■	■														
A3)	***Selezione degli xenobiotici***					■	■	■	■	■	■	■	■	■	■	■	■		
	1) Food contaminants					■	■	■	■										
	2) Food additives									■	■	■	■						
	3) Food pathogens													■	■	■	■		
A4)	***Valutazione degli effetti sulla microflora intestinale sul modello in vitro***						■	■	■	■	■	■	■	■	■	■	■	■	
A5)	***Preparazione della tesi e di articoli***						■	■	■	■	■	■	■	■	■	■	■	■	■

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Idle, Jeffrey R., and Frank J. Gonzalez. "Metabolomics." Cell metabolism 6.5 (2007): 348-351.

Nissen L, Casciano F, Chiarello E, Di Nunzio M, Bordoni A, Gianotti A. Colonic In Vitro Model Assessment of the Prebiotic Potential of Bread Fortified with Polyphenols Rich Olive Fiber. Nutrients. 2021 Feb 27;13(3):787.

Nissen, L, Casciano F, Di Nunzio M, Bordoni A, Gianotti A. Protein enrichment of gluten free bakery products by Arthrospira platensis (spirulina): in vitro study of the effect of formulation and sourdough process on colon microbiota through MICODE gut model. In FoodMicro 2022 Next Generation Challenges in Food Microbiology (pp. 93-93).

Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 2014, 514:181–186.

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Poultry meat downgrading: Insights on the underpinning factors to improve the sustainability of the production

Emilia Luigia Antenucci (email: emilia.antenucci2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Water-Food-Energy-Sustainable Agriculture Nexus; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Dott.ssa Francesca Soglia; Co-tutor: Prof. Massimiliano Petracci

1. Curriculum

Emilia Luigia Antenucci was born in Lanciano (Chieti) in 1995. In 2020 she graduated in Food Science and Technology at the Marche Polytechnic University (Ancona) discussing a thesis on the Nanocellulose-polymer composites for application in food packaging. In 2023 she obtained the Master's degree in Food Science and Technologies (Alma Mater Studiorum – Univeristy of Bologna) discussing a thesis entitled "Evaluation of the quality traits of chicken meat belonging to light conventional and free-range broilers".
In June of the same year, she got a research fellowship at the Department of Agricultural and Food Sciences (Alma Mater Studiorum – University of Bologna). Research activities carried out during those months dealt with the study of muscle abnormalities in poultry meat (i.e., white-striping, wooden breast, and spaghetti meat) with particular reference to the evaluation of their technological properties and composition. Since November 2023, she is a PhD student in Agricultural, Environmental and Food Science and Technology (XXXIX) at the same Department conducting a research project entitled "Poultry meat downgrading: Insights on the underpinning factors to improve the sustainability of the production".

2. State of the art

Meat is the most important source of animal protein for the human diet (Marchewka et al., 2022). In this context, the poultry sector has experienced significant growth over the past 50 years and currently represents the most consumed meat type globally (FAO, 2023). The poultry meat production chain is currently the most efficient and sustainable of the livestock sectors. The carbon footprint, consumption of natural resources, and greenhouse gas emissions per unit of production are remarkably lower than those measured for all other production chains (Tallentire et al., 2018). These results are the outcome of intense selection programs implemented over the past eighty years to develop commercial hybrids characterized by high growth rates and breast yields (Tixier-Boichard, 2020). These selection programs have led to substantial improvements in the production performance and yields, to the point where modern commercial hybrids reach their slaughter weight in about forty days, with a breast proportion exceeding one-fourth of the carcass (Aviagen, 2022). The high breast yield of modern commercial hybrids is also the result of selection that has led to a hypertrophic development of the fibers composing the *Pectoralis major* (PM) muscle. From one side, these practices have allowed for the development of hybrids with high yields in the pectoral muscles, but on the other side, they resulted in a greater susceptibility to the development of muscle abnormalities and defects associated with stress phenomena (i.e., PSE-like, Oregon myopathy) and/or growth-related, including white striping (WS), wooden breast (WB) and spaghetti meat (SM) abnormalities (Petracci et al., 2017). WS is easily recognized by the occurrence of white striations following the same direction of the muscle fibers in poultry breast (Petracci et al., 2019). In contrast, PM exhibiting features ascribable to WB, are characterized by a hardened consistency, out-bulging and pale appearance and myodegenerations with fibrosis at the histological level (Kuttappan et al., 2012a). In the end, the increasing tendency towards separation of the muscle fiber bundles with subsequent degeneration of the PM muscle is typical of SM (Sihvo et al., 2014). The onset of these defects is not a health problem and is not related the safety of meat, even though it is often downgraded due to its compromised sensory and technological properties (Petracci et al., 2019). Considering the incidence rates, which can reach up to 30% in some flocks, the onset of muscle abnormalities in broilers is a significant problem for the sustainability of the poultry sector. Although it is now recognized that the onset of muscle abnormalities is somehow related to the selection programs implemented to improve breast yield, it is possible to hypothesize that some peri-mortem factors may influence the incidence and severity of these quality defects in chicken breast meat. There are no available studies in the literature that have evaluated the contribution of peri-mortem phases to the development of myopathies, while it is generally recognized that they can influence post-mortem processes and the final quality of meat.

3. Objectives and expected results

The present research project deals with the evaluation of the main quality traits and technical properties of poultry meat affected by muscle abnormalities with the aim of improving the current knowledge concerning the causative mechanisms likely involved in their occurrence and the eventual role of peri-mortem factors in exacerbating their severity. That would allow to provide people involved in the poultry sector with guidelines that they can adopt to reduce the economic losses and the resulting environmental impact.

This PhD thesis project can be subdivided into the following activities according to the Gantt diagram reported in Table 1.

A1) Bibliographic research and study of the literature.

A2) Assessment of the potential involvement of collagen type IV in onset of the growth-related abnormalities (WS and WB) affecting broilers' PM by investigating its possible role as one of the primary causes underlying their occurrence.

A3) Evaluation of the effect of pre-slaughter and slaughtering factors' to determine the consequence on the main quality characteristics of poultry meat and to verify their possible involvement in the development and/or heightening of the severity of breast meat abnormalities (e.g. SM).

A4) Molecular analysis to investigate the possible involvement of endogenous enzyme systems of the Pectoralis major muscle in the development of the Spaghetti meat abnormality.

A5) Statistical analysis: univariate and multivariate analysis.

A6) Dissemination, writing scientific papers and final thesis.

Tabella 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)	***Investigating COL IV involvement in the onset of WS and WB***			■	■	■	■	■	■										
	Classification and meat quality analysis			▒	▒														
	COL4 quantification (Western Blot analysis)					▒	▒	▒	▒										
A3)	***Evaluation of the effect of pre-slaughter and slaughtering factors'***				■	■	■	■											
	1)Evaluation of abnormalities				▒	▒													
	2)Qualitative analysis and technological properties					▒	▒	▒											
A4)	***Molecular analysis***							■	■	■	■								
	1)Evaluation of collagenase activity							▒	▒										
	2)Extraction and characterization of collagen								▒	▒	▒								
A5)	***Statistical analysis***													■	■	■	■	■	■
	1) Univariate and multivariate approaches													▒	▒	▒	▒	▒	▒
A6)	***Dissemination, writing scientific papers and final thesis***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Bibliography

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Marchewka J, Sztandarski P, Solka M, Louton H, Rath K, Vogt L, Horbańczuk JO (2023) Linking key husbandry factors to the intrinsic quality of broiler meat. *Poultry Science*, *102*(2), 102384.

Michèle Tixier-Boichard (2020) From the jungle fowl to highly performing chickens: are we reaching limits? World's Poultry Science Journal, 76:1, 2-17.

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Tallentire CW, Leinone I, Kyriazakis I (2018) Artifical selection for improved energy efficiency is reaching its limits in broiler chickens. *Sci Rep* **8**, 1168.

Antiossidanti naturali tailor made: valutazione comparativa di antiossidanti nell'elaborazione di alimenti, mangimi e pet food e determinazione delle condizioni d'uso ottimali

Valentina Antonioni (mail: valentina.antonioni@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Maria Fiorenza Caboni; Co-tutor: Sauro Passeri, Federica Pasini, Silvia Marzocchi

1. Curriculum

Ottobre 2022: Laurea Magistrale in Scienze e Tecnologie Alimentari, Università degli studi di Bologna. Titolo tesi: "Valutazione dello stato dell'acqua nei formaggi a diverso grado di salatura e stagionatura mediante TD-NMR". Relatore: Prof. Laghi Luca, Correlatori: Dott.ssa Pasini Federica & Dott.Ravagli Cesare. Votazione: 107/110.
Ottobre 2019: Laurea in Tecnologie Alimentari, Università degli studi di Bologna. Titolo tesi: "Analisi sensoriale del miele: aspetti operativi". Relatrice: Prof.ssa Caboni Maria Fiorenza, Correlatori: Dott. Marcazzan Gian Luigi & Dott.ssa Pasini Federica. Votazione: 103/110.
Luglio 2016: Diploma di Istituto Tecnico Agrario, Istituto "A. Cecchi", Pesaro.

2. Stato dell'arte

L'irrancidimento ossidativo dei lipidi rappresenta una delle principali cause di riduzione della *shelf life* degli alimenti trasformati (compresi *pet food* e mangimi) contenenti grassi. Anche quando i lipidi sono presenti in piccole quantità la loro ossidazione può essere determinante per la qualità del prodotto. Infatti, questa modificazione chimica non pregiudica solo la salubrità dell'alimento, ma ne influenza soprattutto le caratteristiche organolettiche (variazioni di colore, produzione di *off-flavors* ecc.) rendendolo scarsamente appetibile, agli esseri umani come agli animali.
I perossidi e i prodotti secondari dell'ossidazione, che hanno origine da queste reazioni, sono i diretti responsabili della durata della *shelf life* dei prodotti e sono un importante indice qualitativo soggetto a specifici limiti di legge. Un altro aspetto non trascurabile è l'impatto che l'ossidazione degli alimenti può avere sulla salute umana, a partire dalla co-ossidazione di vitamine e altre molecole funzionali, causando un abbassamento della qualità nutrizionale dell'alimento, fino ad arrivare alla formazione di composti dannosi per la salute. (Hu & Jacobsen, 2016; Kanner, 2007).
L'ossidazione è una reazione non reversibile e auto-catalitica. Non c'è modo di rimediare o di fermarla una volta che ha avuto inizio; si può agire solamente prevenendola e/o rallentandola, prolungando il più possibile la fase di iniziazione.
Possiamo suddividere i sistemi antiossidanti in tre categorie:
- Tipo 1: gli inattivatori di radicali liberi, cioè molecole naturali o sintetiche, che "neutralizzano" i radicali donando loro un elettrone e quindi stabilizzandoli (*radical scavengers*).
- Tipo 2: molecole che prevengono la formazione di radicali liberi, per esempio chelando i metalli che ne catalizzano la sintesi (*metal scavengers*)
- Tipo 3: la gestione dei fattori ambientali che circondano il prodotto, come la temperatura, la luce o l'abbassamento della pressione dell'O_2. Si può agire su questi aspetti durante la produzione o scegliendo un packaging opportuno.

Per quanto riguarda gli additivi per alimenti, *pet food* e mangimi soggetti all'ossidazione lipidica, gli additivi antiossidanti principalmente impiegati si possono suddividere in:

- *Tipo 1 (radical scavengers)*
  - *Sintetici*
    - BHA (butilidrossianisolo) – E320
    - BHT (butilidrossitoluene) – E321
    - TBHQ (terzbutilidrochinone) – E319
    - Propil gallato – E310
    - Ascorbil palmitato – E304
  - *Naturali*
    - Tocoferoli – E306, E307, E308, E309.
    - Composti fenolici del rosmarino (in particolare l'acido carnosico) – E392
- *Tipo 2 (metal scavengers)*
    - Acido citrico - E330

Per quanto riguarda i carotenoidi, l'acido ascorbico e altri composti fenolici da fonti vegetali, la loro scarsa lipofilia ne causa, attualmente, un minor impiego come additivi "tecnologici" in questo genere di prodotti, limitando spesso la loro funzione a nutrizionale-bioattiva. Al contrario, i tocoferoli e alcuni composti fenolici del rosmarino interagiscono senza problemi con la fase lipidica dei prodotti dimostrando, talvolta, di essere maggiormente performanti degli antiossidanti sintetici; questi risultano infatti già abbondantemente impiegati nell'industria *food* e *feed* (Valenzuela & Nieto, 1996; Santos-Sánchez et al., 2017; Gutiérrez del Rio et al., 2021).

3. Obiettivi e risultati attesi

L'obiettivo finale della ricerca è quello di individuare nuovi possibili antiossidanti naturali da impiegare in matrici *food* e *feed*, ma soprattutto quello di definire i parametri ottimali per l'uso concreto di questi antiossidanti e/o di quelli di cui è già consolidato l'impiego nell'industria. Infatti, la letteratura scientifica esistente scarseggia di indicazioni precise sugli antiossidanti più adatti alle singole matrici, sulle dosi di impiego e sulle modalità di inclusione durante il processo produttivo. Lo scopo sarà quindi quello di compiere una valutazione delle performances di anti-ossidazione in funzione delle condizioni d'uso: abbinamento antiossidante-matrice, struttura dell'alimento e strutturazione del grasso, eventuali trattamenti termici, condizioni di stoccaggio, composizione e stato fisico dei blend di antiossidanti, dosi d'impiego, fase del processo e modalità di aggiunta ecc., tramite la misura dei relativi parametri di *shelf life*.
Il progetto di dottorato può essere suddiviso nelle seguenti attività, riepilogate nel diagramma di Gantt in tabella 1:
A1) Studio della letteratura scientifica e della legislazione europea e nazionale riguardante gli antiossidanti naturali e il loro impiego in alimenti e mangimi, con maggiore enfasi su quelli che potrebbero presentare un impatto ambientale ed etico particolarmente ridotto (es. estraibili convenientemente da sottoprodotti) e/o che potrebbero avere effetti positivi sulla salute del consumatore.
A2) Individuazione di un prodotto o una categoria di prodotti target altamente soggetti al fenomeno dell'irrancidimento ossidativo. Studio della composizione e del processo di produzione. Individuazione di molecole antiossidanti con caratteristiche compatibili a tale prodotto e il suo processo. Produzione dei *blend* da testare in scala di laboratorio.
A3) Produzione dei prodotti finali in impianti sperimentali, additivandoli con le miscele antiossidanti in diverse quantità, con diverse modalità e in diversi momenti del processo produttivo. Produzione parallela con l'impiego di additivi sintetici "classici" per ottenere un confronto sull'efficacia e di un campione di controllo non additivato.
A4) Valutazione della *shelf life* dei prodotti additivati. Valutazione delle caratteristiche reologiche, sensoriali e nutrizionali. Confronto con il prodotto non additivato e con quelli additivati con antiossidanti sintetici.
A5) Valutazione critica dei metodi analitici e delle molecole target dell'ossidazione per individuare i più adatti al caso specifico e i più ragionevolmente applicabili anche in una realtà aziendale di piccole-medie dimensioni.
A6) Valutazione della fattibilità e della sostenibilità ambientale ed economica dei *blend* che hanno dato i risultati migliori, sia dal punto di vista dell'azienda produttrice di *blend* antiossidanti, sia dal punto di vista dell'azienda produttrice di prodotti finiti suscettibili all'ossidazione. Studio delle eventuali modalità di *scale-up.*
A7) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici e presentazione orale.

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

<table>
<tr><td>Attività</td><td>Mese</td><td>2</td><td>4</td><td>6</td><td>8</td><td>10</td><td>12</td><td>14</td><td>16</td><td>18</td><td>20</td><td>22</td><td>24</td><td>26</td><td>28</td><td>30</td><td>32</td><td>34</td><td>36</td></tr>
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<tr><td>A2)</td><td>Studio prodotti target e molecole antiossidanti</td><td></td><td></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
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<tr><td></td><td>3) produzione di blend di antiossidanti in scala di laboratorio</td><td></td><td></td><td></td><td></td><td></td><td style="background:#aaa"></td><td style="background:#aaa"></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>A3)</td><td>Prove di additivazione sui prodotti in scala di laboratorio</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td style="background:#555"></td><td style="background:#555"></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>A4)</td><td>Valutazione della shelf life dei prodotti additivati</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td>1) analisi chimiche, reologiche e sensoriali</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td style="background:#aaa"></td><td style="background:#aaa"></td><td style="background:#aaa"></td><td style="background:#aaa"></td><td style="background:#aaa"></td><td></td><td></td><td></td><td></td></tr>
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<tr><td>A7)</td><td>Preparazione della tesi e di articoli</td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td><td style="background:#555"></td></tr>
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Strategie di autenticazione di bevande spiritose e di prodotti del settore vitivinicolo

Silvia Arduini (email: silvia.arduini2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Prof. Fabio Chinnici; Co-tutor: Prof.ssa Alessandra Bendini

1. Curriculum

Laureata in Chimica nel 2003 presso l'Università "La Sapienza" di Roma, consegue, nello stesso anno, l'abilitazione all'esercizio della professione di chimico, e, nel 2021, presso l'università "Alma Mater Studiorum" di Bologna, il master universitario di II livello in "Analisi chimiche e chimico-tossicologiche forensi" con un progetto di lavoro finale dal titolo "Valutazione dell'autenticità di una grappa: approccio univariato e classificazione mediante analisi statistica multivariata".
Dal 2006 svolge la professione di chimico presso il laboratorio chimico di Bologna dell'Agenzia delle Dogane e dei Monopoli dove ha acquisito un'esperienza approfondita delle metodologie di analisi chimica e chimico-fisica nel settore dei prodotti alcolici principalmente bevande spiritose, vini, birre e sottoprodotti della vinificazione con la finalità di determinarne la classificazione doganale nonché la conformità ai regolamenti comunitari vigenti e la presenza di eventuali frodi. Oltre a svolgere l'attività di analisi si occupa dello sviluppo, validazione, implementazione ed accreditamento di nuovi metodi analitici secondo la norma UNI CEI EN ISO/IEC 17025.

2. Stato dell'arte

Allo stato attuale i metodi di autenticazione delle bevande spiritose e dei prodotti del settore vitivinicolo sono essenzialmente di due tipologie. Il primo tipo si basa sulla presenza o sull'assenza di composti definiti "marcatori chimici". Questi composti marker possono essere volatili o elementari e possedere un diverso livello di potenzialità discriminatoria. Per questo motivo, spesso ne viene utilizzato più di uno. La loro identificazione e quantificazione può essere ottenuta mediante tecniche come la cromatografia liquida ad alta prestazione o la gascromatografia, ma anche con l'uso di tecniche più sofisticate come la spettroscopia di risonanza magnetica nucleare o la spettrometria di massa a rapporto isotopico e altre ancora. I marcatori chimici maggiormente utilizzati con le tecniche cromatografiche sono composti del gruppo dei polifenoli (cromatografia liquida) o dei costituenti volatili (gascromatografia). In aggiunta all'analisi dei componenti organici come i composti aromatici e aromatizzanti, l'analisi della composizione elementare, che si può effettuare con varie tecniche quali la spettrofotometria di assorbimento atomico (AAS), il plasma accoppiato induttivamente (ICP)-MS e la spettroscopia di emissione ottica (OES) ICP, è un importante parametro di valutazione della qualità. Un secondo approccio metodologico mira ad autenticare il prodotto in base al suo profilo compositivo generale. I metodi di analisi del profilo sono per lo più basati su tecniche di spettroscopia accoppiata alla chemiometria e vengono utilizzati per autenticare il prodotto soprattutto in base alla varietà e all'origine. La classificazione in base a questi parametri o in base all'età e alla qualità è un compito complesso, soprattutto perché non esiste un singolo composto, o addirittura un gruppo specifico di composti, che sia direttamente collegato a ciascun parametro. Per questo motivo, specialmente nel settore vitivinicolo, tecniche come la spettroscopia infrarossa a trasformata di Fourier (FT-IR), la spettroscopia Raman e la NMR, che producono una rappresentazione grafica di ciascun campione, hanno fatto grandi progressi negli ultimi anni. Allo stesso tempo, c'è anche un rinnovato interesse per le tecniche che, pur non potendo produrre un'identificazione accurata dei composti, come la spettroscopia UV-Vis, possono essere utilizzate in modalità di scansione.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di sviluppare metodi di analisi, quanto più rapidi ed economicamente sostenibili, che permettano di individuare adulterazioni in bevande spiritose ed in prodotti vitivinicoli con particolare interesse nei confronti di prodotti italiani a denominazione di origine ed a indicazione geografica, che, come tali, sono tenuti a rispettare determinate specifiche di produzione, nonché trasformazione e preparazione descritte nei vari disciplinari di produzione. Per perseguire l'obiettivo della ricerca si prevede di utilizzare varie tecniche analitiche, sia cromatografiche che spettroscopiche abbinate a metodi di analisi statistica multivariata.
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 tecniche di analisi per l'autenticazione di bevande spiritose e di prodotti del settore vitivinicolo.

A2) Individuazione matrici e tecnologie produttive da investigare, nell'ambito delle categorie merceologiche relative ai distillati, ai vini ed ai prodotti della filiera vitivinicola il cui processo produttivo sia disciplinato in modo riconosciuto ed accettato, anche a seguito di previsioni che discendono dalla legislazione nazionale e/o europea. Dovranno essere prese in considerazione anche le modalità di campionamento delle suddette matrici che oltre ad essere rappresentative di una certa categoria dovranno essere accompagnate da informazioni che ne descrivano quanto più dettagliatamente la tecnologia di produzione utilizzata.
A3) Analisi dei campioni mediante tecniche analitiche "classiche" quali GC-MS ed HPLC ed elaborazione statistica dei dati ottenuti.
A4) Analisi dei campioni mediante tecniche analitiche "rapide" di tipo "fingerprint" tra cui NIR e Flash-GC ed elaborazione statistica multivariata dei dati sperimentali.
A5) Confronto tra le varie tecniche di analisi al fine di individuare la metodica e/o le metodiche con caratteristiche di rapidità e semplicità di esecuzione, che consentano la migliore discriminazione in ambito controllo qualità/verifica di genuinità, anche in relazione alle differenze conseguenti le possibili varianti tecnologiche produttive.
A6) Periodo di ricerca presso istituzione internazionale
A7) Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici

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) tecniche di analisi "classiche"	▒	▒	▒															
	2) tecniche di analisi "rapide" di tipo "fingerprint"		▒	▒															
A2)	***Individuazione matrici e tecnologie produttive da investigare***		■	■	■	■	■							■	■				
	1) individuazione matrici/tecnologie di interesse economico-produttivo		▒	▒	▒	▒								▒	▒				
	2) valutazione modalità di campionamento presso aziende		▒	▒	▒	▒	▒							▒	▒				
A3)	***Analisi dei campioni mediante GC-MS ed HPLC***		■	■	■			■	■	■									
	1) ottimizzazione metodica di estrazione (per analisi delle sostanze volatili)		▒					▒											
	2) determinazione sostanze volatili e composti fenolici		▒	▒				▒	▒										
	3) analisi statistica dei dati sperimentali			▒	▒				▒	▒									
A4)	***Analisi dei campioni mediante tecniche di tipo "fingerprint"***						■	■	■					■	■	■			
	1) ottimizzazione analisi strumentale per tipologia di matrice						▒							▒					
	2) determinazione segnale delle varie matrici						▒	▒	▒					▒	▒	▒			
	3) elaborazione statistica multivariata dei dati sperimentali						▒	▒	▒					▒	▒	▒			
A5)	***Confronto tra le varie tecniche di analisi***									■	■	■	■	■	■	■	■	■	■
A6)	***Periodo di ricerca presso istituzione internazionale***										▒	▒	▒						
A7)	***Scrittura e pubblicazione della tesi di dottorato, poster, articoli scientifici***			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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Development and optimization of new processing and packaging technologies for fresh-cut fruit

Giulio Giannini (giulio.giannini3@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Marco Dalla Rosa; Co-tutor: Pietro Rocculi

1. Curriculum

Work:
Internship at Dolcezze Savini. (Jul 2020 – Oct 2020)
Internship at Leibniz Institute of Agricultural Engineering and Bio-economy, Potsdam, Germany (Oct 2022 – Feb 2022)
Studies:
MSc in Food Science and Technology, UniBo, Italy (2020-2023), thesis: "Effects of High Hydrostatic Pressure and Antioxidant Blends on Lipid Oxidation of Tenebrio Molitor Paste"
Erasmus exchange in Portugal at the "Instituto Politécnico de Beja" (Aug 2019 – Feb 2020)
BSc Food Technology, UniFi, Italy, 2017-2020, thesis: "MAP packaging and its application on par-baked bread"
Date of birth: 19/01/1998, Bagno a Ripoli, Italy

2. State of the art

Nowadays, consumer habits are changing in terms of food consumption and the way food is consumed; consequently, the way food is offered must also change. The average European citizen is evolving towards an increasingly fast-paced and intensive lifestyle; the time spent preparing food is decreasing and workers are increasingly taking their meals outside home (Testa et al., 2021). Consequently, ready-to-eat fruits, which are also categorized as modern convenience foods, are receiving considerable attention from the market as they are designed to save time and preparation work (Contini et al., 2018). Fresh-cut or minimally processed products are defined by IFPA (2002) as fruits or vegetables that undergo minimal physical changes, such as trimming, peeling and/or slicing into a packaged product that is 100% edible and provides high nutritional value, convenience and flavor while retaining its freshness.
The main problem associated with fresh-cut is rapid spoilage, resulting in a shelf life of less than two weeks. The main factors responsible for the quality of fresh-cut fruits include the variety, ripeness at harvest, physiological state of the raw material, storage conditions, preparation steps, packaging characteristics, and processing and storage temperatures (Beaulieu, 2016). The loss of quality of minimally processed fruit is the result of complex chemical and biochemical mechanisms that influence color, texture and organoleptic properties (Sucheta et al., 2019).
For this reason, the fruit undergoes a washing/sanitizing treatment after slicing to improve the deactivation of enzymes and microorganisms. This phase can be carried out by immersion, brushing, spraying or by using new technologies. The classic immersion treatment combines antimicrobial, anti-browning and texturizing substances (Iturralde-García et al., 2022). These include all chlorine derivatives, cysteine, ascorbic acid, citric acid, sorbic acid, sulphites and calcium forms (ascorbate, chloride and calcium lactate). New formulations include the elimination of sulphites and the reduction of chlorine. Examples are concentrated fruit extracts, essential oils, resorcinol, nisin, melatonin (Iturralde-García et al., 2022).
To improve the shelf life of these products, modified atmosphere packaging is also a valid option (Belay et al., 2019).
New techniques for stabilizing minimally processed fruit include edible coatings, electrolyzed oxidizing water (EOW), ozone, ultrasound, ionizing radiation and ultraviolet light (De Corato, 2020; Maringgal et al., 2020)

3. Objectives

The present research project aims to identify, develop and optimize different technologies for the stabilization of fresh-cut fruits in order to slow down the occurrence of microbiological and physicochemical phenomena responsible for the loss of safety and quality of the products during shelf life. In addition, the project focuses on the elaboration of operating procedures to be developed and implemented in an industrial context by carrying out experimental tests at laboratory/pilot scale up to pre-industrial/industrial tests in the plant, also with the possible support of equipment manufacturers.
The doctoral thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:
A1) Study and formulation of dipping treatments in order to create new blends or optimize the old ones to be integrated them in the company processes.
A2) Study e and formulation of edible coatings to improve product quality and sensorial acceptance by incorporation of active molecules.

A3) Study and application of emerging sanitizing treatments such as plasma activated water, electrolyzed water, ozone treatments.
A4) Conducting preliminary tests with high-pressure CO2 treatments in order to explore this new processing technology and its feasibility on fruit.
A5) Transfer of knowledge gained in the laboratory to industrial scale in order to significantly improve process quality of the company
A6) Thesis preparation and publication of scientific articles

Table 1: Gant 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**	**36**
A1	**Study and formulation of dipping treatments**	■	■	■	■													
	1) Trials with different solutions for preventing browning and improving texture	▒	▒															
	2) Formulation of effective dippings and testing in the company			▒	▒													
A2	**Study e and formulation of edible coatings**					■	■	■	■									
	1) Formulation of coatings with different materials					▒	▒											
	2) Formulation of coatings incapsulated with active molecules							▒	▒									
A3	**Study and application of emerging sanitizing treatments**									■	■	■	■					
	1) Trials with different non-thermal technologies for improving microbial quality									▒	▒							
	2) Testing in the company											▒	▒					
A4	**Conducting preliminary tests with high-pressure CO2 treatments**														■	■	■	
	1) Assessing the feasibility of the technology for fresh-cut fruit														▒	▒	▒	
A5	**Transfer of knowledge gained in the laboratory to industrial scale**			■	■	■	■	■	■	■	■	■	■	■	■	■		
A6	**Thesis preparation and publication of scientific articles**	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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Determination of New Types of Food Coloring and Application in Food Products with a Biotechnological Basis

Sona Hajiyeva (email: sona.hajiyeva2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Assoc. Prof.Francesca Patrignani; Co-tutor: Prof. Rosalba Lanciotti, Asst. Prof. Saida Aliyeva

1. Curriculum

I am Sona Hajiyeva from Azerbaijan, currently pursuing a Ph.D. studies in the Food Science and Technology program at the esteemed Alma Mater Studiorum, University of Bologna in Italy. My academic journey is rooted in a robust foundation, having successfully completed my Master of Science degree with Merit at the Department of Applied Science, Eskisehir Technical University, Turkey. Throughout my master's studies, I had the privilege of serving as an assistant to a distinguished professor and contributing as a lab researcher at the Microbiology Laboratory of ESTU. This experience enriched my understanding of biological sciences, biotechnology, microbiology, molecular biology, genetics, and allied disciplines, fostering a holistic approach to my academic pursuits. My professional identity is further defined by my role as a dedicated biotechnologist. My research interests span a spectrum of disciplines, with a keen focus on advancing knowledge and innovation in the aforementioned scientific domains. I am honored to have been an active participant in various scientific conferences, where I had the opportunity to present my research papers at international seminar. Currently, I am working on my research project at the University of Bologna, Department of Agricultural and Food Sciences. This research aims to identify new food coloring from biotechnological sources. It involves screening microorganisms, extracting colorants, and evaluating their safety and technological features. The study will also assess the applicability of these biotechnological colors in various food products, focusing on sustainability and innovation in food coloring.

2. Stato dell'arte

The use of microbial pigments in food coloring has gained significant attention in recent years. Consumer demand for natural microbial pigments such as β-carotene, riboflavin, and C-phycocyanin is increasing in niche markets (Ramesh et al., 2019). These pigments not only provide vibrant colors to food products but also serve as preservatives and antioxidants (Ramesh et al., 2019). Synthetic colorants, on the other hand, have been found to have potential health risks, making natural pigments highly preferred (Ramesh et al., 2019). Microbial pigments offer several advantages over synthetic colorants. They are cost-effective, scalable, and can be produced through microbial fermentation processes (Sen et al., 2019). These processes offer higher yields, lower production costs, and easier extraction compared to traditional methods (Sen et al., 2019). In addition to this, microbial pigments can be produced throughout the year without being affected by seasonal variations (Poorniammal et al., 2021). The use of microbial pigments in food coloring aligns with the growing consumer demand for clean label and natural food products (Ramesh et al., 2019).
The significance of color in food consumption is indisputable. In particular, shelf-life of foodstuffs is influenced by the changes in the colors. These changes are attributed to poor production processing, and faulty transport and storage methods. Color changes during food storage can mainly be due to oxidation phenomena and microbial spoilage. By contrast, microbial pigments have gained increasing attention as natural and sustainable alternatives to synthetic food dyes due to their safety, environmental friendliness, and potential health benefits. This study aimed to investigate the use of microbial pigments in food products to increase their visual appeal while ensuring consumer safety and regulatory compliance (Kumar et al., 2022).
In this research, different microbial strains, known for their pigment production abilities, will be selected among molds, yeasts, bacteria. Several species will be considered to select potential color producers including ***Monascus ruber***, ***Metschnikowia pulcherrima*** and ***Rodothorula sp.*** To maximize pigment synthesis, these strains are intended to be grown in controlled environments. The appropriate nutritional mediums for microbial pigment production will next be provided, along with a carefully designed habitat to encourage microorganisms to produce pigment.
The study will also focus on the characterization and use of these microbial pigments in foods. By understanding their stability, color intensity, and compatibility with different food matrices, we aim to develop effective strategies for their incorporation into various food products. This includes evaluating sensory attributes, shelf-life, and regulatory compliance to ensure successful integration into the food industry.

3. Obiettivi e risultati attesi

The demand for natural and safe food colorants has increased significantly in recent years due to growing consumer health and environmental awareness. Microbial pigments, derived from microorganisms like bacteria, fungi, algae, and yeast, have emerged as promising alternatives to synthetic food dyes. This project explores the feasibility, safety, and effectiveness of using microbial pigments in food applications. To reach this purpose, the doctoral thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:

A1) Microorganism Selection for Biotechnological Food Coloring. In the research, a Central Composite Design (CCD) will be used to screen microorganisms to identify optimal pigment production conditions. The principal variables which will be considered will regard the temperature, pH, carbon, and nitrogen sources to establish the most suitable conditions for efficient food colorant production.

A2) Pigment Production and Extraction. Selected microorganisms will be cultured in optimized conditions, resulting from the CCDs, for pigment synthesis. Pigment extraction will be performed by using green solvent or enzymatic methods to obtain pure extracts. These optimized processes aim for sustainable, environmentally-friendly food coloring solutions with minimal waste.

A3) Purification and Characterizations. In the purification process, a chromatographic approach, to isolate the pigment and characterize it using techniques like UV-vis, and FTIR-will be used. The goal is to achieve successful isolation of a pure pigment with stable color properties suitable for food applications. Through comprehensive characterization, a deeper understanding of the pigment's chemical and biological properties will be reached, ensuring their safety and quality for use in food products.

A4) Biological Activities Determination: The research will evaluate the biological activities of microbial pigments, focusing on their antioxidant, antimicrobial, and anti-inflammatory properties. This assessment aims to uncover their potential health benefits, emphasizing their value in functional foods and nutraceuticals.

A5) The utilization of microbial pigments in foods. This project will optimize microbial pigments through safety and shelf-life studies, evaluating their impact on food safety, shelf-life, and sensory properties. The goal is to enhance their application, validate their safety, and improve both sensory and functional attributes in food products.

A6) Writing and publication of the doctoral thesis, posters, scientific articles and oral presentation

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)	***Microorganism Selection for Biotechnological Food Coloring***																		
	1)Literature review and microorganism selection																		
	2)Central Composite Design (CCD) for pigment production related to growth conditions																		
A2)	***Pigment Production and Extraction***																		
	1) Extracellular pigments analysis																		
	2) Intracellular pigments analysis																		
A3)	***Purification and Characterizations***																		
	1) Purification of pigment by column chromatography																		
	2) Characterization of pigment by UV-vis, and FTIR																		
A4)	***Biological Activities Determination***																		
	1) Minimal bactericidal concentration (MBC) assay																		
	2) Antioxidant activity determined by DPPH/ABTS assay																		
	3) Total Phenolic Content (TPC)																		
A5)	*The Utilization of Microbial Pigments in Foods.*																		
	1) Sensory evaluation by consumer acceptance of the colored food products																		
	2) Stability studies evaluated under different conditions																		
A6)	***Preparation of the thesis and articles***																		

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Emerging processing and packaging technologies for sustainable production of innovative foods

Busra Oktar (busra.oktar@unibo.it)
Department of Agro-Food Sciences and Technologies, *Alma Mater Studiorum* - University of Bologna
Doctoral Course: Agricultural, Environmental and Food Sciences and Technologies
Topic: Food Science and Biotechnology; Doctoral cycle: XXXIX; Year of attendance: I
Tutor: Pietro Roccoli ; Co-tutor: Ana Cristina De Aguiar Saldanha Pinheiro

1. Curriculum

Studies:

MSc: University of Parma, Parma, Italy Food Safety And Food Risk Management (2021-2023) Master's thesis "Effect of High Pressure Homogenization on Rheological and Functional Properties of Chickpea Flour"
BSc: Gazi University, Ankara, Turkey, Nutrition and Dietetics (2016-2020) Bachelor's thesis "Comparison of Added Sugar Consumption of 1st and 4th Grade Students of The Health Sciences Faculty"

-Health Effects of Monosodium Glutamate From Food Additives (Seminar, 2020)
-Effects of Intermittent Fasting on Weight Loss And Human Metabolism (Seminar, 2020)
-Molecular Nutrition and Nutrigenomics (Seminar, 2020)

Nationality: Turkish
Date of birth (day, month, year): 17.04.1998

2. State of the art

The human population is growing day by day. The United Nations predicts that the world's population will reach about 10 billion people by 2050. The demand for food will increase in direct proportion to the increase in population, and in particular, the need for protein will increase. Because of the increasing demand for protein, the consumption of animal protein is also increasing. Annual meat production is expected to increase from 218 million tons in 1997-1999 to 376 million tons by 2030. In order to reduce the damage that of animal-based foods cause to environmental sustainability, the consumption of protein-rich foods of plant origin has increased and various plant-based foods have entered the market(1). The market for plant-based meat analogs is booming and has developed from niche to mainstream. Since 2015, over 6485 new products have been launched on the market worldwide(2).
Proteins are modified primarily by altering their structure (secondary and tertiary rearrangement and subunit disaggregation) and then changing their primary molecular characteristics to enhance or alter their techno-functional properties when used as a food ingredient (3). There have been many different modification techniques investigated, which, depending on the method, can be divided into physical, chemical, and biological. Physical alterations primarily include creating conformational changes in the protein structure without using particular chemicals. The water-holding capacity (WHC) is a measure that represents the amount of water that a food material can retain and it is a significant quality measure for food. Protein solubility is an important parameter for sensorial and functional properties, and stability of the food products. WAI is an essential feature that influences food cooking properties. (4).
Pulse Electric Fields (PEF), High Pressure Processing (HPP), Ultrasound (US), and Cold Plasma (CP) are some of the techniques which makes physical alterations on food. Modification of the structure could have an impact on the techno-functional properties as well as allergenicity and nutritional changes (5). The PEF technique is an emerging technique for the extraction of proteins from raw biomaterials due to its non-thermal and chemical-free processing advantages (6). Ultrasonication is generally used as a pretreatment method in traditional protein disruption protocols as it can disrupt the cell matrix to improve extractability. In addition to simultaneous extraction and modification, ultrasonication is also used to alter the physical, structural and functional properties of protein-based ingredients (7).
In order to create a sustainable, healthy and tasty novel product formulation, all these functional changes, the mechanisms and effects of the technologies should be considered holistically and the applications should also be evaluated taking into account the consumer's diet. In this way, food and nutritional alternatives can be created that have a positive effect on both the consumer and the environment.

3. Objectives

This research project primarily aims to evaluate the benefits of new technologies such as pulsed electric fields and ultrasound and to increase the efficiency of protein extraction from by-products such as rapeseed meal, sunflower meal, peas and chickpeas. Secondly, the modification of the extracted proteins by cold plasma technology will be evaluated.

After evaluating all functional, rheological and structural properties of the extracted protein, meat and dairy products will then be produced. The project will conclude with a sustainable, nutritious, non-ultra-processed end product.

The doctoral thesis project can be divided into the following activities, summarized in the Gantt chart shown in Table 1:
A1) Bibliographic research on analysis techniques on protein extraction and optimization techniques to be subjected to analysis.
A2) Application of protein extraction and characterization and finding the most repeatable and suitable technique for this research.
A3) Treatment with emerging technologies on plants and by-products to increase the protein extraction yield modification of functional, rheological, and structural properties.
A4) The extracted and/or isolated protein will be evaluated in terms of its functional, rheological and structural properties to identify its possible usages in new food formulations
A5) According to the obtained protein's functional, rheological, and structural behavior try to improve a final product with the aim of partial replacement of meat and dairy products.
A6) Writing and publication of the doctoral thesis, posters, scientific articles, and oral presentation

Table 1. Gantt chart of the doctoral research activity

Time (months)	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
A1 Bibliographic research	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
A2 Plant-based protein extraction and characterization				X	X	X	X	X	X	X	X	X						
A3 Set up of non-thermal processing for protein yield and modification							X	X	X	X	X	X	X	X	X			
A4 Quality evaluation and identification of possible usages for the product								X	X	X	X	X	X	X	X	X		
A5 Optimization of the meat and dairy analogs formulation											X	X	X	X	X	X	X	X
A6 Dissemination of results, preparation of poster, article, and thesis			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

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Application of high-resolution nuclear magnetic resonance (HR-NMR) to evaluate the antioxidant properties of natural compounds used to extend the shelf life of food products rich in unsaturated lipid components

Lorenzo Oliverio lorenzo.oliverio2@unibo.it
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Francesco Capozzi; Co-tutors: Elena Babini, Carlo Mengucci

1. Curriculum

Lorenzo Oliverio was born in Teramo in 1999. In 2023 he obtained his Single Cycle Master's degree in Chemistry and Pharmaceutical Technologies at the Alma Mater Studiorum – Univeristy of Bologna, discussing a thesis on the synthesis of zwiterrionic squaric-acid derivatives, and their possible application as Boolean calculation tools.

Since November of the same year, he is a PhD student in Agricultural, Environmental and Food Science and Technology (XXXIX) at the Department of Agricultural and Food Sciences conducting a research project entitled "Application of high-resolution nuclear magnetic resonance to evaluate the quality of ingredients used to increase sustainability of used products".

2. Stato dell'arte

For a long time, the lipid oxidation process has been and remains a subject of great interest in the field of food science and technology, mainly due to its economic implications. Moreover, considering that it is well known that several compounds with potential adverse health effects could be generated during the oxidative degradation of food lipids, this issue is a matter of great concern from a food safety point of view, and by extension, in the global context of health and diet. Traditional methods to assess lipid oxidation usually monitor the formation of primary and secondary oxidation products, which is not an accurate enough method, as there are many oxidation products formed during the reactions. For this reason, tracking the formation of a single oxidation product cannot be acceptable.
For instance, peroxide value, one of the most widely used analytical methods, quickly reaches a peak, and subsequently declines and, therefore, once the value reaches the maximum, this method cannot be used to monitor the oxidation status of lipid. One of the most reliable methods to measure the oxidation of frying oil is the analysis of total polar compounds (TPC), that however requires a column chromatography, implying use of large quantities of solvents, intensive labour, and long analysis times.
One of the best methods to assess lipidic oxidation is represented by nuclear magnetic resonance.
In general, the highest reliability is observed when the analytical method measures:
1) major products (e.g., polymers/oligomers and TPC) produced in high concentrations, rather than minor products;
2) products continuously generated (e.g., volatile compounds);
3) disappearance of starting materials (e.g., fatty acid level).
Since the NMR technique also measures disappearance of specific protons of the starting material, it has been found to be very reliable. Furthermore, unlike some other methods using reagents to produce new substances that absorb at certain wavelength, the NMR method does not rely on any chemical reaction for the analysis.
Nonetheless, those analysis approaches are still underutilized, because of expensiveness of high-resolution NMR instruments, which consequently are not available in the majority of the food research labs.
NMR spectroscopy is a powerful tool to elucidate the chemical structures of lipid oxidation products, making significant contributions to understanding lipid oxidation processes and mechanisms. In addition, 1H NMR has also been examined as a tool to assess lipid oxidation status in which proton signals at reactive sites of lipid molecules are monitored during the oxidation process. The results generated by this method correlate well with results from traditional methods.
Using the proposed methodology will allow to evaluate conditions of optimal use of natural antioxidants, exploiting the knowledge on the reaction mechanisms that only a complete framework including reagents, intermediate and final products can provide through kinetic studies. In this way, it is possible to optimize the chemical environment within which the molecules of antioxidants and lipid substrates diffuse, controlling the concentrations of pro-oxidant species, such as metal ions, through sequestration with natural chelating agents.

3. Obiettivi e risultati attesi

The research aims at a double result: the analytical methodological one and the one of support to the development of food products. The first consists in the generation of analysis procedures aimed at the kinetic study of the oxidation phenomena of foods rich in unsaturated fats. The deliverable, in this case, consists of an analysis protocol for at least three food products, of which at least one is of animal origin.
The second result is the creation of a kinetic data analysis system to optimize the formulation of a food product with a precisely characterized shelf life. In this case, the deliverable consists of a data analysis system, possibly based on machine learning algorithms, to make decisions supported by kinetic data on the optimal formulation for food products with the longest shelf life possible.
This PhD thesis project can be subdivided into the following activities according to the Gatt diagram reported in Table 1.
A1) Bibliographic research and study of the literature.
A2) Different sample-preparation methods evaluation to identify the method that provides the best repeatability on a large scale.
A3) NMR Analysis involving the acquisition of mono- and bi-dimensional spectra, by different experiments for the purpose of qualitative and quantitative analysis.
A4) Application of statistical multivariate analysis to establish an holistic approach to the research.
A5) Writing and publishing doctoral thesis, posters, scientific papers and oral presentation

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)	***Bibliographic research***	■	■	■															
	1) NMR analysis of lipid-rich compound from food matrices	▒	▒	▒															
	2) Biological pathways of lipid oxidation	▒	▒	▒															
A2)	***Different sample-preparation methods evaluation***			■	■	■	■	■											
A3)	***NMR Analysis***								■	■	■	■	■	■	■				
	1) Analysis of lipidic compounds								▒	▒									
	2) Analysis of the lipidic oxidation									▒	▒	▒	▒	▒	▒				
A4)	***Application of statistical multivariate analysis***															■	■	■	■
	1) PCA analysis															▒	▒	▒	▒
A5)	***Writing and publishing doctoral thesis, posters, scientific papers and oral presentation***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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Approcci innovativi per la messa a punto e la valutazione delle performance di nuove miscele di frittura attraverso analisi chimico-fisiche e sensoriali

Claudia Troisi (email: claudia.troisi3@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum*-Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXIX; Anno di frequenza: I
Tutor: Prof.ssa Maria Teresa Rodriguez Estrada; Co-tutor: Prof.ssa Tullia Gallina Toschi, Dott. Dario Mercatante, Dott.ssa Matilde Tura, Dott.ssa Daniela Natale

1. Curriculum

Istruzione:
A.A. 2023/24-in corso: Dottorato di Ricerca presso l'*Alma Mater Studiorum*-Università di Bologna XXXIX ciclo, progetto di ricerca intitolato: "Approcci innovativi per la messa a punto e la valutazione delle performance di nuove miscele di frittura attraverso analisi chimico-fisiche e sensoriali".
A.A. 2022/23: 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: "Valutazione della stabilità ossidativa di tre oli destinati all'alimentazione animale addizionati con antiossidanti sintetici e naturali". Votazione: 110/110 *cum Laude.*
A.A. 2019/20: Laurea Triennale (L-26) in Scienze e Tecnologie Agro-Alimentari, Università degli Studi di Teramo, Dipartimento di Bioscienze e Tecnologie Agro-Alimentari. Titolo tesi sperimentale: "Transizione dal metodo Kjeldhal al metodo Dumas per la determinazione del contenuto proteico in campioni di grano e pasta in un sistema di controllo qualità aziendale: il caso De Cecco". Votazione: 110/110 *cum Laude*.

2. Stato dell'arte

Negli ultimi anni, l'impegno sempre crescente dei consumatori verso la scelta di alimenti più salutari e di qualità ha portato a porre l'attenzione anche verso la scelta dei migliori metodi di preparazione e cottura degli alimenti, poiché questi possono avere un impatto rilevante sulle caratteristiche nutrizionali e sensoriali del prodotto finito. La frittura rappresenta uno dei metodi di cottura più antichi e semplici, poiché prevede l'immersione di un alimento in un olio o grasso commestibile, riscaldato a temperature comprese generalmente trai i 170°C e 180°C. Si tratta di un processo di cottura che, con il passare del tempo, ha acquisito crescente popolarità a livello industriale, domestico e della ristorazione sia per la velocità con la quale un alimento può essere cotto, sia per le proprietà sensoriali peculiari del prodotto fritto, in termini di flavour, colore e consistenza, che rendono di fatto l'alimento maggiormente appetibile e desiderabile (Pedreschi et al., 2005). Sebbene da un punto di vista pratico la frittura risulti essere uno dei metodi di cottura più convenienti e semplici, ciò che avviene da un punto di vista chimico/fisico è, invece, di più difficile interpretazione. Questo perché, durante il processo di frittura, l'olio e gli alimenti subiscono una serie di complessi cambiamenti chimico-fisici, come ad esempio l'ossidazione ed idrolisi dei lipidi, la gelatinizzazione dell'amido, la denaturazione delle proteine, la reazione di Maillard, ecc. Inoltre, se le condizioni di frittura non sono adeguatamente controllate, tale processo può portare anche alla produzione di sostanze dannose, come ad esempio acrilammide ed ammine eterocicliche, derivanti dalla reazione di Maillard (Gertz, 2014). In particolare, l'ossidazione lipidica è molto accelerata dalla presenza di acidi grassi insaturi, per cui la scelta migliore da un punto di vista tecnologico sarebbe quella di ricorrere all'utilizzo di oli monoinsaturi, ponendo attenzione al livello di acidi grassi saturi il cui consumo può avere un impatto sulla salute umana, in termini di incremento delle lipoproteine a bassa densità (colesterolo LDL) e rischio di malattie cardiovascolari (Anushree et al., 2017). Allo stesso tempo però, prediligere una frittura con un olio ad alto grado di insaturazione promuove la formazione di composti volatili derivanti dalla loro ossidazione e responsabili dell'irrancidimento del prodotto. Per tutti questi motivi, si stanno diffondendo proposte alternative di oli di semi e loro miscele che siano più stabili. In particolare, il miglioramento genetico delle piante ha permesso la selezione di colture di semi oleaginosi particolarmente ricche in acidi grassi monoinsaturi, migliorando da un lato il valore nutrizionale dell'olio e fornendo dall'altro importanti proprietà funzionali richieste dal settore alimentare (Liu *et al*., 2002). Inoltre, al fine di migliorare la stabilità ossidativa degli oli da frittura, studi recenti si stanno concentrando sul potenziale utilizzo di estratti vegetali come antiossidanti naturali, allo scopo di ridurre la produzione di sostanze nocive e preservare il colore e il *flavour* degli alimenti sottoposti a frittura. Tuttavia, la tipologia e la dose degli estratti vegetali utilizzati, i tempi e le temperature di lavorazione, la composizione in acidi grassi dell'olio e il tipo di alimento sottoposto a frittura influenzano in maniera significativa l'attività antiossidante di questi estratti (Li *et al.,* 2023). Pertanto, risulta determinate l'utilizzo di tecniche analitiche e strumentali che possano aiutare ad ottimizzare il processo di frittura, definendo i migliori estratti naturali da impiegare e individuando gli oli e grassi con le migliori performance, in grado di rispondere ai requisiti legislativi e tecnologici richiesti dal settore alimentare. Per avere una caratterizzazione completa degli oli e grassi da frittura è fondamentale accoppiare le analisi chimico-fisiche ad una valutazione sensoriale degli oli e degli alimenti fritti in essi. L'analisi sensoriale permette infatti di valutare il profilo dell'olio o grasso da frittura la cui qualità avrà poi un impatto sul prodotto fritto, in quanto l'olio viene assorbito dall'alimento e quindi diventa parte integrante di quest'ultimo; pertanto, se il gusto e il *flavour* dell'olio non sono accettabili, i prodotti sottoposti a frittura non saranno ritenuti idonei da chi li consuma. Per tale motivo, risulta necessario condurre dei test sui consumatori che consentano di definire le caratteristiche di gusto, le preferenze e i requisiti di accettabilità dell'alimento fritto. D'altro canto, però, nonostante l'importanza che l'analisi sensoriale riveste, ad oggi ci sono ancora pochi studi che supportano le informazioni sensoriali riportate in etichetta per gli oli /miscele da frittura ed i prodotti fritti.

3. Obiettivi e risultati attesi

Il presente progetto di ricerca si propone di valutare le performance in frittura di oli di semi e miscele di oli attraverso un approccio integrato fondato sulla correlazione di dati ottenuti da analisi chimico-fisiche e sensoriali. A ciò si aggiunge, una valutazione in termini di qualità e sicurezza di alimenti preselezionati da sottoporre a frittura. Il presente progetto di ricerca si pone, inoltre, l'obiettivo di evidenziare le correlazioni tra i dati analitici, strumentali e sensoriali al fine di ottenere una caratterizzazione completa degli oli e degli alimenti preselezionati, focalizzandosi in particolare sul difetto di rancido delle miscele di oli e degli alimenti e sull' attributo positivo "croccantezza" degli alimenti.
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) Messa a punto del disegno sperimentale
A3) Campionamento degli oli
A4) Analisi chimico-fisiche delle miscele da frittura e degli alimenti fritti
A5) Analisi sensoriale delle miscele da frittura e degli alimenti fritti
A6) Elaborazione e correlazione dei dati
A7) Stesura della tesi di dottorato, di report, articoli scientifici e presentazioni a convegni

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 disegno sperimentale***	■	■	■	■	■	■												
	1) Selezione delle miscele e dei prodotti da valutare in frittura	░	░	░	░														
	2) Definizione delle condizioni di frittura		░	░	░	░	░												
A3)	***Campionamento degli oli***		■	■	■	■	■	■	■	■									
A4)	***Analisi chimico-fisiche delle miscele da frittura e degli alimenti fritti***				■	■	■	■	■	■	■	■	■	■					
A5)	***Analisi sensoriale delle miscele da frittura e degli alimenti fritti***						■	■	■	■	■	■	■	■	■	■			
	1) Test descrittivi						░	░	░	░	░								
	2) Test sul consumatore											░	░	░	░	░			
A6)	***Elaborazione e correlazione dei dati***														■	■	■	■	■
A7)	***Stesura della tesi di dottorato di report, articoli scientifici, presentazioni a convegni***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

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DOTTORANDI ISCRITTI AL II ANNO (XXXVIII CICLO)

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

Solidea Amadei (e-mail: solidea.amadei2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Rosalba Lanciotti; Co-tutor: Francesca Patrignani, Davide Gottardi

1. Stato dell'arte

L'aumento della popolazione mondiale e la crescente esigenza di garantire sicurezza alimentare stanno determinando, negli ultimi decenni, la necessità di esplorare e sfruttare nuove fonti proteiche. La FAO ha previsto che nei prossimi anni la produzione di proteine animali rimarrà costante, mentre la domanda crescerà a causa dell'incremento demografico (FAO, 2021). Di conseguenza, c'è un crescente interesse, da parte della ricerca e dell'industria, nello sfruttare proteine da fonti alternative come legumi, cereali, e scarti vegetali (Molfetta et al., 2022). Queste matrici possono essere trattate attraverso processi biotecnologici, basati su microrganismi per i quali l'Autorità Europea per la Sicurezza Alimentare (EFSA) ha riconosciuto la qualificata presunzione di sicurezza (QPS), o processi non termici, come le alte pressioni di omogeneizzazione (HPH) e i campi elettrici pulsati (PEF), che consentono di modificare le caratteristiche tecnologiche e funzionali delle proteine. Ad esempio, microrganismi come lieviti e batteri vengono impiegati per fermentare scarti alimentari e fonti proteiche alternative, producendo composti a valore aggiunto. Batteri lattici, tra cui specie appartenenti ai generi *Lactococcus* e *Lactobacillus*, sono stati utilizzati per fermentare gli scarti dell'industria di trasformazione di cereali, vegetali e legumi, incrementando significativamente il contenuto in peptidi funzionali nei prodotti ottenuti (Martí-Quijal et al., 2021). L'utilizzo di lieviti non convenzionali, come *Yarrowia lipolytica* e *Debaryomyces hansenii*, è stato esplorato per valorizzare fonti proteiche alternative, tra cui scarti e sottoprodotti dell'industria agro-alimentare e farine di insetti (Gottardi et al., 2021; Patrignani et al., 2020). Questi processi biotecnologici permettono di ottenere prodotti con un migliore profilo nutrizionale e funzionale. Alcune proteine alternative ottenute da fonti vegetali e scarti vegetali fermentati sono state riconosciute come novel food da EFSA e possono essere utilizzate come ingredienti innovativi in prodotti alimentari, contribuendo così a perseguire agli obiettivi di sostenibilità e di economia circolare.

2. Bibliografia

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3. Obiettivi

Per il raggiungimento degli obiettivi del progetto della tesi di dottorato il lavoro è stato suddiviso nelle seguenti attività secondo il 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, 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. Stato di avanzamento della ricerca e principali risultati

Il presente progetto di ricerca si propone di mettere a punto ingredienti innovativi, 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, in combinazione o meno con trattamenti non termici. Tali ingredienti verranno poi utilizzati in formulazione di alimenti innovativi in modo da migliorare aspetti nutrizionali e funzionali del prodotto finale.

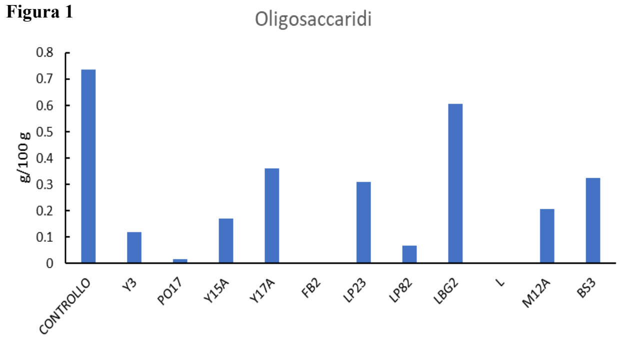
Durante il primo anno di dottorato, a seguito di ricerca bibliografica, sono state individuate alcune fonti proteiche alternative promettenti, come la farina di ceci, le trebbie di birra e le vinacce. Per la valorizzazione di tali matrici sono stati selezionati alcuni ceppi microbici sicuri e con status di QPS, precedentemente caratterizzati per le loro caratteristiche funzionali e tecnologiche e scientificamente noti per le loro capacità proteolitiche e lipolitiche.

Per quanto concerne la farina di ceci, sono stati allestiti dei processi biotecnologici utilizzando ceppi di batteri lattici, tra cui *Lactiplantibacillus plantarum* (LP23, LP82), *Lactococcus lactis* (LBG2), *Lactiplantibacillus paracasei* (L), *Latilactobacillus sakei* (M12A) e *Latilactobacillus curvatus* (BS3) e ceppi di lieviti quali *Yarrowia lipolytica* (Y3, PO17), *Debaryomyces hansenii* (Y15A, Y17A) e *Saccharomyces cerevisiae* (FB2), con l'obiettivo di selezionare i ceppi più promettenti per l'ottenimento di un ingrediente a valore aggiunto da utilizzare nella formulazione di prodotti alimentari innovativi. A questo scopo, le condizioni del processo biotecnologico sono state definite in funzione delle necessità dei singoli ceppi microbici e i campioni ottenuti a seguito dell'incubazione, sono stati caratterizzati per quanto riguarda la loro composizione (proteine e peptidi, zuccheri, acidi grassi e composti anti-nutrizionali) e funzionalità (attività antiossidante, contenuto in fenoli totali e attività prebiotica). Inoltre, sono stati caratterizzati da un punto di vista sensoriale, tramite la valutazione del profilo in composti volatili, e l'accettabilità da parte di consumatori.

I risultati principali hanno evidenziato come la farina di ceci sia una matrice favorevole per lo sviluppo microbico; infatti, tutti i microrganismi sono stati in grado di crescere, comportando, principalmente nei campioni inoculati con batteri lattici, un abbassamento del pH, che ha reso meno favorevole lo sviluppo di *Enterobacteriaceae*. Inoltre, il processo biotecnologico operato dai microrganismi ha comportato differenze nella composizione chimica rispetto alla farina di ceci non inoculata. Per quanto riguarda il contenuto proteico, tutti i campioni inoculati hanno evidenziato un decremento della concentrazione di proteine solubili (valutata tramite il saggio Bradford) e un aumento del contenuto in peptidi (testato tramite il saggio OPA). Ciò può essere considerato un risultato positivo in quanto i peptidi sono molecole di minore dimensione rispetto alle proteine, più facilmente digeribili ma anche potenzialmente più funzionali.

Nel profilo in acidi grassi, analizzato mediante GC/MS, non sono state osservate differenze significative tra i vari campioni. Tuttavia, nei campioni inoculati con i microrganismi è stato registrato un lieve decremento nell'abbondanza di acidi grassi polinsaturi, come il linoleico e l'α-linolenico, accompagnato da un aumento degli acidi grassi monoinsaturi, come l'oleico, e saturi come il palmitico.

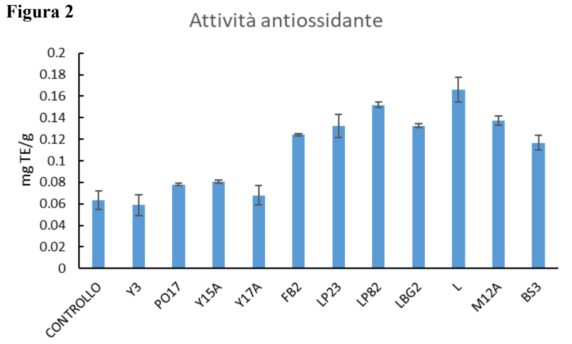
Figura 1



In seguito al processo biotecnologico, si sono verificate modifiche nel contenuto in zuccheri. In particolare, il contenuto in glucosio e saccarosio è diminuito in tutti i campioni inoculati con i microrganismi. Anche il contenuto in oligosaccaridi appartenenti alla famiglia del raffinosio, inizialmente stimato intorno a 0.74 g/ 100 g di prodotto, si è ridotto fino al limite di quantificazione nei campioni inoculati con *S. cerevisiae* FB2 e *L. paracasei* L (Figura 1). Tale riduzione è da considerarsi positiva dato che questi oligosaccaridi possono causare gonfiore e flatulenza durante la digestione.

Uno dei composti anti-nutrizionali più abbondanti nella farina di ceci è l'acido fitico. Durante questo studio è emerso che alcuni microrganismi, in particolare i lieviti, sono stati in grado di consumarlo, riducendo fino al 20% il contenuto nella matrice alla fine del periodo di incubazione.

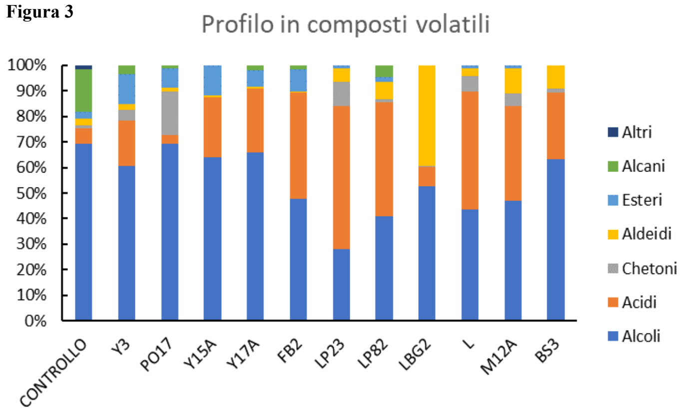
Figura 2



Inoltre, i campioni sono stati valutati per la loro capacità di fungere da radical scavenger. Sia tramite il saggio ABTS che DPPH, è stato evidenziato l'incremento dell'attività antiossidante dei campioni inoculati rispetto al controllo. Dai risultati del saggio DPPH (Figura 2), espressi in mg di Trolox equivalenti su grammo di campione, si può notare che l'attività antiossidante dei campioni inoculati con *S. cerevisiae* FB2 e con i batteri lattici sia significativamente superiore rispetto a quella del controllo e dei campioni inoculati con i lieviti. Tali risultati sono in linea con il contenuto in fenoli totali testato tramite il saggio di Folin-Ciocalteau.

L'attività prebiotica dei campioni è stata testata sui ceppi probiotici *Bifidobacterium longum* subsp. *infantis* DSM 20088 e *Lactobacillus rhamnosus* GG ed è stato riscontrato che sono cresciuti di circa due cicli logaritmici in 3 ore di incubazione nei campioni inoculati con *Debaryomyces hansenii.*

Figura 3



Il profilo in composti volatili si è modificato in conseguenza del processo biotecnologico. L'abbondanza delle classi di composti volatili si è rivelata differente in funzione del ceppo inoculato (Figura 3). In generale, nei campioni inoculati con lieviti, la classe di composti volatili più abbondanti era quella degli alcoli mentre in quelli inoculati con batteri lattici era quella degli acidi. La quantità di 1-esanolo, aroma tipico di legume, è stata ridotta, così come quella di alcune aldeidi, tra cui l'esanale, che è associato all'aroma di fagiolo e grasso. D'altro canto, alcuni composti volatili sono risultati più abbondanti nei campioni inoculati con i microrganismi, per esempio gli esteri, tra cui l'etil-acetato che apporta un aroma di fruttato.

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

Atti di convegno - poster:

Amadei S, Gottardi D, Rossi S, Braschi G, Siroli L, Lanciotti R, Patrignani (2024) Biotechnological valorization of chickpea flour for the production of value-added ingredients for innovative food formulations, in: FoodOmics 2024: fifteen years on from. Where are we now, what's next, Cesena, Italy.

Rossi S, Amadei S, Gottardi D, Braschi G, Lanciotti R, Patrignani (2023) Biotechnological valorisation of chickpea flour for the production of innovative added value ingredients for food formulation, in: 7th International Conference on Microbial Diversity 2023 - Agrifood microbiota as a tool for a sustainable future, Parma, Italy.

Amadei S (2023) Valorisation of alternative protein sources by biotechnological processes and non-thermal technologies to obtain new ingredients to be used in the formulation of innovative foods, in: XXVII Workshop on the developments in the Italian PhD research on food science technology and biotechnology, Portici, Napoli, Italy.

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

Chiara Angelucci (email: chiara.angelucci3@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof. Massimiliano Petracci; Co-tutor: Giulia Tabanelli, Rudi Magnani, Francesca Soglia

1. State-of-the-Art

Traditional heat treatments applied to food often result in chemical, physical, nutritional, and sensory alterations that can compromise the overall quality of the product. Consequently, there is a growing consumer demand for more natural food products with minimal processing. This trend reflects consumers' desire to preserve the perceived safety, nutritional integrity, and sensory characteristics of food items (Valdramidis & Koutsoumanis, 2016).
Achieving a safe food matrix is important to ensure the overall safety of a food product, with the primary challenge being the prevention of pathogenic microorganisms' presence. (Rosario *et al*., 2021). In this contest my PhD research focuses on exploring the application of non-thermal treatments for enhancing the safety and extending the *shelf-life* of sausages and soft cheese. The perishability of sausages and the consequent limited *shelf-life* pose significant challenges for market expansion. Therefore, considerable effort has been directed towards identifying methods to delay microbial growth and chemical processes that compromise product quality.
The use of non-thermal treatments becomes essential since heat treatment is not practical for certain products. High hydrostatic pressure processing (HPP) is, among these techniques, one of the most promising. When food is treated with HHP, it is packed in appropriate pressure-resistant materials and exposed to hydrostatic pressure of several hundred megapascals (MPa). In commercial applications, pressures between 200 and 600 MPa are applied for a maximum of five minutes at room temperature or below (Aganovich *et al.,* 2021).

2. References

Aganovic K, Hertel C, Vogel RF, Johne R, Schlüter O, Schwarzenbolz U, Jäger H, Holzhauser T, Bergmair J, Roth A, Sevenich R, Bandic N, Kulling SE, Knorr D, Engel KH, Heinz V (2021) Aspects of high hydrostatic pressure food processing: Perspectives on technology and food safety. Compr. Rev. Food Sci. Food Saf. 20, 3225-3266.
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. Crit. Rev. Food Sci. Nutr. 61(7), 1163-1183.
Valdramidis VP, Koutsoumanis KP (2016) Challenges and perspectives of advanced technologies in processing, distribution, and storage for improving food safety. Curr. Opin. Food Sci. 12, 63-69.

3. Objectives

The present research project aims to evaluate impact of the application of non-thermal treatments on 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) Bibliographic research 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, texture, and microbial growth.
A3) 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.
A4) HPP application on fermented sausages to evaluate their quality and *shelf-life* extension.
A5) HPP application on dairy products to evaluate their safety and extension of *shelf-life*.
A6) Writing and publication of the doctoral thesis, posters, scientific papers, and oral presentations.

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) Colour measurement by colorimeter		▒	▒	▒	▒	▒	▒	▒	▒									
	2) Evaluation of lipid oxidation (TBARS)		▒	▒	▒	▒	▒	▒	▒	▒									
	3) Texture Profile Analysis test		▒	▒	▒	▒	▒	▒	▒	▒									
	4) Microbiological analysis and evaluation of *shelf-life*		▒	▒	▒	▒	▒	▒	▒	▒									
A3)	***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									▒	▒	▒	▒	▒	▒	▒	▒	▒	
A4)	***HPP application on fermented sausages***										■	■	■						
	1) Evaluation of product quality										▒	▒	▒						
	2) Microbiological analysis and evaluation of *shelf-life*										▒	▒	▒						
A5)	***HPP application on softshel cheeses***													■	■	■	■	■	
	1) Evaluation of product quality													▒	▒	▒	▒	▒	
	2) Microbiological analysis and evaluation of *shelf-life*													▒	▒	▒	▒	▒	
A6)	***Preparation of manuscripts, presentations, and thesis***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research status and main results

The purpose of the PhD research was to assess the potential of different treatments of HPP in extending the *shelf-life* of pork fresh sausages packed under vacuum and produced in a pilot plant. Treated and non-treated samples have been compared with regard to their microbial population composition and dynamics both with culture-dependent and independent methods (*i.e.* metagenomic analysis) to study the spoilage patterns in relation to the treatment applied. In addition, attention has been posed to the color of the sausages as an important product feature assuring the acceptability of consumers.
Four different meat mixtures were produced in an industrial plant (CLAI, Cooperativa Lavoratori Agricoli Imolesi, Imola, Italy). The ratio lean meat/fat was approx. 90:10. The raw meat was coarsely ground (4-5 mm) at 0°C and mixed with NaCl 2.2%, grounded black pepper 0.1% and ascorbate 0.02%. This mixture was divided into 4 batches, that differed for the pH value (5.8 or 5.4) and the presence (100 ppm) or the absence of nitrites. Each meat mixture was portioned in aliquots of about 100 g, vacuum packed and stored under refrigeration conditions (4°C) overnight. The samples of each batch were divided into three groups: the control (not-treated), HHP-treated samples at 300 MPa and HHP-treated samples at 600 MPa. The samples were stored at 4°C and monitored during the *shelf-life*. The initial microbial load of the fresh sausages was relatively low (approx. 4 log CFU/g).
The growth kinetics showed that all the not pressurized samples were spoiled after 18 days of storage at 4°C, even if the samples at pH 5.8 without nitrite showed a higher microbial growth rate. In general, the application of a pressure of 300 MPa caused a small reduction of the initial microbial counts, less than 1 log cycle. However, a slow increase was observed during storage, reaching the initial cell concentration only after 20 days and allowing a considerably prolongation of product shelf-*life*. As expected, the treatment at 600 MPa had a more relevant effects on the initial microbial counts. Interestingly, this effect was more pronounced in the sausages at pH 5.4, being total microbial counts lower than others at the end of storage, in the presence of nitrite. On the other hand, the sausages at pH 5.8 without nitrite showed the faster growth dynamics.
Regarding the results about the lightness (L*), the control group exhibited lower values compared to the treated samples at 300 MPa and 600 MPa. In general, L* value is higher in samples without the addition of nitrate, independently on the pH, compared to samples with the addition of this additive. For the redness parameter (a*), regardless of the level of pressure applied, the samples with the addition of nitrate showed higher values, thus confirming the key role of nitrate in enhancing the red color of meat product. This is due to the formation of nitrosomyoglobin a compound formed when nitrite react with myoglobin. As for yellowness (b*), treated samples with lower pH and without nitrates consistently exhibit higher b* values compared to the control, regardless of the pressure level applied.

5. List of publications as part of the PhD activity

Angelucci C, Barbieri F, Baños A, Garcia Madero JM, Montanari C, Tabanelli G, Gardini F (2023) Anti-listerial activity of autochthonous lactic acid bacteria in fresh pork sausages. Proc. of the 69th International Congress of Meat Science and Technology, from Tradition to Green Innovation, 20-25 August 2023, Padova, p. 292-293.

Angelucci C, (2023). Use of non-thermal treatments to improve the quality, safety, and shelf-life of products of animal origin. Proc. of the 27thWorkshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, 13-15 September 2023, Naples, p. 40.

Angelucci C, Tabanelli G, Gardini F, Barbieri F, Soglia F, Magnani R, Gardini G, Petracci M (2024) Omics techniques for assessing the potential of high hydrostatic pressure to improve the shelf-life of fresh sausages. Book of abstracts of the 7th International Conference on Foodomics 2024, 14-16 February 2024, Cesena (Italy), p. 66.

Microbial Biopolymers for Innovative Packaging to Increase Food Shelf-life and Safety

Marianna Ciccone (email: marianna.ciccone2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Water-Food-Energy-Sustainable Agriculture Nexus; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof. Francesca Patrignani; Co-tutors: Dr. Lorenzo Siroli, Dr. Davide Gottardi

1. State of the art

In response to environmental concerns, there is a growing interest in sustainable alternatives to petroleum-based films for food packaging. While petrochemical polymers are cost-effective and have excellent properties, their non-degradability and dependence on finite resources pose an environmental challenge. The development of biodegradable polymers for sustainable packaging aims to reduce the accumulation of plastic and reduce dependence on non-renewable materials (González-López *et al.*, 2023). According to the literature, natural polymers such as polysaccharides, proteins and lipids are used in the development of biodegradable food contact materials. Although various materials are promising as biopolymers, they are still under-researched. One such material is yeast biomass, which is characterised by cell walls made of glucans, mannoproteins and chitin. For example, β-glucans, constituting 55-65% of yeast cell walls, have demonstrated efficacy in forming multicomponent films alongside cell wall proteins. Several non-thermal technologies such as high-pressure homogenisation (HPH) and pulsed electric fields (PEF) are currently available to destroy yeast cell walls and isolate components, offering sustainable alternatives to thermal methods. In addition to yeast cell walls, some biopolymers such as pullulan produced by *Aureobasidium pullulans* or bacterial cellulose produced by various acetic bacteria such as *Komagataeibacter xylinus* could also be viable alternatives for obtaining biopolymers of microbial nature for use in food packaging (Saharan *et al.*, 2024). Moreover, biopolymers are gaining importance in food packaging as they can act as carriers for other molecules with antioxidant and antimicrobial properties. Active packaging systems with functional additives interact with food surfaces or the headspace of the packaging to prevent the growth of pathogenic or spoilage microorganisms. When antimicrobial agents such as essential oils or bacteriocins are incorporated directly into the food, the efficacy can be reduced due to diffusion. Conversely, incorporating these actives into packaging materials offers a more efficient method of preservation, with controlled diffusion ensuring residual activity during storage and distribution. Nisin, a bacteriocin from *Lactococcus lactis* subsp. *lactis*, is known to inhibit pathogenic microorganisms and retain its activity after processing, making it a promising candidate for antimicrobial packaging in biopolymer matrices (Bukvicki *et al*., 2023). The microbial fermentation of agricultural and food waste offers a promising solution for the production of biopolymers and thus contributes to sustainability. The utilisation of waste and by-products from the agricultural and food industry as culture substrates for microbial growth, such as whey, molasses and by-products from the wine and beer industry, represents an opportunity for the sustainable use of resources (Horue *et al.*, 2021). However, while biopolymers made from renewable raw materials offer low-carbon alternatives, ensuring their safety for food contact applications is crucial. In this context, comprehensive risk and safety assessments throughout the production process are essential, addressing potential contaminants, non-intentionally added substances (NIAS), and microbial hazards (EFSA, 2023). The inherent complexity of these biopolymers requires continuous assessment to ensure the safety and quality of biopolymer materials for food packaging. To summarise, biopolymers derived from waste through microbial fermentation represent a promising route for sustainable food packaging. Optimising production processes, ensuring safety through risk assessments and balancing economic, environmental, and social considerations are key aspects for realising this vision.

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3. PhD thesis Objectives and Milestones

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

A1) **Bibliographic research** to identify the most suitable microorganisms for the recovery of biopolymers.
A2) **Screening of selected strains and optimisation of microbial performance** on agri-food industry by-products.
A3) **Selection of the most suitable method and technologies** for recovering of the biopolymers of interest.
A4) **Characterisation of biopolymers** in terms of their antioxidant and antimicrobial properties and film formulation.
A5) **Evaluation of the shelf life and safety of food matrices** in relation to selected packaging conditions.
A6) **Writing and Editing** of the PhD thesis, scientific papers, and oral and/or poster communications.

Table 1. Gantt diagram related to this PhD project.

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1) Identification of suitable microorganisms	▒	▒	▒	▒	▒	▒												
A2)	***Screening of the selected strains and optimization of microbial performance***			■	■	■	■	■	■	■									
	1)Selection and technological characterisation of selected microorganisms			▒	▒	▒	▒	▒	▒	▒									
	2) Optimisation of growth conditions for microorganisms on agri-food industry wastes and by-products			▒	▒	▒	▒	▒	▒	▒									
A3)	***Development of biotechnological processes for the recovery of biopolymers of interest***						■	■	■	■	■	■	■	■	■				
	1) Application of microbial enzymes to obtain cellular compounds						▒	▒	▒	▒	▒								
	2) Application of high-pressure homogenisation, pulsed electric fields											▒	▒	▒	▒				
A4)	***Antimicrobial and technological characterisation of the obtained biopolymers and film formulation***												■	■	■	■	■		
	1) Antimicrobial and antioxidant characterisation of biopolymers												▒	▒	▒	▒	▒		
	2) Film formulation and characterisation														▒	▒	▒		
A5)	***Evaluation of the shelf life and safety of food matrices in relation to selected packaging conditions***														■	■	■	■	■
A6)	***Thesis and Paper Preparation***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research progress and main results

The main objective of this PhD project is to develop biopolymers for food packaging using components derived from bacteria or yeast cells, either enriched or not with antimicrobial compounds. Comprehensive literature research identified biopolymers obtainable from microbial cultures and methods for their extraction, characterization, and application in food packaging. Preliminary trials at the lab scale evaluated the release of β-glucans from yeast cell walls using a baker's and a brewer's spent strain of *Saccharomyces cerevisiae*. Two non-thermal alternative technologies (HPH 125 MPa for 3 passes and PEF 1.5 kV/cm for 45 pulses), combined or not with a thermal treatment (TT 90°C for 20 minutes), were applied to enhance the release of these biopolymers. The β-glucans content in the supernatants was dependent on the treatment applied, and all samples subjected to HPH (in combination or not with other treatments) had significantly higher β-glucans content than the others. The supernatants of the HPH-treated baker's yeast samples showed concentrations from 2.1 to 3.9 g/100g dry matter, while the other samples remained below 0.15 g/100g dry matter. The brewer's spent yeast showed a higher β-glucans content in all samples compared to commercial yeast: the HPH+TT and TT+HPH samples showed the highest β-glucan content in the cell supernatants, followed by the HPH+PEF and PEF+HPH samples, with values of 13.10 g/100g and 9.44 g/100g dry matter, respectively (**Figure 1**). HPH and PEF represent a useful strategy to promote the release of β-glucans from yeast cells, which can be used in the formulation of biodegradable polymers and in the production of new sustainable films for food packaging. Among the substances that could potentially be used as microbial biopolymers, there is pullulan, a microbial exopolysaccharide obtained by fermentation of the yeast like fungus *Aureobasidium* spp. Pullulan has various applications in the food industry, including as thickening agents and stabilizers, alongside emerging roles in edible coatings and films for food packaging. Two *Aureobasidium* strains (*A. pullulans* var. *melanogenum* DSM 2404 and *A. pullulans* DSM 3042) were assessed for

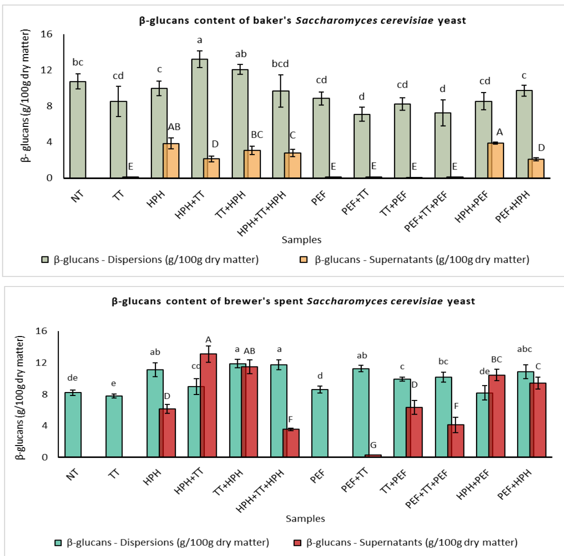

Figure 1: β-glucans content (g/100g dry matter) of dispersions and supernatants obtained after application of the different treatments. NT represents the control sample. *Different letters at the superscripts indicate significant differences for each series ($p < 0.05$).*

their ability to produce pullulan from agri-food by-products, including cheese whey, olive pomace, grape pomace, brewer's spent grain, and beet molasses. The results showed that the two tested *Aureobasidium* strains were able to produce pullulan in all the tested by-products. However, the production rate was strongly affected by the strain and the substrate. Both the strains showed the highest pullulan production on cheese whey followed by beet molasses, brewer's spent grain, olive pomace and grape pomace. Nonetheless, the strain *A. pullulans* DSM 3042 showed the highest pullulan rate in all the considered substrates and conditions. Using food industry by-products as a carbon source for pullulan production by *A. pullulans* strains is not only sustainable but also holds promising implications for the food packaging industry. However, further studies in order to characterize the quality and purity of the pullulan obtained by the various by-product should be performed. Concerning the production of bacterial cellulose (BC), the growth of five different acetic acid bacteria (AAB) strains belonging to the species *Komagataeibacter xylinus*, *Komagataeibacter rhaeticus* and *Komagataeibacter hansenii* and the ability to produce BC on various by-products of the agri-food industry (beet molasses, brewer's spent grains and cheese whey) were evaluate. The evaluation of growth and BC production was carried out during incubation of the samples at 28°C for 10 days. The results obtained indicate that all AAB strains were able to grow on the agri-food by-product substrates. The ability to produce bacterial cellulose depended on the AAB strain and the agri-food by-product substrate. The brewer's spent grains showed good yields of bacterial cellulose from all the strains considered. In contrast, on beet molasses, the highest yields were obtained with the strain *K. xylinus* DSM 2004 (3 g/L of bacterial cellulose). Cheese whey and beet molasses were the most selective substrates in terms of bacterial cellulose yields: only *K. xylinus* DSM 2004, *K. rhaeticus* LM4 and *K. rhaeticu*s SM1 strains were able to produce significant amounts of BC. Furthermore, an initial characterization of the bacterial cellulose was caried out. The cellulose films were characterized for their physical and chemical properties through infrared spectroscopy (IR analysis), differential scanning calorimetry (DSC), and thermogravimetric analysis (TGA). Thermogravimetric analysis shows a high degradation temperature for the cellulose material, increasing the possibilities for processing. Tensile tests highlight the significant influence of water on bacterial cellulose: the wet sample exhibits higher plastic deformation than the dried one. Infrared spectroscopy (IR analysis) reveals that bacterial cellulose has a high liquid absorption capacity due to its porous structure. The cellulose obtained can be utilized for labelling by adhering it to a PET film using a bio-based and biodegradable polymer adhesive. Additionally, it can be employed in container fabrication through meticulous control of the drying process. Moreover, its absorbent properties enable it to absorb chemically unrelated liquids, facilitating applications such as incorporating food colouring and extra virgin olive oil. Based on the results obtained, both yeast biomass and bacterial cellulose represent promising alternatives to conventional sources of biodegradable polymers for the development of a sustainable packaging films. However, further experiments and studies are necessary to achieve films with the desired properties for food contact materials.

5. List of publications produced during the PhD activities

Ciccone M, Barthélémy E, Tsochatzis E, Rainieri S, Njieukam JA, Siroli L, Gottardi D, Lanciotti R, Patrignani F (2024) Approach to perform the Safety Assessment of Cellulose and Nisin Biopolymers obtained from Bacterial Fermentation as Innovative Food Contact Materials Aimed to Extend Food Shelf Life. 11th Shelf-Life International Meeting - SLIM2024.

Ciccone M, Njieukam JA, Leone G, Siroli L, Gottardi D, Lanciotti R, Pizzichini D, Patrignani F (2024) Characterization and utilization of brewer's spent yeast for sustainable packaging innovations. 22nd World Congress of Food Science and Technology.

Ciccone M, Njieukam JA, Hernandez JBM, Brashi G, Siroli L, Gottardi D, Lanciotti R, Rocculi P, Patrignani F (2024) High pressure homogenization and pulsed electric fields for favouring the release of β-glucans and mannoproteins from yeast cell walls. 22nd World Congress of Food Science and Technology.

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Njieukam JA, Siroli L, Ciccone M, Gottardi D, Lanciotti R, Patrignani F (2024) Bacterial cellulose production by selected acetic acid bacteria on different agri-food by-products. 7th The International Conference on Foodomics.

Ciccone M, Siroli L, Gottardi D, Njieukam JA, Braschi G, Lanciotti R, Patrignani F (2023) Application of non-thermal technologies for the recovery of β-glucans from yeast biomass. 7th International Conference on Microbial Diversity.

Njieukam JA, Siroli L, Ciccone M, Gottardi D, Patrignani F, Lanciotti R (2023) Selection of bacterial cellulose producing acetic acid bacteria from kombucha tea and evaluation of the influence of culture conditions on bacterial cellulose production. 7th International Conference on Microbial Diversity.

Siroli L, Ghirardelli E, Njieukam JA, Ciccone M, Gottardi D, Ricci A, Parpinello GP, Lanciotti R, Patrignani F (2023) Microbiological and chemical-physical composition of kombucha in relation to starter cultures. 7th International Conference on Microbial Diversity.

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

Federico Drudi (email: federico.drudi3@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Urszula Tylewicz; Co-tutor: Silvia Tappi

1. 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, more and more companies are launching such products on the market, 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 et al., 2021). Unfortunately, proteins derived from plant sources differ not only in terms of their nutritional value but also in terms of their technological properties. Solubility and gelling properties are generally lower than those of animal origin (especially at pH close to neutrality), making their use in formulations more complex (Akharume 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, making 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 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).

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3. Objectives

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 subdivided 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) Properties evaluation of different legume flours with the aim of identifying one or two specific legumes to work on.
A3) Treatment application to the selected flours, aiming at studying the effects on functional properties. PEF, US, HPP and CP effects will be evaluated and optimisation of parameters for the best treatment will also be studied.
A4) Properties evaluation of the modified flours in terms of 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) Production of a final bakery, snack or dairy replacement product based on the ingredients obtained, aiming to understand if the functionalisation process can improve the performance on a final product.
A6) Assesment of sustainability, from an environmental perspective, of selected functionalization methods. LCA methodology will be used taking into account other, more conventional functionalisation systems.
A7) Writing poster, articles and final dissertation, along with conferences attendance.

Table 1. Gantt diagram of the planned activities for the PhD

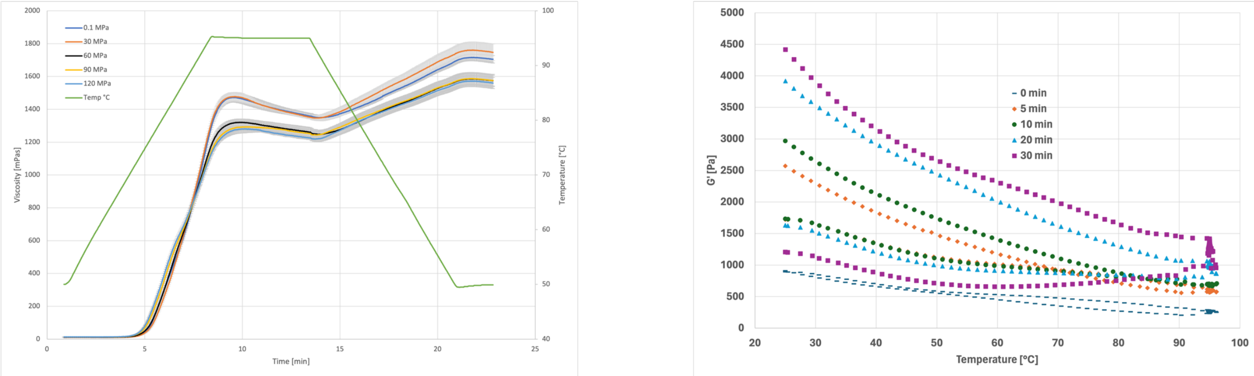
		Time [month]																	
RESEARCH ACTIVITY		**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
A1	Literature review and study	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2	Flour characterization and identification of target characteristics	■	■	■	■														
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 of developed processes												■	■	■	■	■		
A7	Writing dissertation, posters, scientific papers				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research advancement and main results

As indicated in the Gantt chart, the work in the first half of the first year focused mainly on studying the literature on the effects of various non-thermal technologies on plant protein, with an emphasis on understanding the operating principles of these techniques. This work led to the publication of a chapter entitled "Conventional and unconventional modification of plant protein" in a review article (Nowacka et al., 2023).

In addition to the literature review, the second main activity was dedicated to establishing and gaining confidence in repeatable protocols for analyzing protein and flour functionality. Part of this activity was carried out during my one-month stay at the Wroclaw University after winning a scholarship from the Polish International Scholarship Exchange program (PROM). As a result, a list of internal methods for evaluating functional properties such as water holding capacity (WHC), oil holding capacity (OHC), water solubility index (WSI), protein solubility, foaming (FC) and emulsifying capacity was developed, as well as a set of methods for evaluating rheological and textural properties. All these tests were carried out specifically for the evaluation of whole chickpea flour and protein isolates.

The initial treatment trials focused on the high pressure homogenization (HPH) of a slurry of chickpea flour and water (1:2 ratio) treated at different pressures, namely 0, 30, 60, 90 and 120 MPa for one cycle. The results (Tab.2) showed that some functional properties were significantly improved, namely solubility, WHC and OHC. The viscosity profile was also affected (Fig. 1a), with an overall decrease in viscosity and a strong decrease in breakthrough viscosity. This result can mainly be explained by three phenomena: reduction in particle size, partial protein denaturation and partial starch damage. However, when evaluating the starch damage on the treated flours, the low values found (never more than 2%) indicate that the effects on the functional properties are not due to starch modifications. The denaturation of the proteins, on the other hand, was improved (measured indirectly by solubility and directly by the sulfide bridges), especially at a treatment pressure of 30-60 MPa. The results of this research are the subject of a manuscript that will be submitted to an scientific indexed journal.



(a) ***(b)***

Figure 1. Pasting profile of chickpea flour treated with HPH *(a)*. Temperature sweeps of pea protein isolate after cold plasma treatment *(b)*.

Table 2. Selected functional properties evaluated for the HPH treated chickpea flour.

Sample	Control	30 MPa	60 MPa	90 MPa	120 MPa
WHC [g/g db]	1.11 ± 0.02 [bc]	1.06 ± 0.04 [c]	1.11 ± 0.01 [bc]	1.18 ± 0.04 [a]	1.35 ± 0.02 [a]
OHC [g/g db]	0.89 ± 0.02 [c]	1.09 ± 0.06 [a]	1.06 ± 0.06 [ab]	0.98 ± 0.02 [bc]	0.99 ± 0.01 [ab]
WAI [g/g db]	5.47 ± 0.07 [d]	5.59 ± 0.18 [cd]	5.83 ± 0.14 [bc]	6.14 ± 0.11 [ab]	6.46 ± 0.06 [a]
WSI [g/100g db]	39.95 ± 1.07 [b]	43.50 ± 0.38 [a]	42.70 ± 0.50 [a]	40.07 ± 0.07 [b]	39.44 ± 0.15 [b]
Foam capacity [%]	19.33 ± 1.15 [d]	26.00 ± 0.00 [c]	29.33 ± 2.13 [ab]	28.00 2.00 [bc]	32.00 ± 0.00 [ab]
Foam stability after 30 min [%]	86.30 ± 5.48 [a]	69.23 ± 7.69 [b]	63.69 ± 7.22 [b]	66.89 ± 6.06 [b]	68.75 ± 0.00 [b]

Different letters in rows indicate statistically significant ($p<0.05$) differences between samples.

Following this initial test, a more in-depth study was carried out. Specifically, the protein and starch fractions were extracted from the chickpea flour and then treated separately with HPH. The control and treated samples were analyzed for the following properties: WHC, OHC, WAI, WSI, emulsion and foam stability, pasting and rheological characterization. The results of this study are currently being analyzed. Unfortunately, it seems that the denaturation process occurring on the isolation step is limiting the possibility to identify any modification after the HPH treatment, probably as a result of the denaturation induced by isoelectric precipitation, nevertheless a better evaluation of the results is needed before any conclusion can be drawn.

Currently also a new cold plasma source is being tested, specifically designed to treat powders and preliminary trials are being carried out on pea protein isolate (PPI). Specifically, treatment times of 0, 5, 10, 20 and 30 minutes have been applied and the isolate assessed in term of solubility, SDS-PAGE, and rheology.

Results (Fig. 1b) show a marked G' increase proportional to the time of treatment, indicating that PPI suspension at 15% are being able to form stronger gels after the application of plasma. FTIR result show a minimal secondary structure modification after the treatment and the absence of a clear band shift on SDS-PAGE analysis suggest no effect on the primary structure of the protein. Thus, more studies are needed to assess if the cold plasma treatment is affecting the tertiary or quaternary structure of the protein, in order to link the observed rheological modification to a structural or chemical effect of the plasma on the protein molecules.

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

Nowacka M, Trusinska M, Chraniuk P, Drudi F, Lukasiewicz J, Nguyen NP, Przybyszewska A, Pobiega K, Tappi S, Tylewicz U, Rybak K and Wiktor A (2023) Developments in plant proteins production for meat and fish analogues. Molecules 28.7, 2966. (DOI: 10.3390/molecules28072966)

Combining instrumental and sensory methods to assess food products of animal origin

Mara Antonia Gagliano (email: maraantonia.gagliano@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Enrico Valli; co-tutor: Francesca Soglia, Francesca Patrignani

1. State of the art

Human sense perception can be combined with "artificial senses" based instruments, which have been applied for e.g. quality control, freshness monitoring, shelf-life study and authenticity evaluation of food products. These tools may show many advantages, such as they are fast, efficient, non-destructive, and sustainable with respect of environment (Ali et al., 2020). Among the artificial senses, e-noses and e-tongues based on hybrid or electronic sensors, are being investigated. 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. Electronic noses can be used e.g. to assess quality of beef fillets (Sun et al., 2024), measure flavour quality changes of oxidized chicken fat (Song et al., 2013), verify the authenticity of Parmigiano-Reggiano cheese (Becchi et al., 2024) and identification of botanical origin of honey (Huang et al., 2016). Another rapid analytical and non-destructive instrument is represented by the computer vision system (CVS) (electronic eye), which consists in an illumination device, a camera, a computer with a high-resolution monitor. CVSs are important to classify food products into specific grades, detect visual defects and estimate properties such as colour, shape, size, surface defects; examples are estimation of fat content in poultry products (Chmiel et al., 2016), or predicting colour grade in beef meat (Sun et al., 2018) and honey characterization with different botanical and geographical origin (Shafiee et al., 2014). The application of instrumental methods and microbiological analyses in addition to sensory ones are beneficial to investigate how different animal farming systems can affect the quality of meat and dairy products (Costa et al., 2024; Kasapidou et al., 2023; El-Deek et al, 2016). Therefore, this PhD research project is focused on investigating the effect of animal farming systems and origin on the quality of selected food products of animal origin by applying multi-analytical techniques to assess their quality; a joint discussion of sensory, microbiological and instrumental data will be herein carried out.

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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.

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3. Objectives

In order to achieve the objectives of the PhD thesis, the project was divided into the following activities reported in the Gantt diagram below (Table 1).

A1) Bibliographic research: research in the literature focused on image analysis, analytical methods to determine volatile profile, sensory analysis on food products of animal origin, and how different animal farming systems and origin can affect their quality.
A2) Aroma and visual analysis: set-up and application of HS-Flash GC-FID, HS-GC-IMS, SPME-GC-MS and electronic eye-based methodologies (dairy and meat products, and honey).
A3) Sensory evaluation: descriptive analysis (e.g., QDA®, Flash Profile, etc.) and consumer tests on cheese, milk and meat and measuring of emotional response to such food products.
A4) Microbiological analysis: microbiological analyses (total mesophilic bacteria, *Lactobacillus* spp., *Lactococcus* spp., yeast and moulds, *Staphylococcus* spp., total Enterococci) on food products of animal origin, including cheese.
A5) Statistical analysis: univariate and multivariate data analysis. Joint statistical analyses of the results obtained from sensory, microbiological tests and instrumental analysis.
A6) Writing of the oral and/or poster communications, scientific papers and PhD final thesis.

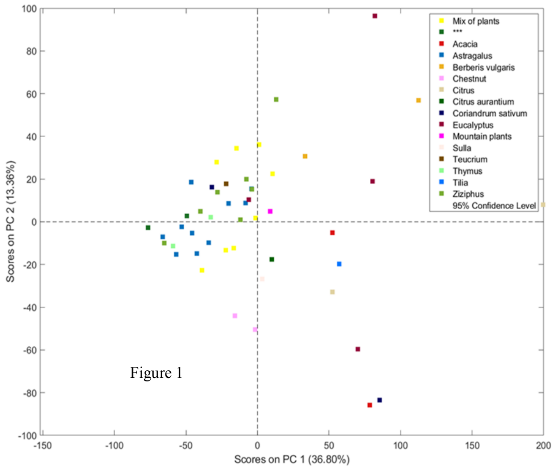
Table 1. Gantt diagram showing the PhD activities.

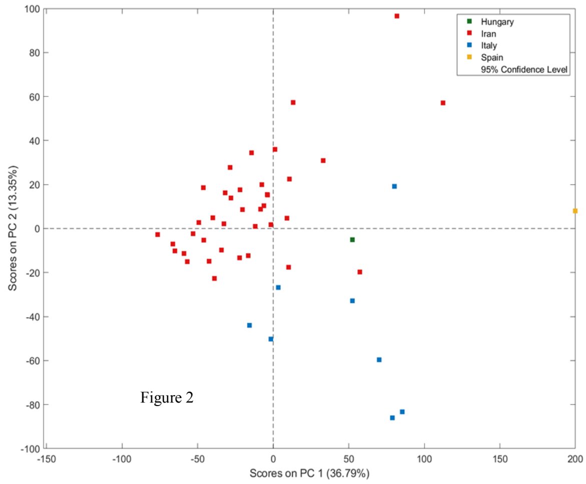
Activities	Month	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Bibliographic research***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2)	***Aroma and visual analysis***		■	■	■	■	■	■	■	■	■	■	■	■	■				
	1) Set-up of analytical methodologies		▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒				
	2) Methods application on food products				▒	▒	▒	▒	▒	▒	▒	▒	▒	▒	▒				
A3)	***Sensory evaluation***		■	■	■	■	■	■	■	■	■	■	■	■	■				
	1) Quantitative descriptive analysis (QDA®)		▒	▒	▒	▒	▒	▒	▒	▒	▒	▒							
	2) Consumer test				▒	▒	▒				▒	▒	▒	▒	▒				
	3) Emotional response to animal food products										▒	▒	▒	▒	▒				
A4)	***Microbiological analysis***					■	■	■	■	■									
A5)	***Statistical analysis***					■	■	■	■	■	■	■	■	■	■	■	■	■	■
	1) Data elaboration					▒	▒	▒	▒	▒	▒					▒	▒	▒	▒
	2) Univariate and multivariate approaches							▒	▒	▒							▒	▒	▒
A6)	***Dissemination, writing scientific papers and final thesis***				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research progress and main results

This PhD project is focused on evaluating the quality of different food products of animal origin (poultry and beef meat, dairy products, and honey) by using multi-analytical instrumental and sensory approach, to investigate the effect of the farming system, as well as the botanical and geographical origin. Moreover, microbiological analyses have been carried out to investigate possible relations between dairy cows' diet and cheeses microbiome and to assess samples safety. During the first part of the PhD research project, aroma and image analyses were conducted on food products, i.e. Parmigiano Reggiano cheeses and honeys. Regarding the first food matrix, 10 samples of Parmigiano Reggiano cheese obtained from different farming systems were analyzed (6 samples from milk obtained from cows fed dry hay, coded as PDH, and the others from milk obtained from cows fed with fresh herbage supplementation, PFF). To investigate the possible relationships between dairy cow diets and the microbiome of the samples, microbiological analyses were carried out. Instrumental, microbiological and sensory data were discussed jointly. Specifically, no sensory differences were found among the samples in terms of aroma, taste and texture attributes, considering the different feeding of the cows, while a greater intensity of yellow color was observed in samples belonging to the PFF group; this difference in color was determined by both sensory and instrumental methods (image analysis) and can be related to the cows' diet rich in carotenoids (Manzocchi et al.,2021). HS-Flash GC-FID volatilome analysis showed differences in the chromatographic profiles of the two experimental groups currently under evaluation. In addition, all samples met the microbiological quality and safety parameters by showing non-significant differences between samples in terms of total microbial and lactic acid bacteria cell loads. The joint instrumental, sensory and microbiological characterization allows obtaining an analytical profile of the analysed cheeses potentially useful for quality control and contributing to the authenticity of this high-value typical food product.
Regarding the honey, the volatile profile of 50 honey samples belonging to different botanical and geographical origin was analysed by HS-Flash GC-FID and HS-GC-IMS. The untargeted statistical elaboration of the results showed a good discrimination of the samples based on their aromatic profile, according to their botanical and geographical origin. In particular, in the PCA a clustering of the honey samples was observed, considering their botanical origin, especially for those belonging to astragalus, thyme, eucalyptus and coriander (figure 1). Finally, in figure 2, it can be observed that Italian honeys discriminate well from those originating from Iran.

Figures 1 and 2. biplots of HS-Flash GC-FID results considering botanical origin (figure 1) and geographical origin (figure 2) as variables. These elaborations were made considering the honey samples volatile compounds (peak areas).





In the coming months, during the PhD research period abroad, the measuring of emotional responses to food products of animal origin will be investigated. The volatile compounds of milk and beef samples from different farming systems will be evaluated by several instrumental methods (HS-Flash GC-FID, HS-GC-IMS, SPME-GC-MS), visual analysis with electronic eye, microbiological analysis and sensory tests.

5. List of publications during doctoral activities

Baldi G, Gagliano MA, Soglia F, D'Elia F, Laghi L, Rocculi P, Petracci M (2023) High-Intensity Ultrasonication as an Innovative Approach for the Softening of Wooden Breast Meat in Broiler, Meat Muscle Biol.7(1): 1-10.

Gagliano MA, Baldi G, Soglia F, Mancinelli AC, Petracci M (2023) Qualitative characterization of chicken meat according to the main Italian commercial categories, Proc. of the 25th ASPA Congress, pp. 214-215.

Gagliano MA, Soglia F, Zampiga M, Sirri F, Petracci M (2023) Comparison of meat quality traits among chickens' genotypes with different growth-rates, Proc. of the 25th ASPA Congress, p. 214.

Gagliano MA, Soglia F, Tura M, Zampiga M, Valli E, Gallina Toschi T, Sirri F, Petracci M (2023) Quality traits of thigh meat from the more promising medium-growing genotypes to be used for European broiler production, Proc. of the 69th International Congress of Meat Science and Technology from Tradition to Green Innovation, pp 422-423.

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Gagliano MA, Tura M, Soglia F, Cevoli C, Barbieri S, Braschi G, Patrignani F, Bendini A, Gallina Toschi T, Petracci M, Valli E (2024) Multi-analytical characterization of Parmigiano Reggiano PDO cheeses produced with milk from different dairy cows feeding, Book of abstract "FOODOMICS 2024 - 7TH INTERNATIONAL CONFERENCE ON FOODOMICS 2009-2024, fifteen years on from. Where are we now, what's next", pp. 113-115.

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

Yogesh Kumar (email: yogesh.kumar3@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Andrea Versari; Co-tutor: Giuseppina Paola Parpinello, Arianna Ricci

1. 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% 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.

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Saliba AJ, Ovington LA, Moran CC (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

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3. Obiettivi

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 (i) Dealcoholized Wine: A Scoping Review of Volatile and Non-Volatile Profiles, Consumer Perception, and Health Benefits,
(ii) Mechanisms of the initial stage of non-enzymatic oxidation of wine: A mini review.
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 using different techniques.
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***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

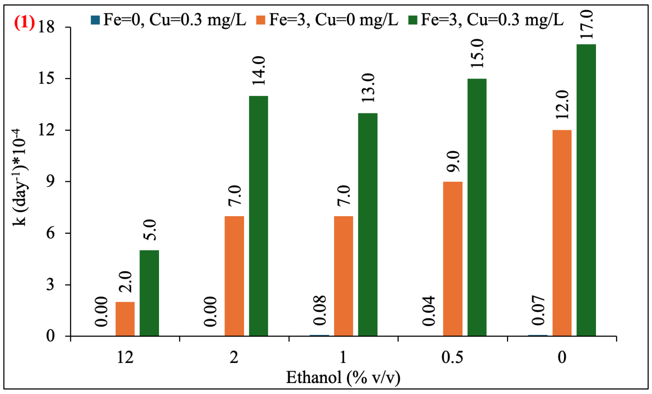
4. Stato di avanzamento della ricerca e principali risultati

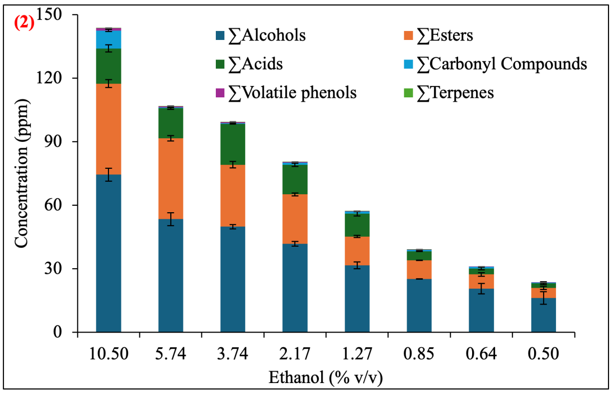
The research so far has yielded interesting results for the following research works: (i) Ethanol, metal and SO_2 interplay: Browning kinetics in model wine (ii) Enhancing ethanol removal from commercial wines: A comparative study of RO, NF, and osmotic distillation (OD).
(i) The aim of this research is to investigate the rate of browning (measured at A420 nm) within a model wine solution. The goal is to understand the mechanisms influencing real-world scenarios, including low- and no-alcohol wines. Model wine samples were prepared by incorporating polyphenols (caffeic acid and catechin) into an aqueous ethanol solution containing transition metals (iron and copper) along with SO_2.
The results showed that model wines without ethanol demonstrated the highest browning rates, both in the presence ($k = 0.0022$ day^{-1}) and absence ($k = 0.0035$ day^{-1}) of SO_2. Notably, ethanol concentration exhibited a negative correlation with kinetic rates in both scenarios: with SO_2 ($r = -0.9317$) and without SO_2 ($r = -0.9667$). The addition of iron and

copper separately resulted in a slight increase in browning, particularly noticeable with iron, whereas adding only copper showed a non-significant impact. However, when iron and copper were combined, a significant synergistic effect was observed, with the rate notably sensitive to copper concentration (Fig1).

(ii) This research aimed to assess the effectiveness of Reverse Osmosis (RO), Nanofiltration (NF), and Osmotic Distillation in achieving ethanol reduction while preserving the characteristics of wine and understanding the mass transfer phenomena during the process. Initially, sets of ethanol removal experiments were conducted using RO (RO-SE, RO-SG, RO-SC) and NF (TS 40, NF99, HL, NF-DK) membranes on hydroalcoholic solutions and commercial white wine (SanCrispino) at a volume reduction factor of 4. Concurrently, a separate set of experiments involving osmotic distillation (OD) with varied feed and stripping parameters was conducted. All experiments were performed at 21 ± 1 °C. The produced wines were compared with the original wine for physicochemical composition (FTIR Bacchus), color profiles (by CIELab instrument), and volatile profiles (36 compounds by GS-MS).

The removal of ethanol from wine ranged from 0.5 to 10.5% v/v, and the reduction efficiency depended on operating conditions such as time, membrane type, and operating temperature. The choice of membrane had a significant impact, leading to various modifications in the wine. Membranes NF-DK, HL, and NF 99 exhibited higher ethanol rejection (95-98%) and significantly higher retention percentages of reducing sugars (91-93%), glucose (about 80%), fructose (89-91%), and tartaric acid (89-91%). On the other hand, membranes RO-SC and RO-SG retained almost 100% of citric and tartaric acid. Color parameters such as intensity, tone, chroma, hue, L^*, and b^* increased, while the a^* value decreased. In the case of OD, the removal of ethanol to 50% showed minor changes in physicochemical properties, whereas wine with 0.5% ethanol exhibited significant alterations in physicochemical parameters. When examining volatile compounds, a general decrease was observed with ethanol removal, especially in dealcoholized wine (0.5%), which showed higher losses of volatile compounds such as esters (89%), volatile alcohols (78%), acids (86%), carbonyl compounds (96%), and volatile phenols (88%) (Fig2). The reduction of total volatile correlated well (r = 0.93) with the reduction of ethanol





Figures: (1) Browning kinetic (k) rate as influenced by varying concentrations of iron and copper across different ethanol concentrations (2) Total aroma (ppm) and chemical classes (ppm) for dealcoholized wines with respect to the original wine (10.5% v/v).

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

Yogesh K, Ricci A, Parpinello GP, Versari A (2024) Dealcoholized Wine: A Scoping Review of Volatile and Non-Volatile Profiles, Consumer Perception, and Health Benefits. Food and Bioprocess Technology. doi: 10.1007/s11947-024-03336-w.

Guanghao W, and Kumar Y (2024) Mechanisms of the Initial Stage of Non-enzymatic Oxidation of Wine: A Mini Review. Journal of Food Science 1750-3841.17038. doi: 10.1111/1750-3841.17038.

Kumar Y, Cassano A, Conidi C, Ricci A, Parpinello GP, Versari A (2024) Evaluating Membrane Behavior to Ethanol-Water Mixtures and Wine: A Comparative Investigation. *LWT:* Food Science & Technology (Under review)

Kumar Y, Ricci A, Guanghao W, Parpinello GP, Versari A (2024) Ethanol, metal and SO_2 interplay: Browning kinetics in model wine. *Food Chemistry: X* (Under review).

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

Joel Armando Njieukam (email: joelarmando.njieuk2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof.ssa Rosalba Lanciotti; Co-tutor: Dott. Lorenzo Siroli e Claudio Dall'Agata

1. Stato dell'arte

La ricerca di soluzioni alternative per sostituire i materiali sintetici di origine fossile in molti settori rappresenta un tema di grande attualità. Infatti, negli ultimi anni, vari studi sono stati fatti per valutare l'utilizzo di risorse rinnovabili e biodegradabili quali, i biopolimeri come risposta a tale sfida. Tra i biomateriali, la cellulosa di origine microbica, prodotta principalmente da batteri acetici, ha suscitato un enorme interesse grazie alle sue eccellenti e peculiari proprietà fisico-chimiche, l'abbondanza in natura, la non tossicità, la purezza e la sua natura ecosostenibile (biodegradabile e biocompatibile) le quali, hanno favorito la sua applicazione in molti settori industriali, tra cui quello degli imballaggi alimentari (Yanti et al., 2021). Tuttavia, l'elevato costo di produzione, le rese in cellulosa non sempre elevate e l'assenza di proprietà funzionali quali attività antimicrobiche o antiossidanti ne ostacolano notevolmente l'espansione. Sebbene siano stati fatti tanti studi sullo sviluppo di nuove strategie per una produzione più efficiente e sostenibile di cellulosa, ulteriori ricerche sono necessarie al fine di ottimizzare le condizioni di produzione da parte di ceppi di batteri acetici appartenenti al genere *Komagataeibacter*. Per aumentare la sostenibilità della produzione di cellulosa batterica e favorirne l'impiego a livello industriale, sono necessari sforzi sostanziali che includono l'ottimizzazione dei protocolli di fermentazione, lo sviluppo di nuovi reattori, l'utilizzo di enzimi (Ullah et al., 2015), la selezione di ceppi più performanti (Buldum G, Bismarck A & Mantalaris A, 2018), l'utilizzo di substrati a basso costo (Jozala et al., 2015). Inoltre, tali biomateriali destinati a venire a contatto con gli alimenti possono essere funzionalizzati attraverso l'incorporazione di componenti ad attività antimicrobica o antiossidante che vengono 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 (Almasi, Oskouie & Saleh, 2021).

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Jozala AF, Pértile RAN, Dos Santos CA, Santos-Ebinuma V, Seckler MM, Gama FM, Pessoa AJ (2015) Bacterial cellulose production by *Gluconacetobacter xylinus* by employing alternative culture media, *Appl Microbiol Biotechnol*. 99:1181–1190.

Ullah MW, Ul-Islam M, Khan S, Kim Y, Park JK (2015) Innovative production of bio-cellulose using a cell-free system derived from a single cell line, *Carbohydr. Polym.* 132:286-294.

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3. Obiettivi

Il progetto di ricerca può essere suddiviso nelle attività riepilogate nel diagramma di Gantt in **Tabella 1** e descritte di seguito:

A1) **Ricerca bibliografica**

A2) **Produzione film bio-based di cellulosa batterica:** isolamento e caratterizzazione di ceppi di batteri acetici produttori di cellulosa a partire da matrici alimentari quali kombucha e kefir (A2.1), ottimizzazione delle rese in cellulosa batterica da parte dei ceppi più performanti in mezzi sintetici attraverso modulazione di diverse variabili culturali (A2.2), utilizzo di scarti e sottoprodotti dell'industria alimentare (melasso, siero, trebbie di birra) come substrati di crescita dei ceppi selezionati al fine di ridurre i costi di produzione (A2.3).

A3) **Selezione di antimicrobici naturali per la funzionalizzazione di cellulosa batterica:** selezione di antimicrobici naturali tra cui oli essenziali ad elevata attività antimicrobica nei confronti di microrganismi degradativi e patogeni di prodotti alimentari e, in particolare, di prodotti ortofrutticoli (A3.1).

A4) **Attivazione e caratterizzazione della cellulosa batterica**: definizione di protocolli per la produzione di biopolimeri integrati con antimicrobici naturali (A4.1) e caratterizzazione del film attivato con valutazione delle

proprietà meccaniche e termiche e valutazione dell'attività antimicrobica *in-vitro* (A4.2).
A5) **Applicazione su cartone ondulato e valutazione dell'effetto su prodotto confezionato**: La cellulosa batterica attivata sarà accoppiata a imballaggio in cartone ondulato secondo il protocollo definito (A5.1) e verrà valutato l'effetto del packaging attivo sulla shelf-life, sicurezza e proprietà organolettiche di un prodotto ortofrutticolo ad alto valore (A5.2) definito insieme al partner industriale "CONSORZIO BESTACK".
A6) **Preparazione della tesi, di articoli, poster 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	***Produzione film bio-based di cellulosa batterica***			■	■	■	■	■	■	■	■	■							
	1) Isolamento, screening e caratterizzazione batteri acetici			▒	▒	▒													
	2) Ottimizzazione performance e definizione protocolli di produzione					▒	▒	▒	▒	▒	▒	▒							
	3) Produzione di cellulosa batterica su scarti e sottoprodotti alimentari					▒	▒	▒	▒	▒	▒	▒							
A3	***Selezione di antimicrobici naturali per la funzionalizzazione di cellulosa batterica***				■	■	■	■	■	■	■	■	■						
	1) Screening, caratterizzazione e selezione di antimicrobici naturali				▒	▒	▒	▒	▒										
A4	***Attivazione e caratterizzazione della cellulosa batterica***									■	■	■	■	■	■				
	1) Definizione di protocolli per l'integrazione di antimicrobici naturali									▒	▒	▒	▒						
	2) Caratterizzazione antimicrobica e tecnologica del film attivo											▒	▒	▒	▒				
A5	***Applicazione su cartone ondulato e valutazione dell'effetto su prodotto confezionato***														■	■	■	■	
	1) Definizione del protocollo per il rivestimento di cartone ondulato con film bio-based														▒	▒	▒		
	2) Valutazione dell'effetto del packaging attivo sulla shelf-life di prodotti confezionati															▒	▒	▒	
A6	***Preparazione della tesi, di articoli, poster e presentazione orale***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Stato di avanzamento della ricerca e principali risultati

Il presente progetto di dottorato 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 prodotta da ceppi di batteri acetici selezionati, e funzionalizzato grazie all'addizione di composti antimicrobici naturali in grado di ridurre la proliferazione microbica e di incrementare la shelf-life di prodotti ortofrutticoli confezionati.
Nel primo anno, le attività si sono concentrate sull'isolamento, identificazione e caratterizzazione di ceppi di batteri acetici produttori di cellulosa batterica a partire da alimenti fermentati quali kombucha e kefir. In particolare, sono stati isolati 62 ceppi di presunti batteri acetici di cui 20 hanno mostrato una buona capacità di produrre cellulosa su terreni di coltura sintetici. Tutti i ceppi produttori di cellulosa batterica sono stati identificati come appartenente al genere *Komagataeibacter*. Il successivo screening ha permesso di selezionare i 5 ceppi con le più alte rese in cellulosa batterica su mezzi di coltura sintetici da impiegare nelle fasi successive del progetto: *Komagataeibacter hansenii* 6DB, *Komagataeibacter rhaeticus* LM2, LM4, SM1 e *Komagataeibacter xylinus* DSM 2004 (usato come ceppo di riferimento).
A seguito della ricerca bibliografica, diversi parametri colturali (terreno di coltura, fonte di carbonio e relativa concentrazione) sono stati valutati con lo scopo di definire le condizioni ottimali per l'ottenimento di elevate rese in cellulosa usando terreni sintetici. Altri parametri quali, la temperatura, il tempo di incubazione, la quantità di inoculo, sono stati mantenuti costanti. I 5 ceppi sono stati, inizialmente, testati su 5 terreni commerciali (**Tabella 2**) incubandoli a 28 °C per 10 giorni in condizioni statiche. Oltre alla produzione di cellulosa, altri parametri quali il carico cellulare, il pH, la produzione di acidi organici e, in particolare, di acido gluconico, il consumo di zuccheri sono stati monitorati durante il periodo di incubazione.

GET	HSM	GYC	GY	AAM
5.0% glucose, 0.1% yeast extract, 0.7% peptone, 0.8% disodium phosphate, 1.4% (v/v) ethanol	5.0% glucose, 0,5%peptone, 0.5% yeast extract, 0.27% disodium phosphate, 0.115% citric acid	5.0% glucose, 1.0% yeast extract, 2.0% calcium carbonate	5.0% glucose, 1.0% yeast extract	5.0% glucose, 1.5% peptone, 0.8% yeast extract, 0.5% (v/v) ethanol, 0.3% (v/v) acetic acid

Tabella 2: Composizione terreni di coltura (g/L).

Come evidenziato in **Fig. 1**, le maggiori rese in cellulosa sono state ottenute usando i terreni AAM e GYC per tutti i ceppi considerati. La composizione del terreno di coltura è risultata fondamentale per l'ottenimento di buone rese: il carbonato di calcio presente nel brodo GYC ha impedito il calo di pH dovuto alla produzione e accumulo di acidi

organici durante i processi fermentativi. Dall'altra parte, l'acido acetico ed etanolo aggiunti al terreno AAM hanno consentito una maggiore produzione di cellulosa in quanto questi rappresentano fonti addizionali di carbonio favorendo, quindi, l'utilizzo del glucosio per la produzione di cellulosa, principalmente. Anche sul terreno GY, una buona resa in cellulosa batterica è stata ottenuta anche se la rapida acidificazione del mezzo ha, considerevolmente, limitato le performance dei ceppi considerati. In tutti i campioni, è stata osservata una correlazione positiva tra il contenuto di acido gluconico e la diminuzione del pH e una correlazione negativa tra accumulo di acidi organici e resa in cellulosa.
Le successive analisi sono state svolte impiegando solo il terreno GY e facendo variare la fonte di carbonio (glucosio, mannitolo, fruttosio, saccarosio, lattosio e glicerolo) e la relativa concentrazione (1, 2, 3 e 5%). Anche se i più alti livelli di produzione di cellulosa sono stati ottenuti usando glucosio, fruttosio e mannitolo come fonte di carbonio e a concentrazioni pari a 3 e 5%, è stato evidenziato un comportamento ceppo-dipendente e, in molti casi, non è stata osservata linearità tra l'incremento di cellulosa prodotta e l'aumento della concentrazione zuccherina.

Al fine di ridurre il costo di produzione e nell'ottica di una produzione ecosostenibile di cellulosa, è stata valutata la capacità dei 5 ceppi di batteri acetici selezionati, di sviluppare e produrre cellulosa su differenti sottoprodotti dell'industria agroalimentare utilizzati come substrato di crescita (melasso, trebbie e lievito esausto dall'industria birraria e siero derivante dalla lavorazione del formaggio).

I substrati naturali sono stati utilizzati tal quali o con aggiunta di soluzione tampone. Oltre alla determinazione della resa in cellulosa, sono stati valutati la cinetica di crescita, la produzione di cellulosa batterica, il pH e il consumo di carboidrati in 4 tempi diversi: il giorno dell'inoculo (T0), dopo 4 (T4), 7(T7) e 10 (T10) giorni di incubazione. In assenza di soluzione tampone è stata registrata una limitata produzione di cellulosa a causa della rapida diminuzione di pH dopo 4 giorni di incubazione, soprattutto nei campioni di trebbie e di melasso. Utilizzando, invece, una soluzione di tampone fosfato (pH 5.8) è stato possibile tenere sotto controllo il pH ed incrementare la resa in cellulosa.
Infatti, seppur con diverse cinetiche, tutti i ceppi considerati sono stati in grado di produrre cellulosa e sviluppare incrementando costantemente il carico cellulare nei 3 substrati analizzati (Fig. 2A, Fig. 2B e Fig. 2C). Anche in questo caso, la cinetica di crescita è risultata ceppo dipendente. Inoltre, non è stata osservata una correlazione diretta tra il consumo di zuccheri e la resa in cellulosa.
La produzione di cellulosa è stata osservata su tutti i substrati considerati seppure con rese molto variabili e dipendenti dal ceppo. Su trebbie tutti i ceppi sono stati in grado di produrre cellulosa batterica dando rese maggiori di 1 g/L (Fig. 2C). Per quanto riguarda il siero (Fig. 2B) le maggiori rese in cellulosa sono state registrate dai ceppi DSM 2004 e LM4 (1,68 e 1,59 g/L, rispettivamente). Anche su melasso, questi due ceppi hanno dato le più alte rese: 2,88 g/L per DSM 2004 e 1,70 g/L per LM4 (Fig. 2A). Complessivamente, il melasso ha dimostrato di essere la matrice più addata per una produzione efficiente di cellulosa e inoltre 7 giorni di incubazione sembrano sufficienti per ottenere le più alte rese in cellulosa nelle condizioni adottate.
Le analisi condotte, finora, hanno permesso di ottenere cellulosa batterica di elevata purezza sia su terreni sintetici che su substrati naturali. I prossimi step riguarderanno l'individuazione di composti antimicrobici naturali e i protocolli per la loro integrazione nella cellulosa ottenuta al fine produrre un packaging attivo ed ecosostenibile.

Fig. 2A

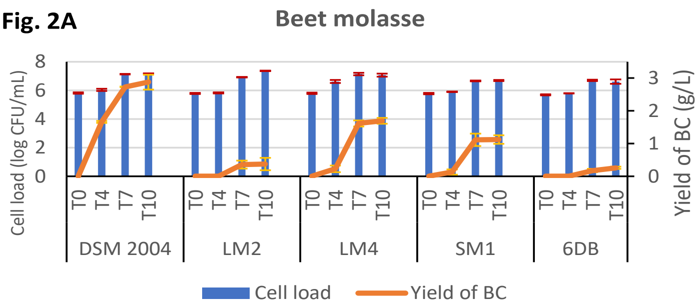


Fig. 2B

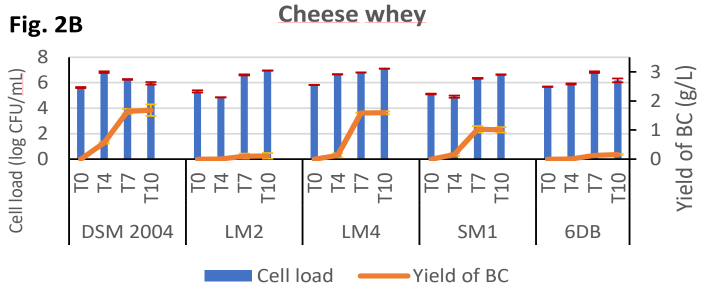
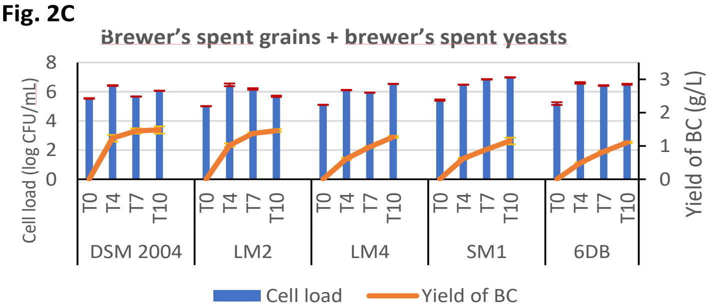


Fig. 2C



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

Njieukam JA, Siroli L, Ciccone M, Braschi G, Gottardi D, Patrignani F, Lanciotti R (2024) Optimization of the production of bacterial cellulose by *Komagataeibacter* spp. on brewer's spent grains. 28th International ICFMH Conference Food Micro (Spain, 8-11 July).

Njieukam JA (2023) Development of an active bio-based coating for corrugated cardboard with waterproofing and antimicrobial properties. 27° Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology (Portici, 13-15 September).

Njieukam JA, Siroli L, Ciccone M, Gottardi D, Patrignani F, Lanciotti R (2023) Selection of bacterial cellulose-producing acetic acid bacteria from kombucha tea and evaluation of the influence of culture conditions on bacterial cellulose production. 7th International Conference on Microbial Diversity. (Parma, 26-29 September).

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

Giulia Salvatori (email: giulia.salvatori5@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum*-Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof.ssa Maria Teresa Rodriguez Estrada; Co-tutor: Dott. Dario Mercatante.

1. Stato dell'arte

Negli ultimi anni, le limitazioni legislative all'utilizzo di lipidi ricchi in acidi grassi *trans* (TFA) e saturi (SFA), 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, più salutari ed in grado di apportare agli alimenti caratteristiche tecnologico-sensoriali simili o persino migliorative rispetto a quelle generalmente fornite da SFA e TFA come plasticità, sapore, *texture, mouthfeel* (Li et al., 2022). Una delle principali alternative oggetto di ricerca negli ultimi anni è rappresentata dagli organogels (OGs), una classe di gel in cui una fase organica liquida (come, ad esempio, un olio vegetale), viene immobilizzata e intrappolata all'interno di una rete tridimensionale termo-reversibile tramite l'impiego di organogelatori non-trigliceridici (Bascuas et al., 2020). Tale meccanismo di strutturazione 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 (idrogenazione ed interesterificazione), ampiamente diffusi nell'industria alimentare. Inoltre, diversi studi hanno valutato positivamente gli OGs come sistemi "*carrier*" per il trasporto di composti bioattivi (lipofili e/o idrofili) per i quali potrebbero rappresentare un sistema in grado di aumentare la loro dispersibilità nella matrice alimentare e biodisponibilità nel tratto gastro-intestinale, controllandone il rilascio, proteggendoli dall'ossidazione e dalla perdita di funzionalità (Orhan & Eroglu, 2022). In questo contesto, i sottoprodotti della filiera agro-alimentare si presentano come una notevole fonte di composti bioattivi ad elevato valore biologico con comprovate proprietà antiossidanti, antimicrobiche e salutistiche. I principali sottoprodotti incidenti a livello nazionale ed europeo sono quelli derivanti dalla filiera dell'olio di oliva, del pomodoro, cerealicola, delle patate, ecc., i quali sono ricchi di composti bioattivi come carotenoidi, composti fenolici, beta-glucani (Fritsch et al., 2017). L'inclusione di tali composti nella formulazione di OGs consentirebbe la valorizzazione di suddetti sottoprodotti, aderendo a concetti di economia circolare, *green economy* e sviluppo sostenibile. Tra gli organogelatori (o agenti gelanti), le cere sono considerate molto promettenti in quanto presentano un'elevata capacità di legare e strutturare l'olio a basse concentrazioni di utilizzo; inoltre, sono ampiamente diffuse, di grado alimentare, poco costose e possono essere impiegate facilmente con approcci di "dispersione diretta" nell'olio vegetale liquido per la produzione di OGs (Silva et al., 2021). Nella realizzazione di OGs è altresì opportuno considerare che la forza del gel varia in funzione di diversi fattori ed è strettamente correlata alla concentrazione di organogelatore/i impiegata per realizzarlo (Principato et al., 2021). Tuttavia, l'aumento della concentrazione di cera nella formulazione potrebbe influenzare negativamente le caratteristiche sensoriali degli OGs e quindi del prodotto alimentare finale nel quale si intende impiegarli. Ne consegue che una delle sfide principali per la formulazione di tali strutture lipidiche consiste nel migliorare le proprietà meccaniche impiegando al contempo una concentrazione ridotta di agente gelante. A tal proposito, le fibre alimentari sono ben note per le loro eccezionali proprietà come agenti addensanti in sistemi a base acquosa, nonché per la loro capacità di aumentare il profilo nutrizionale dei prodotti finali (Principato et al., 2021). La presenza di gruppi idrossilici liberi a livello molecolare che presentano una buona affinità sia per fasi idrofile che lipofile, rende le fibre vegetali componenti potenzialmente impiegabili come agenti strutturanti e/o stabilizzanti per la formulazione di OGs. In base alle attuali conoscenze, finora solo Principato et al. (2021) hanno investigato l'impiego di fibra alimentare (fibra di bambù, ad elevata concentrazione di fibre insolubili) per realizzare OGs tramite approcci di dispersione diretta nella fase oleosa, dove è stato evidenziato che la loro inclusione può avere effetti sulla struttura e sulla stabilità complessiva dei gel, anche in presenza di fluttuazioni di temperatura. Ad oggi, non sono presenti studi finalizzati alla realizzazione di OGs utilizzando sottoprodotti come fonti di agenti gelanti, di composti bioattivi e come agenti strutturanti e/o stabilizzanti la struttura del *network* formato dagli organogelatori.

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3. Obiettivi

Il presente progetto di ricerca si propone di sviluppare OGs con adeguate caratteristiche di stabilità chimico-fisica, ossidativa, proprietà nutrizionali, sensoriali e di *texture*, attraverso la valorizzazione e l'impiego di diversi sottoprodotti della filiera agro-alimentare come fonte di composti bioattivi, organogelatori e agenti strutturanti/stabilizzanti al fine di fornire una soluzione innovativa e sostenibile ai grassi saturi e/o *trans* attualmente impiegati nelle formulazioni alimentari. L'impiego di tali OGs per lo sviluppo di prodotti alimentari innovativi (convenzionali e/o *plant-based*) permetterebbe di rallentare e/o ridurre processi ossidativi e idrolitici a carico della frazione lipidica e proteica, ottenendo prodotti sicuri, stabili da un punto di vista ossidativo e con almeno le stesse, se non superiori, caratteristiche di conservabilità, organolettiche e nutrizionali. Per il raggiungimento degli obiettivi del progetto della tesi di dottorato il lavoro è stato suddiviso nelle seguenti attività secondo il diagramma di Gantt riportato in Tabella 1:

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 degli ingredienti***	■	■	■	■	■	■	■	■										
	1) Caratterizzazione dell'olio di girasole alto oleico (HOSO)	▒	▒	▒	▒	▒	▒	▒											
	2) Essicamento e caratterizzazione delle trebbie di birra (BSG)	▒	▒	▒	▒	▒	▒	▒											
	3) Essiccamento e caratterizzazione delle bucce di pomodoro				▒	▒	▒	▒	▒										
A3)	***Formulazione di OGs e valutazioni preliminari***	■	■	■	■	■	■	■	■	■	■	■							
	1) Valutazione del metodo di produzione per dispersione diretta	▒	▒																
	2) Valutazione degli effetti dovuti all'eventuale aggiunta di un agente surfattante e/o strutturante		▒	▒	▒														
	3) Inclusione di composti bioattivi, valutazione della loro ritenzione e delle loro proprietà					▒	▒	▒	▒	▒	▒	▒							
A4)	***Analisi della stabilità ossidativa e chimico-fisica degli OGs***						■	■	■	■	■	■	■	■					
A5)	***Analisi delle proprietà meccaniche, reologiche e termiche degli OGs***						■	■	■	■	■	■	■	■					
A6)	***Formulazione e studio di shelf-life di alimenti innovativi (convenzionali e/o plant-based)***											■	■	■	■	■	■		
A7)	***Scrittura e redazione della tesi di dottorato, di articoli scientifici e/o di poster e comunicazioni orali***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Stato di avanzamento della ricerca e principali risultati

Al fine di perseguire gli obiettivi del progetto di ricerca è stata effettuata un'ampia ricerca bibliografica finalizzata alla valutazione e messa a punto del lavoro, in funzione degli obiettivi prefissati e delle lacune esistenti ad oggi nel campo degli OGs. Come fase liquida per la costituzione degli OGs è stato selezionato olio di girasole alto oleico (HOSO) in virtù della sua elevata concentrazione in acidi grassi monoinsaturi (MUFA), in particolare acido oleico (C18:1 *n*-9), che lo rende meno sensibile a fenomeni ossidativi rispetto a oli vegetali caratterizzati da un maggior contenuto in acido linoleico (C18:2 *n*-6) come componente principale. Come organogelatore è stata selezionata una cera vegetale, nota come cera di crusca di riso (RBW), un sottoprodotto della produzione dell'olio di riso. In seguito, si è proceduto con la caratterizzazione delle materie prime che verranno utilizzate per la costituzione di OGs. In particolare, sono state eseguite analisi su HOSO finalizzate a caratterizzarne il profilo in acidi grassi (FA) e a valutarne lo stato di ossidazione, i cui risultati sono riportati in Tabella 2.

Dai dati riportati si evince che i valori relativi al PV (1,16 mEq O_2/kg olio) e agli acidi grassi principalmente presenti in tale materia prima (80,25% acido oleico, 10,88% acido linoleico, 4,32% acido palmitico e 2,76% acido stearico) sono in accordo con i risultati riscontrati, per HOSO raffinato, da Romano et al. (2021). Anche i dati relativi ai principali composti volatili (VOCs), composti polari, dieni coniugati e indice di stabilità ossidativa (OSI time) sono in linea con quelli riscontrati da studi precedenti condotti da Petersen et al. (2012) e Merrill et al. (2008). Nel complesso, la caratterizzazione completa della materia prima di partenza, permetterà di valutare nel dettaglio le caratteristiche degli OGs futuri e di monitorarne la stabilità (chimico-fisica, ossidativa e idrolitica) durante la conservazione.

Tabella 2. Risultati preliminari caratterizzazione HOSO: analisi di stabilità ossidativa e profilo in acidi grassi (FAME).

Status ossidativo e idrolitico				**FAME (%)**	
Parametri	**Risultati**	**VOCs**	**Area del picco x 10^3**	**FA**	**% area**
PV (mEq O_2/kg olio)	1,16±0,06	Eptano	5929	C16:0	4,32±0,04
Dieni e trieni coniugati		Ottano	5112	C18:0	2,76±0,04
268	0,37±0,00	2-propenale	1099	C18:1 *n*-9	80,25±0,12
264	0,59±0,00	Pentanale	362	C18:2 *n*-6	10,88±0,04
232	2,57±0,01	Esanale	1639	SFA	8,08±0,07
ΔK	0,09±0,00	1-pentanolo	243	MUFA	80,62±0,11
OSI time (h)	15,22±0,14	(*E*)-2-eptenale	250	PUFA	11,28±0,04
Composti polari (%)	8,42±1,63	Acido acetico	859	PUFA *n*-6	10,95±0,04
		Acido butanoico	50	PUFA *n*-3	0,06±0,01
		2,4-decadienale	1557		
		Acido esanoico	482		
		Acido eptanoico	201		
		Acido ottanoico	622		
		Acido nonanoico	527		

Risultati preliminari dei metodi di produzione per dispersione diretta: sono state effettuate prove preliminari a diverse concentrazioni di RBW provenienti da due diversi fornitori (RBW-1 e RBW-2). In particolare, sono state testate le concentrazioni (p/v) del 3% per RBW-1 e del 3% e 4% per RBW-2. Nel caso di RBW-2 è stato necessario eseguire una prova al 4% in quanto a concentrazioni inferiori non si osservava formazione di gel. Sono stati quindi preparati 10 g di OGs per dispersione diretta a 80°C, sia tramite agitatore magnetico scaldante sia a bagnomaria (WB) in costante agitazione. Gli OGs sono stati trasferiti in provette Sovirel da 5 mL e in tubi Eppendorf da 2 mL, precedentemente pesate. Tutti i campioni sono stati lasciati a temperatura ambiente per un'ora e successivamente conservati in frigorifero per 48 ore. La *oil loss* (OL%) e la *oil binding capacity* (OBC%) dei gel ottenuti, sono stati valutati come media di 3 repliche, sottoponendo gli OGs a 4 cicli di centrifugazione (30 min a 14.000 rpm) come descritto da Blake e Marangoni (2015). I valori di OL sono stati calcolati come percentuale di olio decantato dopo il primo (OL_1) e l'ultimo ciclo di centrifugazione (OL_4). I risultati delle prove sopra descritte sono riportati nella Tabella 3.

Tabella 3. OL e OBC di OGs formulati con cera di crusca di riso di due diversi fornitori (RBW-1 e RBW-2) in funzione del metodo di produzione (piastra scaldante o bagnomaria).

OGs	**Piatra scaldante**	**Bagnomaria**	**OL_1**	**OL_4**	**OBC (%)**
3% RBW-1	X		3,47±0,54**	14,90±1,94**	85,55±1,88*
3% RBW-1		X	50,81±5,52*	64,45±5,08*	37,48±4,92**
4% RBW-2	X		67,49±0,45	74,80±0,22^{++}	28,19±0,22^{+}
4% RBW-2		X	68,70±2,09	76,90±0,06^{+}	26,17±0,06^{++}

*-**, +-++ medie statisticamente significative (test t-Student p<0,01) tra i campioni formulati con la stessa tipologia di cera e diverso metodo di produzione (piastra scaldante o bagnomaria).

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

Presentazione del progetto nell'ambito di un ECS meeting PNRR Spoke 4, in data 19/12/2023: "Development of alternative lipid structures containing bioactive compounds from agri-food by-products and their application for innovative and sustainable foods formulation".

Salvatori G, Mercatante D, Rodriguez-Estrada MT "Agri-food by-products as ingredients for the formulation of sustainable food organogels". *Review in corso di stesura sulla rivista Foods.*

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

Giovanni Selva (email: giovanni.selva2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: I
Tutor: Francesco Capozzi; Co-tutor: Stanislav Sykora

1. 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 PDO 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.

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3. Goals

An application 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.

Tabella 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. State of research progress and main results

During the starting period of this PhD, I did a bibliographical research on Low Resolution NMR applications in food science, which cover many categories, such as the distinction and quantification of different components or phases (solid/liquid, muscle/fat…), the monitoring of food ageing (stocked food) or ripening (cheese, ham…), and many more. The bibliographic research has the purpose to select some foodstuff on which to focus my studies. Cereals, meat, and even meat substitutes seem to be the most important food today and in the next future in the global industry market. In particular, NMR relaxometry seems to be a promising tool to contribute increasingly within the field of meat processing, not only in the elucidation of dynamic changes during cooking, salting, storage, but also to evaluate the quality attributes.
In the while, the main activities focused on the development of a method to classify samples of Pecorino Sardo cheese through the evaluation of NMR relaxometry data. The classification is carried out in function of the ripening time. Also, we explored the possibility to discriminate between PDO (Protected Denomination of Origin) samples and those which are not prepared according to the procedural guidelines, which forbid the use of heat-treated milk. NMR relaxometry measurements are sensitive to those conditions, such as ripening or heat treatment, that affects the properties related to molecular dynamics. For this reason, it seems to be a perfect experimental technique for this purpose. The project has been proposed by the Italian Inspectorate for food frauds repression (ICQRF), which also provided us with some samples for the preliminary studies.
Samples have been cored and measured on a 20 MHz Bruker Minispec relaxometer, where the CPMG sequence has been executed in order to acquire the NMR transverse relaxation signal. Then, a software tool has been developed to evaluate the raw data, and to perform the Inverse Laplace Transform on the relaxation curves. The ILT is a fitting routine which carries out the distribution of the components relaxation times. We found that the discrete ILT, which consists in a multi-exponential fitting, returned results which are most sensitive to those properties respect whom we want to classify the samples. In fact, either the relaxation time and the intensity of the components obtained with the fitting describe the changing in the samples ripening time and milk coagulation temperature. Then, we followed a multivariate statistical approach to analyse the fitting parameters which led to successfully distinguish fresh and ripened samples and to discriminate suspicious samples that may not be prepared according to the regulation.
So, the method seems to be promising to detect counterfeited products, even though we still need to refine it. Currently, we are acquiring data from more reference samples in order to expand our database, which will strengthen the statistical analysis. Also, I am learning the instrument programming language for writing new sequences in order to explore the

possibilities to optimize the method workflow. At last, I expect to publish an article on this study, whose writing is currently in progress. Next, we would like to explore new approaches for the classification, based on machine learning algorithms.

Improvement of quality and nutritional value of foods using natural compounds and mild biotechnologies

Fatemeh Shanbeh Zadeh (email: fatemeh.shanbehzade2@unibo.it)
Dipartimento di Scienze e Tecnologie agro-alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof.ssa Lucia Vannini; Co-tutor: Prof. Fausto Gardini

1. Stato dell'arte

The need to guarantee an adequate shelf life to foods often relies on heat treatments, which can partially impair their nutritional value, or debated preservatives which are negatively perceived by consumers. Natural alternatives can be based on ingredients derived from plants, e.g essential oils or plant extracts, through green biotechnologies, e.g. based on tailored microbial fermentations, and non-thermal technologies.

Cold plasma technology is increasingly recognized as an innovative non-thermal approach in the realms of food decontamination, processing, storage, and agriculture. This innovative technology excels in generating reactive species with minimal energy input, effectively enhancing the safety and quality of a broad spectrum of food products with limited effects on their nutritional or sensory properties. Cold plasma is not only eco-friendly and cost-effective but also do not require the use of chemicals, making it an ideal choice for improving food quality. The technology is distinguished by the atmospheric pressure within its reactor, which can be either high/atmospheric or low/vacuum. High-pressure or atmospheric cold plasma has been notably successful in controlling microbial contamination and preserving the post-harvest quality of fresh fruits and vegetables, thus ensuring the safety and extending the shelf life of produce without compromising on quality (Saedi *et al*., 2024; Yudhistira *et al*., 2023). In previous studies, the application of cold plasma treatment effectively targeted microbial reduction, significantly inactivating pathogens such as *Escherichia coli* O157:H7, *Enerococcus faecalis*, and *Aspergillus niger* in foods like carrots, white radish, dried figs, and dried peaches, with a notable efficiency ($p < 0.05$). *E. coli* showed a higher susceptibility to cold plasma, with inactivation primarily resulting from DNA damage and cellular leakage. The treatment maintained the sensory and physicochemical qualities of these products, including their texture, colour, and water content, without significant changes ($p > 0.05$). This suggests that atmospheric cold plasma technology can be a valuable tool in reducing microbial threats in perishable and frequently consumed products such as vegetables and dried fruits, while preserving their essential properties (Yarabbi *et al*., 2023). Subsequent studies have shown to significantly impact the nutritional and phytochemical composition of barnyard and pearl millet, effectively reducing microbial contamination and enhancing grain safety and shelf life. Barnyard millet responds well to cold plasma, exhibiting increases in fats, antioxidants, and essential vitamins (B3, B9, B12), alongside decreases in antinutrients like tannin and phytic acid, and an enrichment of iron content. These changes indicate potential of cold plasma to improve the nutritional value of millets (Charu *et al*., 2024).

Traditional fresh produce decontamination methods, involving extensive use of chemicals and water, present considerable environmental and cost concerns. These techniques require a lot of energy and water, leading to potential chemical residues on foods, which can impact both their safety and sensory quality. Cold plasma technology emerges as a sustainable alternative, capable of effectively reducing microbial contamination without heavy reliance on water, chemicals, or high energy consumption. This innovative approach aligns with increasing consumer demands for minimally processed, residue-free foods while potentially improving the sensory qualities of fresh produce. This research aims to assess the potentials of cold plasma in offering a solution to the limitations of traditional decontamination methods, enhancing food safety and the sensory attributes of fresh produce.

2. Bibliografia

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3. Obiettivi

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

A1) **Review of the scientific literature** in the field of emerging non thermal technologies as alternatives to heat treatments, sanitizers and chemical preservatives in relation to different food products, and agri-food waste and by-products as a source of bioactives.

A2) **Assessment of the potential of a selected non-thermal technology** in assuring quality and safety of target products

A3) **Functional characterization of the by-products:** selection of different by-products and testing for some bioactivities, e.g. antioxidant, prebiotic towards some commercial probiotic bacteria, antimicrobial/antifungal against foodborne pathogenic and spoilage microorganisms.

A4) **By-products valorisation through microbial fermentation**: strains of yeasts and lactic acid bacteria will be used to ferment the by-products in order to enhance their bioactivities and use them as functional food ingredients.

A5) **Definition of experimental design** to reformulate selected food products and recalibrate the processes by exploiting the most promising biotechnological solutions outlined by the previous activities. Also the interactive effects with non-thermal treatments, e.g. cold plasma, high pressure, will be assessed to obtain foods with enhanced quality and safety features, prolonged shelf life, improved nutritional and functional features.

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

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)	***Literature review***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
A2)	***Non-thermal technology assessment***	■	■	■	■	■	■	■											
	1)Experimental Setup and Optimization	░	░	░	░														
	2)Microbiological Assessment and Quality Study		░	░	░	░	░	░											
A3)	***By-products characterisation***						■	■	■										
A4)	***By-products valorisation through microbial fermentation***							■	■	■	■	■	■	■	■				
A5)	***Food reformulation and process recalibration***											■	■	■	■	■	■	■	
A6)	***Research Dissemination and Documentation***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Stato di avanzamento della ricerca e principali risultati

This overall aim of this PhD thesis is the development of biotechnological approaches to reformulate food products through new ingredients and recalibrate processes, especially heat treatments. This goal is aimed at innovating traditional foods into products with enhanced quality and safety features, prolonged shelf life, improved nutritional value, organoleptic characteristics and functional features. The activities carried out during the first year were focused on Plasma Activated Water (PAW) to investigate its effectiveness as a sanitizing agent for ready-to-eat vegetables to be used as an alternative to the chemical sanitizers. Baby leaf lettuce was chosen as target product, and comparative treatments included washing the vegetables for 2, 10, and 20 minutes with PAW, alongside a control of a 2-minute wash in 100 ppm sodium hypochlorite solution, mirroring industrial standards. The effects of different treatments on microbiological quality, chlorophyll and volatile compounds were detected immediately after treatments and over a 12-day storage period (4 °C until day 4 and 15 °C until day 12).

The cell counts of coliform bacteria, total mesophilic count, *Pseudomonas* spp., total psychrotrophic bacteria were analysed immediately after washing resulting in relevant reductions in particular for PAW treatment for 10 minutes. Such a condition was effective particularly against *Pseudomonas* spp. and coliform which were not detectable, thus corresponding to ~ 2 Log reductions (fig.1a). On the other hand, an increase in bacterial viability of the surviving microbiota was observed during the storage phase, particularly after the thermal abuse at 15°C (fig.1b). However, this increase was mitigated in samples treated with PAW, which exhibited lower bacterial proliferation compared to the untreated control samples. Total mesophilic bacteria attained a level of 7 Log CFU/g after 6 days, while such a value, which is usually considered a threshold value for shelf-life, was achieved after ~ 10 days in the PAW-washed produce (fig.1b). Moreover, a 10-minute PAW treatment resulted in a near 1.5-logarithmic-unit reduction in coliform bacteria counts, which was observed after a period of 6-7 days (data not shown). In contrast, a 2-minute treatment with sodium hypochlorite resulted in a 2-logarithmic-unit reduction in bacterial counts, also observed after the same period. Consistency in microbial response was further evidenced by the behaviours of psychrotrophic bacteria and *Pseudomonas* spp., which paralleled the response patterns of mesophilic bacteria. Such results underscore a generalized effectiveness of the treatments across different microbial taxa, with discernible impacts manifesting within a week of application. These findings suggest that PAW could be a viable alternative to conventional decontaminants for enhancing the microbial safety of ready-to-eat baby leaf vegetables.

For the physicochemical assessment of baby leaf vegetables treated with PAW and hypochlorite, Gas Chromatography coupled with mass spectrometry and solid phase micro-extraction (GC-MS/SPME) was used to analyse the volatile compounds. Moreover, chlorophyll content was measured to gauge freshness throughout storage. These tests are key to evaluating the effects of the treatments on vegetable quality. To clarify the differences between treatments, Principal

Component Analysis (PCA) of the volatile compounds was conducted (fig.2). The samples, along with their corresponding molecules, were mapped onto factors which accounted for 66.83% of the total data variance, with the first principal component (PC 1) explaining 47.13% and the second (PC 2) capturing 19.70%. In the PCA analysis, the concentration of 3,3,5-Trimethylcyclohexyl acetate was initially higher in baby leaf samples after the PAW washing for durations of 2 and 10 minutes. However, this compound's concentration decreased after one and four days of storage. Similarly, the levels of cis-2-Pentenol were also higher immediately following a 2 and 20-minute PAW treatment, but these levels subsequently reduced over the storage period of one day and four days. This trend suggests that while PAW washing results in initial higher content of certain volatile compounds, their presence diminishes over time during storage. The data indicate that the treatments involving PAW, denoted as P2, P10, and P20, have shown a favourable effect on maintaining chlorophyll content in salad leaves during storage at 4°C. In particular, the PAW treatments exhibited slower rates of chlorophyll degradation when compared to the control and the IPO treatments (data not shown). This suggests that PAW treatments could be more effective in preserving the green colour and possibly the nutritional quality of salad leaves, enhancing their shelf-life and marketability. The robustness of these findings is further supported by the high correlation coefficients, which indicate a good fit for the kinetic model to the experimental data. The observed effectiveness of PAW in microbial decontamination, coupled with its capacity to preserve physicochemical qualities like chlorophyll content, positions it as a promising technology for enhancing the safety and extending the shelf life of leafy greens in future food processing applications.

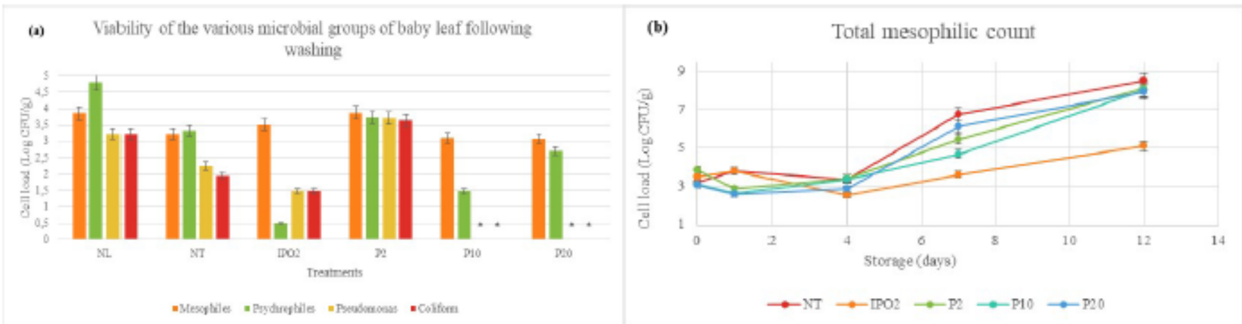

Figure 1: (a) Cell viability of spoilage microbial populations in baby leaf lettuce unwashed (NL), washed with tap water (NT), sodium hypochlorite for 2 minutes (IPO2), or PAW for 2, 10, and 20 minutes (P2, P10, P20, respectively); (b) changes over storage of the total mesophilic bacteria surviving the different wash treatments of baby leaf lettuce. *Below the Limit of Detection (LOD) at less than 30 CFU/g.

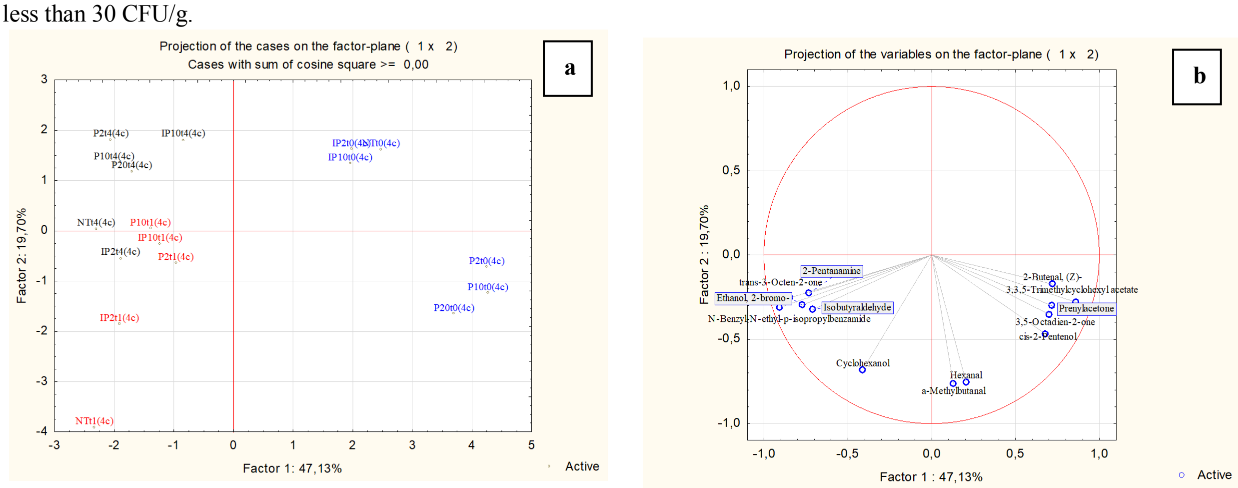

Figure 2: (a) Projection of cases obtained by Principal Component Analysis of the significant volatile molecules detected through one-way ANOVA ($p<0.05$) for the baby leaves washed with different treatments and stored at 4°C. (b) Projection of variables obtained by PCA of the significant volatile molecules detected through one-way ANOVA ($p<0.05$) for the baby leaves.

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

Shanbeh Zadeh F, Vannini L, Gardini F (2023) Improvement of quality and nutritional value of foods using natural compounds and mild biotechnologies. Poster presented at the 27th Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, Portici, Italy, Sept 13-15.

Vannini L, Shanbeh Zadeh F, Cellini B, Gardini F (2023) Screening of microbial yeast and lactic acid bacterial cultures to improve quality and nutritional value of foods. Proceedings of the 9th International Conference on Food Chemistry & Technology, Paris, Nov 27-29, Pages 89-90.

Shanbeh Zadeh F, Drudi F, Giordano E, Laurita R, Capelli F, Gherardi M, Gardini F, Romani S, Vannini L (2024) Cold atmospheric plasma treatments for the decontamination of sliced carrots. Proceedings of the 7th International Conference on FoodOmics, Cesena, Italy, Feb 14-16, Pages 179-181.

Analysis of volatilome of virgin olive oils and flavoured oils: quality grade evaluation and study of modification during storage

Rosalba Tucci (rosalba.tucci2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, Alma Mater Studiorum - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof.ssa Alessandra Bendini; Co-tutor: Dott. Enrico Casadei, Dott.ssa Silvia Mingione

1. State-of-the-Art

In the Mediterranean area, especially in Spain, Italy and Greece, olive oil represents one of the main food products with a world production of 2,511,000 tonnes expected for the 2022/23 olive oil campaign (DG AGRI, 2023). In the European Union (EU), virgin olive oils (VOOs) can be classified into three commercial categories, based on both physicochemical and sensory parameters, such as: extra virgin (EV), virgin (V) and lampante (L) (Reg. EU 2022/2104). Despite several modifications that occurred over the years, the official method for sensory evaluation still shows some weaknesses as it is time-consuming and, in case of a non-correct training of assessors, can be affected by not satisfactory reproducibility of results (Barbieri et al., 2020). Therefore, the identification and quantification of volatile organic compounds (VOCs) in VOOs is of great importance to assess their quality. In fact, targeted and untargeted instrumental methods based on the analysis of these molecules can be considered an interesting tool to support the sensory analysis (Panel test) (Quintanilla-Casas et al., 2020). Specifically, some VOCs have been proposed as markers to detect positive (e.g., fruity) and negative sensory attributes according to their concentrations (Valli et al., 2020). For this purpose, targeted methods based on headspace solid phase microextraction (SPME) with the use of flame ionization detector (FID) or mass spectrometry (MS) are being recently validated (Casadei et al., 2021; Aparicio-Ruiz et al., 2022). Furthermore, rapid instrumental methods concerning rapid 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 (Barbieri et al, 2020; Valli et al., 2020). Finally, in the global economic scenario of olive oil, flavoured oils are becoming increasingly popular. Consumers are attracted by their versatility of culinary use due to the possibility to convey a wide range of aromas to food preparations thanks to the use of different kinds of flavouring matrices such as herbs, spices, fruits, and vegetables (Baiano et al., 2016). The addition of specific flavouring agents to olive oils, depending on the applied technology to produce the flavoured oil (co-extraction, contact and essential oils inclusion), affects the incorporation in the oil matrix of specific bioactive compounds with antioxidant and/or healthy and/or sensory properties. Consequently, analytical evaluation of flavoured oils, taking into account their compositional and sensory characteristics, is essential to verify the quality of the product and to study its performance during storage (Lamas et al., 2022).

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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.

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3. Objectives

To achieve the objectives of the PhD project the following activities, according to the Gantt chart shown in Table 1, are carried out:

A1) **Sampling**: preparation, anonymisation, and shipment of oils to the involved laboratories.

A2) **Definition of instrumental and sensory protocols**: setting up of analytical protocols for GC-IMS and sensory analysis to be applied in a shared modality by the five different laboratories.

A3) **Sensory and instrumental alignment tests**: verification of the degree of analytical alignment of five GC-IMS instruments using specific prepared *ad-hoc* standards. The same analytical protocol has been applied by the five laboratories participating in the trial (A3.1); check of sensory alignment among the five panels participating in the trial by application of a specific decision tree scheme (A3.2).

A4**) Creation of sensory and instrumental datasets**: the dataset will consist of at least 150 samples analysed by both sensory and instrumental analyses (GC-IMS).

A5) **Development of chemometric models**: estimation models (EV vs V) will be built using the dataset (A4).

A6) **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.

A7) **Volatile and sensory analysis of flavoured oils**: flavoured oils marketed by Olitalia company were selected; volatilome and sensory characteristics, as well as possible modifications during storage, will be monitored.

A8) **Analysis of bioactive compounds in monovarietal extra virgin olive oils of *Nostrana di Brisighella* cultivar**: development of a new quality protocol to maximize the content of bioactive compounds in monovarietal *Nostrana di Brisighella* extra virgin olive oil (obtained applying different agronomic and technological variables), focusing on volatile molecules and phenolic compounds.

A9) **Writing of research papers and final thesis**.

Table 1. Gantt diagram related to this PhD project

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1)	***Sampling***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			
A2)	***Definition of instrumental and sensory protocols***			■	■	■													
A3)	***Sensory and instrumental alignment tests***			■	■	■	■	■	■	■	■	■	■						
	1) Instrumental alignment			▒	▒	▒	▒	▒											
	2) Sensory alignment					▒	▒	▒	▒	▒	▒	▒	▒						
A4)	***Creation of sensory and instrumental datasets***				■	■	■	■	■	■	■	■	■	■	■	■			
A5)	***Development of chemometric models***															■	■	■	■
A6)	***Shelf-life study***					■	■	■	■	■	■	■	■	■	■				
A7)	***Volatile analysis of flavoured oils***									■	■	■	■	■	■				
A8)	***Analysis of bioactive compounds in monovarietal extra virgin olive oils of Nostrana di Brisighella cultivar***							■	■	■	■	■	■	■					
A9)	***Writing of research papers and final thesis***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research progress and main results

The main aim of this research project is to develop chemometric models to estimate olive oil commercial categories, in particular, discriminating V from EV olive oils. The 57 samples analysed so far have been evaluated by five different sensory panels and have been classified according to a decision tree, based on the agreement among the panels regarding the commercial category and the main perceived defect (Barbieri et al., 2020). The sensory evaluation resulted in the following classifications: 12 samples as EV, 30 as V, 14 as borderline (BL) (classified between EV and V), and 1 as L. In addition to sensory analysis, the profile in volatile compounds of the same oils were analysed using Headspace Gas Chromatography-Ion Mobility Spectrometry (HS-GC-IMS) technique in five different laboratories equipped with the same type of instrument. Raw data from HS-GC-IMS analysis came from 3D chromatograms (heatmaps) characterized by GC retention time (seconds), IMS drift time (milliseconds), and ion current intensity (millivolt). From the analysis of 15 preselected standard volatile compounds, 3D spectral intensity regions were identified using Vocal software (Gesellschaft für Analytische Sensorsysteme mbh, G.A.S.; Dortmund, Germany). These volatile molecules are known to be markers of specific sensory attributes of virgin olive oils, both positive such as fruity and negative such as the main defects (fusty-muddy, winey-vinegary, musty-humid-earthy, frostbitten olives, rancid) and used to classify the samples. Their intensity values (VOCs) were employed to validate four PLS-DA models (EV vs noEV; L vs noL; Lvs V; EV vs V) as described by Valli et al. (2020). Through the combination of probability data obtained from the application of the four chemometric models, the 57 analysed samples were estimated. In total, 30 samples were predicted as V, 12 as BL, 12 as EV, and 3 as L (Table 2). Of particular interest are BL samples, such as sample 16, which was estimated by the PLS-DA model as V, while it has been classified by all panels involved in the trial as BL, because there was no agreement on the main perceived defect (second type of misalignment). Three out of five panels evaluated the sample with a slight defect of "fusty-muddy sediment", two out of five of "winey-vinegary" and one of "frostbitten olives" and "rancid". Following the decision tree, a blind formative re-assessment was carried out, re-coding the sample as “36” (Table 2). The sample was re-classified as V for both the sensory and the estimation model. Another example concerns sample 6, which was classified as EV by three out of five panels, but evaluated as BL by the decision tree. This represents a first type of misalignment, resulting in the disagreement among the panels on the commercial category. Also, for the PLS-DA model sample 6 turned out to be BL, confirming the impossibility of classifying with certainty the sample as EV. This is because there is a high probability of the sample belonging to both the first estimation model and the fourth. Specifically, the EV-noEV probability is 72%, and the EV-V is 56%. Until now, around 80% of the analysed samples have been correctly classified according to sensory classification, but the dataset will be implemented in the coming months to make the estimation models even more robust. In addition, the volatile profiles of 34 selected samples were also analysed using SPME-GC-MS technique to assess possible qualitative and quantitative modifications during a 12-month storage period. Currently, time zero of these oils has been valued, and the study is still ongoing. The same technique was also used to analyse a specific set of flavoured oils (lemon, basil, rosemary, oregano, truffle, and chili) produced by Olitalia s.r.l. (the co-founding company of this doctoral project). This latest experimentation aims to identify and quantify the volatile markers unique to each flavouring which are important for establishing an internal quality control system. In addition, this study focuses on assessing any changes that may occur during storage (still ongoing).

Table 2. Evaluation of agreement between predicted and sensory commercial category in a set of 57 VOOs.

Sample	Sensory classification	EV_noEV	L_noL	L_V	EV_V	Predicted category	Sample	Sensory classification	EV_noEV	L_noL	L_V	EV_V	Predicted category
1	V	0.05	0.09	0.06	0.13	V	30	V	0.42	0.07	0.06	0.17	V
2	V	0.90	0.07	0.10	0.89	EV	31	V	0.64	0.08	0.14	0.32	V
3	V	0.22	0.38	0.40	0.14	V	32	V	0.30	0.09	0.08	0.17	V
4	EV	0.87	0.28	0.84	0.53	EV	33	EV	0.87	0.08	0.08	0.66	EV
5	EV	0.74	0.18	0.46	0.29	BL (EV/V)	34	BL	0.93	0.08	0.07	0.84	EV
6	BL	0.72	0.07	0.06	0.56	BL (EV/V)	35	V	0.90	0.31	0.18	0.95	EV
7	V	0.58	0.08	0.09	0.29	V	36	V	0.13	0.40	0.24	0.13	V
8	EV	0.74	0.10	0.20	0.28	BL (EV/V)	37	V	0.13	0.22	0.15	0.16	V
9	V	0.16	0.38	0.17	0.13	V	38	V	0.29	1.00	1.00	0.26	L
10	V	0.58	0.10	0.12	0.24	V	39	BL	0.58	0.07	0.07	0.35	BL (EV/V)
11	V	0.67	0.31	0.33	0.40	V	40	V	0.32	0.65	0.54	0.26	V
12	EV	0.69	0.07	0.08	0.25	BL (EV/V)	41	V	0.57	0.25	0.21	0.43	V
13	L	0.29	0.35	0.16	0.17	V	42	BL	0.60	0.08	0.06	0.27	BL (EV/V)
14	BL	0.74	0.12	0.21	0.54	BL (EV/V)	43	V	0.18	1.00	1.00	0.15	L
15	V	0.58	0.12	0.13	0.34	V	44	BL	0.42	0.31	0.18	0.26	V
16	BL	0.15	0.46	0.21	0.13	V	45	EV	0.88	0.07	0.10	0.83	EV
17	EV	0.22	0.08	0.06	0.17	V	46	V	0.56	0.07	0.06	0.36	V
18	V	0.77	0.07	0.06	0.66	EV	47	V	0.50	0.13	0.14	0.50	V
19	BL	0.77	0.07	0.10	0.48	BL (EV/V)	48	BL	0.95	0.07	0.20	0.92	EV
20	BL	0.09	0.65	0.43	0.17	BL (V/L)	49	BL	0.12	0.12	0.09	0.14	V
21	EV	0.82	0.07	0.06	0.65	EV	50	EV	0.96	0.31	0.18	1.00	EV
22	V	0.16	0.09	0.06	0.20	V	51	BL	0.42	1.00	0.99	0.16	L
23	V	0.16	0.07	0.07	0.15	V	52	BL	0.55	0.14	0.11	0.21	BL (EV/V)
24	EV	0.78	0.10	0.06	0.38	BL (EV/V)	53	V	0.46	0.29	0.48	0.19	V
25	V	0.25	0.14	0.12	0.30	V	54	V	0.26	0.12	0.09	0.14	V
26	V	0.06	0.33	0.35	0.22	V	55	EV	0.86	0.27	0.90	0.86	EV
27	V	0.38	0.09	0.17	0.51	V	56	BL	0.78	0.23	0.16	0.47	BL (EV/V)
28	V	0.04	0.22	0.11	0.13	V	57	V	0.59	0.07	0.07	0.40	V
29	EV	0.85	0.10	0.32	0.60	EV							

5. Publications produced during the PhD activities

Casadei E, Valli E, Bendini A, Barbieri S, Tucci R, Ferioli F, Gallina Toschi T (2024) Valorization of monovarietal Nostrana di Brisighella extra virgin olive oils: focus on bioactive compounds, Front. Nutr.11.1353832.

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Fostering sustainability in the olive oil supply chain: valorization of typical virgin olive oils, olive mill by-products and waste

Sofia Zantedeschi (email: sofia.zantedeschi2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVIII; Anno di frequenza: II
Tutor: Prof.ssa Tullia Gallina Toschi; Co-tutor: Prof. Enrico Valli; Dott.ssa Matilde Tura

1. Stato dell'arte

Lo sviluppo sostenibile, definito come "*soddisfare i bisogni attuali senza compromettere quelli delle generazioni future*" (Brundtland, 1987), si fonda su tre pilastri: ambientale, sociale ed economico, finalizzati rispettivamente a proteggere le risorse naturali, promuovere l'economia locale e coinvolgere nel processo decisionale le comunità locali (Menozzi, 2014). La produzione di olio d'oliva, alimento di grande valore economico in quanto principale fonte di acidi grassi monoinsaturi della dieta mediterranea, è in continua crescita a livello mondiale (Guasch-Ferré et al., 2014). È importante garantire e favorire la sostenibilità della filiera dell'olio d'oliva, per fornire un vantaggio competitivo ai produttori e un incentivo ai consumatori, poiché rappresenta un valore aggiunto e un elemento di differenziazione per i prodotti (Lombardo et al., 2021).

Al fine di supportare la sostenibilità socio-economica della filiera olivicolo-olearia, la politica dell'Unione Europea per lo sviluppo rurale promuove la produzione di indicazioni geografiche (IG), ovvero le DOP e le IGP (Menozzi, 2014). Tali certificazioni promuovono la qualità alimentare legata all'origine geografica, generando effetti positivi su aspetti economici, sociali ed ambientali che si rafforzano nel tempo grazie alla preservazione delle risorse locali coinvolte (Vandecandelaere, 2010). Infatti, i consumatori sono generalmente disposti a comprare un prodotto con certificazione IG ad un prezzo più alto rispetto ai prodotti non certificati (Cei et al., 2018). Inoltre, le IG possono salvaguardare metodi tradizionali e patrimoni culturali grazie all'impiego di tecniche di produzione tradizionali (WIPO, 2021). Valorizzare i prodotti alimentari con IG può contribuire a creare posti di lavoro e stimolare la crescita economica, specialmente nelle aree rurali (Deselnicu et al., 2013).

Per rendere la produzione di olio d'oliva sostenibile è necessario intervenire su tutte le fasi della filiera. Un aspetto molto importante, oltre alla valorizzazione della produzione locale di oli extra vergini di oliva di qualità, è l'elevata quantità di sottoprodotti e scarti che si generano in frantoio, quali nocciolino, sansa, acque di vegetazione e foglie. Il nocciolino viene principalmente sfruttato come una fonte di energia rinnovabile e sostenibile attraverso processi di combustione e, potenzialmente, per la produzione di biocarburanti (García Martín, 2020). La sansa, invece, può essere inviata ai sansifici per produrre olio di sansa oppure può essere impiegata come materia prima per il compostaggio, per la produzione di energia tramite processi di combustione o di biogas, per l'estrazione di composti bioattivi, come i polifenoli (Dermeche et al., 2013). In particolare, viene prodotta una ingente quantità di acque di vegetazione, che presentano un problema a livello ambientale, in quanto il loro potenziale inquinante può causare effetti negativi sul suolo e le falde acquifere (Gómez-Caravaca et al., 2014). Tuttavia, essendo ricche di composti bioattivi, le acque di vegetazione possono essere valorizzate: 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, separate dalle olive durante la produzione dell'olio, 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). Considerando la grande importanza dello sviluppo di una filiera dell'olio d'oliva sostenibile e di frantoi virtuosi, risulta necessario investigare la valorizzazione dei sottoprodotti e degli scarti di frantoio finora meno utilizzati, quali le foglie. Queste pratiche favoriscono la sostenibilità delle risorse e la riduzione dell'inquinamento derivante dal settore dell'olio d'oliva, rappresentando anche opportunità economiche (D'Adamo et al., 2019).

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3. Obiettivi

Per il raggiungimento degli obiettivi del progetto della tesi di dottorato il lavoro è stato suddiviso nelle seguenti attività secondo il diagramma di Gantt riportato in Tabella 1:

A1) **Caratterizzazione di oli vergini di oliva albanesi:** caratterizzazione strumentale e sensoriale di due campioni di due frantoi di olio albanese monovarietale Kalinjot.

A2) **Caratterizzazione di oli vergini di oliva portoghesi:** caratterizzazione strumentale e sensoriale di due campioni di oli portoghesi da selezionare.

A3) **Caratterizzazione oli vergini di oliva marocchini**: caratterizzazione strumentale e sensoriale di due campioni di oli marocchini da selezionare.

A4) **Valorizzazione di sottoprodotti del frantoio:** caratterizzazione (percentuale lipidica e di umidità) della sansa, di acque di vegetazione, nocciolino e foglie di ulivo. Prove di produzione di biogas con miscele di acque di vegetazione e foglie di ulivo.

A5) **Scrittura e redazione** della tesi di dottorato, di articoli scientifici e/o di poster e preparazione di comunicazioni orali per workshop e convegni.

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)	***Caratterizzazione di oli di oliva vergini albanesi***	■	■	■															
	1) Analisi e parametri: OSI time, profilo dei composti volatili, perossidi, analisi sensoriale	▒	▒	▒															
A2)	***Caratterizzazione di oli di oliva vergini portoghesi***												■	■					
	1) Analisi e parametri: OSI time, profilo dei composti volatili, perossidi, analisi sensoriale, fenoli totali (Folin-Ciocalteu), fenoli (HPLC)												▒	▒					
A3)	***Caratterizzazione di oli di oliva vergini marocchini***														■	■			
	1) Analisi e parametri: OSI time, profilo dei composti volatili, perossidi, analisi sensoriale, fenoli totali (Folin-Ciocalteu), fenoli (HPLC)														▒	▒			
A4)	***Valorizzazione di sottoprodotti del frantoio***	■	■	■	■	■	■	■	■	■	■	■		■					
	1) Caratterizzazione analitica (lipidi e umidità) dei sottoprodotti	▒	▒				▒	▒	▒				▒	▒	▒				
	2) Potenziale metanigeno di acque di vegetazione in miscela con foglie di ulivo						▒	▒	▒	▒	▒	▒							
A5)	***Preparazione della tesi e di articoli***	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

4. Stato di avanzamento della ricerca e principali risultati

Il presente progetto di ricerca si propone di migliorare la sostenibilità della filiera dell'olio di oliva attraverso la valorizzazione di oli di oliva vergini di aree geografiche specifiche e dei sottoprodotti del frantoio.

Per quanto riguarda la caratterizzazione di oli vergini, sono state eseguite analisi chimiche e sensoriali su due oli monovarietali 100% Kalinjot prodotti da due frantoi albanesi. Lo studio conferma le peculiari caratteristiche qualitative di questi oli, che soddisfano i criteri stabiliti dall'Unione Europea per gli oli extravergini. Gli oli in esame presentano anche un elevato contenuto di composti fenolici, superando la soglia necessaria per l'indicazione salutistica. Essi presentano un OSI time superiore a 20 ore, parametro importante per indicare la stabilità ossidativa nel tempo e dunque avere un'indicazione della shelf-life del prodotto. L'analisi SPME-GC-MS ha rilevato 33 composti volatili, con prevalenza di aldeidi, alcoli e chetoni. In particolare, tra i composti principali sono stati identificati e quantificati le aldeidi (*Z*)-2-esenale, esanale e (*E*)-2-pentenale, gli alcoli (*Z*)-3-esen-1-olo e 1-esanolo e l'1-penten-3-one, associati alla caratteristica sensoriale di verde. I composti volatili associati ai difetti, come l'acido butanoico e l'acido acetico, invece, sono stati rilevati a concentrazioni notevolmente inferiori. La mappa termica generata dall'analisi all'HS-GC-IMS ha evidenziato dodici composti volatili, inclusi quelli associati a note fruttate. Inoltre, l'analisi sensoriale ha rilevato due attributi positivi secondari minori, ovvero il sentore di erba e quello di pomodoro, rilevanti anche per la possibile futura applicazione di una denominazione di origine. Infatti, gli attributi organolettici secondari riflettono le caratteristiche del territorio di produzione, in quanto dipendono da fattori come le condizioni pedoclimatiche e le cultivar degli ulivi (Deiana et al., 2019).

Altri oli di oliva vergini verranno caratterizzati durante il terzo anno di dottorato, durante due periodi all'estero in Portogallo e in Marocco.

I sottoprodotti del frantoio, ovvero sansa, acque di vegetazione, nocciolino e foglie, sono stati campionati presso il frantoio CAB Brisighella e sono stati caratterizzati, in termini di percentuale di umidità e di lipidi, nelle campagne 22/23 e 23/24. I valori percentuali di umidità e lipidi estratti sono rimasti costanti tra le due campagne, con medie di 32.52% nelle foglie, 20.25% e 0.48% nel nocciolino, 78% e 0.62% nelle acque di vegetazione e 42.87% e 2% nella sansa. Questi parametri sono fondamentali poiché influenzano direttamente la resa, la qualità e l'efficienza dei processi di valorizzazione come l'estrazione di composti bioattivi, la produzione di biogas e la produzione di energia termica.

Sono stati misurati, inoltre, i solidi totali e i solidi volatili di acque di vegetazione, sansa e foglie per la determinazione del potenziale metanigeno (BMP). È stata eseguita un'analisi BMP sulle acque di vegetazione in miscela con il 4% di foglie di ulivo. Il gas prodotto è stato, poi, analizzato al microGC per verificarne la composizione. Sono previste ulteriori prove di metanazione sulle acque di vegetazione tal quali e loro miscele con le foglie in diverse percentuali, in base ai risultati ottenuti dalle prove precedenti.

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

Zantedeschi S (2023) Improving sustainability of the vegetable oils supply chains: innovative analytical methods for quality control, valorization of by-products and reduction of waste, in: Proceedings of 27th Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology, pp. 228-229.

DOTTORANDI ISCRITTI AL III ANNO (XXXVII CICLO)

Investigation on colour features and oxidative stability of rosé wines

Federico Baris (e-mail: federico.baris2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVII; Anno di frequenza: III
Tutor: Fabio Chinnici

1. State of art

The characteristic taste, aroma, and texture (sensory characteristics) of wine are largely determined by phenolic compounds, also known as polyphenols. Found throughout the wine, these molecules primarily come in the form of flavonoids. Flavonoids include tannins, which cause a drying sensation, anthocyanins (responsible for the red colour in red wines), flavonols (such as quercetin) and small amounts of flavones (Vidal et al., 2004). Depending on their sources, two main categories of tannins could be defined: hydrolysable and condensed (Versari et al., 2012). Hydrolysable tannins are characterized by a complex structure that revolves around a central unit of glucose that is esterified with various phenolic acids. Examples of hydrolysable tannins come from sources such as walnut, chestnut, cherry and oak galls. On the other hand, condensed tannins, or proanthocyanidins, are natural compounds that can be found naturally in numerous plants, including grapes, quebracho, mimosa, and acacia (Vignault et al., 2018). While their use in red wines is well documented (Vivas & Glories, 1996), their role in rosé wines remains understudied. Oenological tannins act as antioxidants by scavenging free radicals that can damage the wine (Magalhães et al., 2014). This helps to protect the wine from oxidation, which can lead to browning and off-flavours. They can chelate iron, which can speed up oxidation, so they can improve the clarity and stability of the wine's colour (Pascual et al., 2017). Finally, they can co-pigment with anthocyanins intensifying the existing colour and contribute to the formation of new pigments (Trouillas et al., 2016). This results in a richer, more vibrant colour in red and rosé wines. Another critical aspect influencing rosé wine quality is light exposure. Bottled rosé can be exposed to light for extended periods during storage. This exposure can trigger the formation of unpleasant aromas and degrade colour, lastly reducing the wine's shelf life (Dias et al., 2012). Two main reactions represent the core of this process: Fenton reaction and photolysis. The first reaction involves the generation of highly reactive hydroxyl radicals by hydrogen peroxide and metal catalysts like iron. These radicals can oxidize various wine components, including phenolics and amino acids, leading to aroma and flavour decline. (Danilewicz, 2003). Photolysis refers to the light action which can directly break down organic molecules in wine, impacting colour and aroma. When combined with the Fenton reaction, the process becomes even more harmful, accelerating the degradation of the wine (Grant-Preece et al., 2017). These changes depend on the irradiation conditions and the duration of light exposure. Variation in light exposure conditions and duration can lead to differences in sensory characteristics and shelf life among individual wine bottles. Despite the market's preference for clear bottles, some studies have shown that darker coloured glass offers superior protection for rosé wines (Dias et al., 2012, 2013). Understanding the effectiveness of various bottle types can be invaluable for rosé producers.

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3. Expected Results

This project aims to elucidate the mechanisms governing the extraction, transformation, and stabilization of colour pigments in rosé wines. It will further evaluate the efficacy of various cellar practices in managing this crucial quality parameter and ensuring its persistence throughout the wine's shelf life.

To achieve the project's objectives, the research was divided into a series of key activities, detailed within the Gantt chart presented in Table 1:

A1) Bibliographic research on the different and consolidated analytical methodologies for the determination of colour, anthocyanins and tannins in rosé wines.
A2) Evaluation of analytical techniques for colour and tannins suitable for implementation within production settings, utilizing spectrophotometric assessments. These included: a) obtaining 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 replicate observed phenomena. It is deemed imperative to conduct experiments under laboratory conditions, utilizing synthetic matrices that will be subsequently tested in various concentrations to modulate the proportions of pigments, tannins, and phenolic acids.
A4) Development of a colour and tannic profile map through the application of an analytical-sensory screening of Italian rosé wines. They were procured from market sources and regions historically renowned for such products (Bardolino, Valtenesi, Abruzzo, Apulia), as well as emerging areas (Tuscany, Sicily) or regions specializing in sparkling rosé wines (Lambrusco in Emilia, Pinot Noir in Franciacorta or Trento).
A5) Assessment of the influence of oenological tannins from different sources on the evolution of colour over time of a laboratory vinified rosé wine. They were previously characterized to identify a scale of antioxidant effectiveness then added to the rosé to evaluate their effectiveness under realistic conditions.
A6) Research on the role of photofenton on wines oxidation using different colour bottles. Two distinct light sources were used to emulate the radiation of sunlight (D65) and typical supermarket lighting (840P15). This approach examined the potential effects of light on three different nuances of model rosé wine using bottles with different colours (amber, UV-filter, flint).
A7) Investigation of chitosan properties in controlling oxidation in rosé wines compared to sulphite addition. Once characterized, the chitosans were added to the wine which was monitored over time focusing on colour development, iron's oxidation-reduction equilibrium and oxygen consumption. The study also employed the measurement of acetaldehyde formation kinetics to determine the degree of wine spoilage.
A8) Publications and writing final thesis. A comprehensive dissertation will be written, outlining the research conducted and its findings. At least three scientific articles resulting from the research will be published in national and international peer-reviewed journals within the field of food science and technology. At the end of the project, at least 2 oral presentation and 2 poster will be published, concerning the topic of the project carried out during the researches.

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)	***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)	***Assessment of the influence of oenological tannins***							■	■	■	■	■	■						
	1) Characterization of oenological tannins from different origins							▒	▒	▒	▒	▒	▒						
	2) Study on the effects of their addition to a rosé wine									▒	▒	▒	▒						
A6)	***Research on the role of photofenton on wines oxidation***										■	■	■	■					
A7)	***Investigation of chitosan anti-oxidant properties***														■	■	■	■	
	1) Characterization of chitosans from distinct fungoid sources														▒	▒			
	2) Investigation on their role in preserving wine from oxidation															▒	▒	▒	
A7)	***Publications - communications and writing of final thesis***						■	■	■	■	■	■	■	■	■	■	■	■	■

4. Research status and main results

The research conducted thus far has yielded interesting findings regarding the evaluation of the influence of oenological tannins on rosé wines, as well as the effect of light in photofenton conditions.
Eight different commercial oenological tannins, derived from different sources, were previously characterized in a model

solution without anthocyanins and their phenolic richness was evaluated. Table 1 shows their richness expressed as a percentage (g of tannin per 100 g of commercial product) in function of the analysis took out. The different tannins were grouped in 2 families of condensed tannins: procyanidins/prodelphinidins (PC/PD) and profisetinidins/prorobitenidins (PF/PR); and 2 families of hydrolyzable tannins: gallotannins (GT) and ellagitannins (ET). Indeed, PC/PD include tannins from grapes (skin and seed), meanwhile tannins from acacia and quebracho are included in PF/PR. Among hydrolyzables tannins, ellagitannins include tannins from chestnut, oak and cherry, and gallotannins were represented by tara.

	Tannins	**Folin-Ciocalteu**	**TPI (280nm)**	**Bate-Smith**	**DAC**
			Richness (%)		
PC/PD	**Skin**	89.4 ± 1.5 b	92.5 ± 0.7 b	62.4 ± 1.1 a	38.1 ± 0.7 c
	Seed	98.6 ± 0.7 a	99.2 ± 0.2 a	61.0 ± 1.3 a	77.3 ± 0.7 a
	AV PC/PD	**94.0 ± 4.6 A**	**95.8 ± 3.4 A**	**61.7 ± 0.7 A**	**57.7 ± 19.6 A**
PF/PR	**Acacia**	72.9 ± 1.1 c	76.4 ± 0.7 c	38.3 ± 0.4 c	57.9 ± 1.0 b
	Quebracho	91.4 ± 1.3 b	93.1 ± 0.3 b	43.3 ± 1.1 b	8.6 ± 0.1 e
	AV PF/PR	**82.2 ± 9.2 A**	**84.8 ± 8.4 A**	**40.8 ± 2.5 A**	**33.3 ± 24.7 A**
GT	**Tara**	72.2 ± 1.1 c	22.4 ± 0.3 e	2.3 ± 0.1 f	0.5 ± 0.0 f
	AV GT	**72.2 ± 1.1 A**	**22.4 ± 0.3 B**	**2.3 ± 0.1 B**	**0.5 ± 0.0 A**
ET	**Oak**	98.4 ± 0.9 a	39.0 ± 0.3 f	9.2 ± 1.0 e	0.1 ± 0.0 f
	Chestnut	87.5 ± 1.6 b	52.7 ± 0.9 d	3.6 ± 0.4 f	0.1 ± 0.0 f
	Cherry	66.3 ± 2.3 d	38.5 ± 0.4 f	17.3 ± 1.1 d	23.0 ± 0.2 d
	AV ET	**84.1 ± 13.3 A**	**45.5 ± 9.6 B**	**10.0 ± 5.6 B**	**7.7 ± 10.8 A**
	AVERAGE	**84.6 ± 11.8**	**67.6 ± 24.2**	**29.7 ± 23.2**	**25.7 ± 27.6**

Table 1 Chemical characterization of the eight oenological tannins about their phenolic richness. Richness is expressed as a percentage (g of tannin per 100 g of commercial product).
PC/PD = procyanidins/prodelphinidins; PF/PR = profisetinidins/prorobitenidins; GT = gallotannins; ET = ellagitannins.

Their ability to consume oxygen was also investigated during two weeks at room temperature. A non-invasive optical oxygen monitoring system has been used consisting of an internal sensor patch and an external reader to measure the dissolved oxygen levels of the solution. Ellagitannins, such as chestnut and cherry, turned out to be the most efficient oxygen consumers, followed by skin tannins, quebracho tannins, seed tannins, and gallotannins (Figure 1).

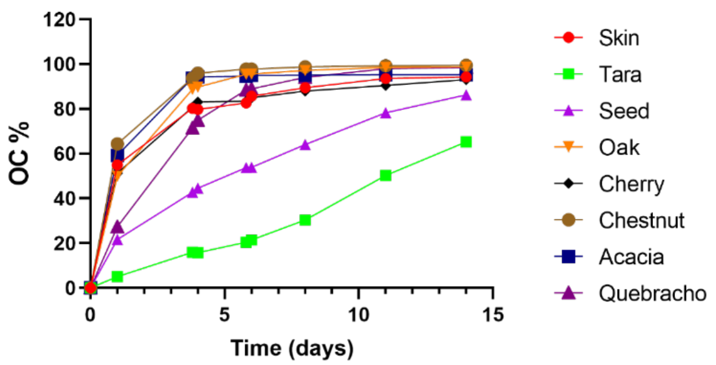

Figure 1. OC = Oxygen consumption kinetics of different oenological tannins in an oxygen-saturated model rosé wine at 20°C

Furthermore, they were added to a laboratory vinified rosé wine This research was focused on how the tannins affected the wine's colour and its overall phenolic content. Their ability to chelate iron has been evaluated in order to slow down the oxidation of wine. Iron contained in wines acts as a catalyst to the Fenton reaction that generates reactive hydroxyl radicals with a risky oxidative power. The chestnut tannin proved to be very efficient also on iron chelation (Figure 2), theoretically offering a better protection for the wine during shelf life. However, data related to the wine's colour (CIELAB parameters, spectrophotometric analyses, total anthocyans and anthocyanins) demonstrated how in fact the colour evolution may depend on complex kinetics and could not be directly linked to the chelation of iron (data not shown).

Another study investigated the impact of prolonged light exposure on anthocyanins preservation over time. Model rosé wine solutions were prepared with distinctive anthocyanins concentrations and placed in bottles of different colours (hence distinct light absorbing features) and positioned in a light testing chamber to simulate both the typical supermarket lighting and sunlight radiation. The prolonged light exposure revealed that among the six categorized main

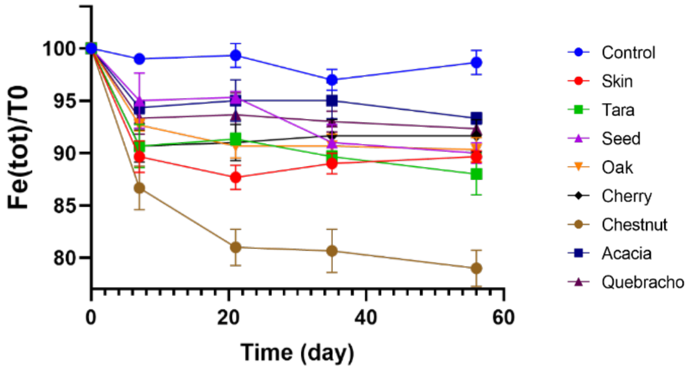

Figure 2. Iron chelation kinetics of the samples containing the different oenological tannins and control over time, compared to T0

families of anthocyanins, amber bottles exhibited superior effectiveness in preserving them, followed by UV filter-coated bottles. Conversely, flint or clear bottles, commonly employed in the market, showed the poorest performance (Figure 3). Notably, the vitisins family demonstrated the highest preservation level, with a reduction of less than 10% after 10 days of light exposure in amber bottles. If clear glass failed to provide sufficient protective effects, transparent UV-blocking films proved to be valuable in preventing the photodegradation of pigments while maintaining the transparency of the bottle. Further researches are required to fully comprehend how different bottle types also preserve the volatile compositions of wines.

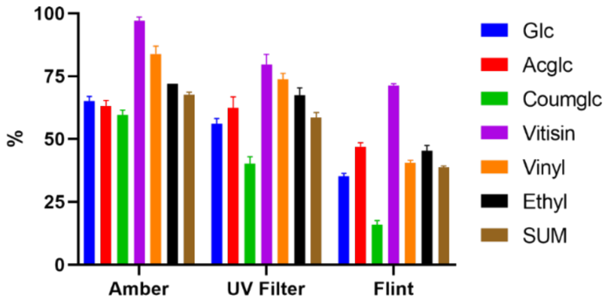

Figure 3. Overall mean residual amount (as percentage) of the different anthocyanins' families after two months in function of the different bottle colours.
Glc = Glucosides; Acglc = acetylglucosides; Coumglc =Coumaroylglucosides; Vynil = Vinylphenol adducts; Ethyl = Ethyl-bridged adducts; Sum = Mean % of the total sum of families

The effects of two fungal chitosans, derived from *Aspergillus niger* and *Agaricus bisporus*, on the oxidative evolution of a rosé wine, are currently under investigation in our laboratory. All of these studies could offer valuable insights for producers and winemakers to guide their selection of strategies to effectively control oxidation in their products. Small changes in supply chain could improve the quality of products that customers can find on supermarket shelves.

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

Baris F, Castro Marín A, De Aguiar Saldanha Pinheiro AC, Tappi S, Chinnici F (2023) Efficacy of fungoid chitosan from *Aspergillus niger* and *Agaricus bisporus* in controlling the oxidative browning of model white wines, Innov Food Sci Emerg Technol 86: 103381.

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.

Baris F, Castro Marín A, Chinnici F (2023) Il chitosano per migliorare le caratteristiche degli spumanti ottenuti con metodo classico, VVQ 6: 63-66.

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

Gebremedhin Gebremariam Gebremical (email: gebremedh.gebremica2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVII; Anno di frequenza: III
Tutor: Prof. Pietro Rocculi
Co-tutor: Dott. Filippo Capelli, Dott. Romolo Laurita, Dott.ssa Silvia Tappi, Prof.ssa 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 a multipurpose food ingredient (de Oliveira et al., 2018). However, native starch presents several drawbacks such as insoluble granules, tendency to syneresis, instability of the gel to heat, acid and shear forces and weak structure (Singh et al., 2011). As a result, starch modification became important to change its functionality. Various modification methods have been investigated (Zia-ud-Din et al., 2017); however, these methods pose problems in terms of environmental pollution, food safety, chemical residues, waste disposal, longer treatment time, and cost. To overcome these issues, physical methods, and in particular emerging technologies such as cold atmospheric plasma (CAP) and plasma-activated water (PAW) are also being explored (Ganesan et al., 2021; Laurita et al., 2021). CAP is defined as the fourth state of matter, and 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 CAP, is characterized by an acidic pH, changes in 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 (CAP and PAW) technology is considered a smart green technology, that does not generate hazardous, chemical waste, is cost effective and therefore is promising for sustainable food consumption patterns and ensuring global food security (Misra et al., 2016). However, there is limited research on its application for starch modification. Therefore, this study aimed to evaluate the effect of CAP and PAW on food modification to facilitare their industrial application.

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3. PhD Thesis Objectives and Milestones

This PhD research project is aimed to investigate the applications of cold plasma technology for food modification and to increase the knowledge of its effects on different food matrices during processing.

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) **Application of Plasma Activated Water (PAW)** to modify the rheological, thermal, hydration, and pasting properties of three types of starch;
A3) **Combination of Plasma Activated Water (PAW) and Annealing** for the modification of functional properties of potato starch;
A4) **Evaluation of Plasma-Activated Water (PAW) and Annealing** effect on sorption and thermodynamic properties of potato starch**;**
A5) **Application of Cold Atmospheric Plasma (CAP) to modify teff flour**, by setting up a plasma device that allows constant mixing of the product;
A6**) Effect of Cold Atmospheric Plasma to inactivate a spore forming fungus from flour;**
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***																		
A4)	***Plasma-Activated Water (PAW) and Annealing Effects on Sorption Isotherms and Thermodynamic Properties of Potato Starch***																		
A5)	***Application of Atmospheric cold plasma (gas) to modify the teff flour***																		
A6)	***Effect of Atmospheric Cold Plasma with Continuous Mixing to Inactivate Fungal from Flour***																		
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, 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. The starches were then evaluated for various characteristics (rheological, thermal, and functional properties) and by Fourier-transform infrared (FTIR) spectroscopy. The average discharge power was ~167.12 W, 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 properties of native and PAW treated potato (P), normal (NM), and waxy maize (WM) are shown in Tables 2 and 3, respectively. The results for the pasting properties of the P sample, and statistical analysis revealed that PAW-treated samples were 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), the 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 reduced ($P < 0.05$) the value of peak viscosity (PV), holding strength viscosity (HSV), SBV, and final viscosity (FV) in WM compared to the native sample.

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 gel hardness is reported in Table 3. PAW treatment was found to significantly ($p<0.05$) change the SP value of the 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 for the PAW treated samples, respectively. The WSI of the PAW treated starches was higher than that of the native ones, but only significant for NM and WM. Concerning gel hardness, similar 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 a decrease was observed for WM starch . In general, even though the consistency coefficient (k) and flow index (n) were different for the three analyzed starches, the flow behavior of all three samples was typical of pseudo-plastic fluids (shear thinning region), as $n<1$. As depicted in Table 3, the correlation coefficient (R^2) was 0.95–0.99 for all samples, indicating a satisfactory fit. PAW treatment resulted in a substantial and significant ($P<0.05$) increase in the viscosity value (k) and a decrease in 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 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 s n)	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 PAW was generated as described above. Then, the potato starch was treated by immersion in the generated plasma-activated water (PAW), by annealing in distilled water (DW-ANN) and by a combination of both; annealing in the generated PAW (PAW-ANN) for 4 h at 55 C°. As shown in Fig. 1, it was found that although there were no significant differences between native and PAW-treated samples, both DW-ANN and PAW in combination with annealing (PAW-ANN) resulted in lower values for paste clarity, swelling, solubility and syneresis.

The higher hydration and paste clarity in native and PAW, probably due to the less cross-linked and amorphous structure and the high content of polar groups. The cross-linking reaction, which probably occurred during DW-ANN and PAW-ANN treatment, prevents the dissociation of starch chains and enhances the interactions between starch functional groups, resulting in clusters of helical amylopectin side chains, leading to lower hydration and paste clarity of potato starch.

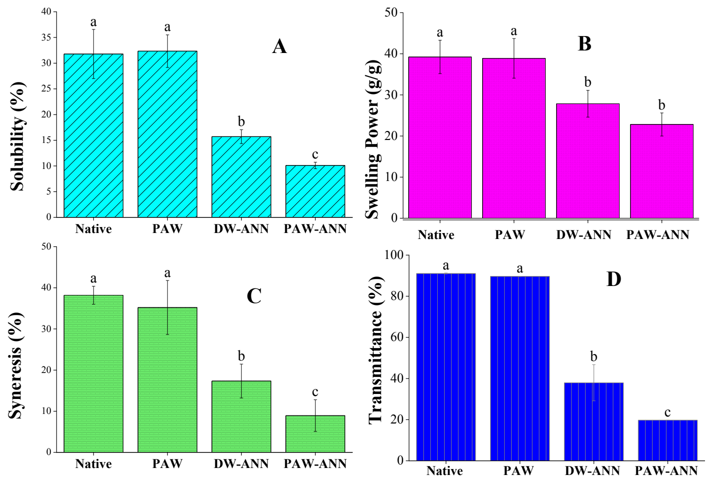

Fig. 1. Solubility (A), swelling power (B), syneresis (C) and paste clarity (D) of potato starch

The experimental results indicates in Table 4, for the equilibrium … DW-ANN, and PAW-ANN potato starch as a function of water activi… , 35, and 45 °C). In the study, a non-linear regression analysis wa… the experimental data to predict the water adsorption kinetics, revealing satisfactory model fits across different temperatures. The GAB model effectively predicted adsorption over a wide range of water activity(0-95 %RH). The parameters derived from the GAB model, including Mo, Cg, K, and SA, were obtained. Native potato starch exhibited higher values for these parameters compared to DW-ANN and PAW-ANN treatments, indicating a more hydrophilic surface and stronger water binding. Conversely, DW-ANN and PAW-ANN treatments showed reduced Mo, Cg, and SA, suggesting weakened water-starch interactions possibly due to cross-linking by PAW incombination of annealing. Increasing temperature led to a decrease in active sorption sites across all samples, likely due to the energy gained by water molecules enabling detachment from sorption sites, resulting in lower Mo, Cg, and SA values. Overall, the combined PAW and annealing treatment resulted in a synergistic reduction in active water sorption sites, highlighting the complex interplay between treatment effects and starch properties.

Table 4. *Estimated values of GAB model coefficients (M_0, C_g, K), surface area (SA) and statistical data of coefficient of determination (R^2) and mean root mean square (% P)*

Samples	Temperature (°C)	M_0	C_g	K	R^2	% P	SA (m^2/g)
	25	0.1	9.54	0.81	0.9998	0.24	356.01±16.38^{g}
Native	35	0.095	8.87	0.8	0.9999	0.93	336.68±6.46^{f}
	45	0.084	8.54	0.8	0.999	0.39	298.77±8.41^{d}
	25	0.095	8.89	0.79	0.999	0.88	338.19±4.14^{f}
PAW	35	0.085	8.38	0.76	0.998	0.26	302.34±8.18^{d}
	45	0.079	7.69	0.76	0.998	1.08	281.82±12.34^{bcd}
	25	0.09	7.32	0.77	0.997	1.4	320.98±0.96^{e}
DW-ANN	35	0.084	7.48	0.78	0.998	0.13	297.17±3.94^{bc}
	45	0.076	6.88	0.79	0.999	1.35	271.17±0.12^{b}
	25	0.082	7.45	0.75	0.997	1.25	290.23±5.29^{dc}
PAW-ANN	35	0.079	6.62	0.73	0.997	0.11	279.83±3.69^{d}
	45	0.072	6.36	0.73	0.998	1.11	255.57±5.46^{a}

A 1st research article has been published, a 2nd work, revised paper has been submitted and a 3rd article is in preparation. In addition, other study has been conducted and manuscripts are in preparation. Further experiments on the application of atmospheric cold plasma for the modification of flour and the inactivation of microorganisms with continuous mixing is also on going.

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

Gebremedhin GG, 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).

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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.

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Production, Composition and Sensory Characterization of New Flavoured Oils: focus on sustainability

Celeste Lazzarini (email: celeste.lazzarini3@unibo.it)
Department of Agricultural and Food Sciences, *Alma Mater Studiorum* - Università di Bologna
PhD Programme: Agricultural, Environmental and Food Science and Technology
Research topic: Water-Food-Energy-Sustainable Agriculture Nexus; Cycle: XXXVII; Year: III
Tutor: Prof. Tullia Gallina Toschi; Co-tutor: Prof. Enrico Valli, Dr Matilde Tura

1. State of the art

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. To increase the affordability of healthy diets, the cost of nutritious foods must decrease while 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 in each food supply chain, through their valorization, with a resulting increase in sustainability and circularity of the whole food sector (FAO, 2020).

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 global food energy intake. 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).

An example is hemp cultivation, which produces not only various end-use products but 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). *Cannabis sativa* L. 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). In addition to the most common hemp products, hemp essential oils have shown many different properties, such as antibacterial against *S. aureus*, *H. pylori*, Candida, and Malassezia spp. enzymes and cancer cells, low toxicity on mammals and beneficial organisms (e.g. pollinators) (Benelli et al., 2018; Zheljazkov et al., 2020).

Consumers are more and more attracted by healthy foods; that is why markets are promoting products added with functional ingredients (Meyerding et al., 2018).

In this framework, the enrichment of foods with bioactive compounds would meet the market demand for healthy products, and proper labelling focusing on geographical origin and health, as well as sustainability claims would be possible drivers also of economic growth for developing countries (Bradley et al., 2011).

The recovery of bioactive healthy compounds, especially using innovative technologies, through the valorization of agro-industrial by-products, is a growing opportunity nowadays (Spaggiari et al., 2021). An example is the recovery of lycopene from tomato by-products; this 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 (Coelho et al., 2023; Eslami et al., 2023).

Sustainable food technologies play a quite relevant role in 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, used on raw materials with thermolabile compounds, which 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, to improve healthy properties and sensory characteristics (Sacchi et al., 2017). The addition of certain vegetable matrices to the oil may also promote its oxidative stability thanks to the presence of antioxidants, such as lycopene, which can derive from tomato by-products (Lamas et al., 2022).

To assess the overall potential of such enriched products, their sensory evaluation is fundamental to characterize them and recognise marketing drivers through consumer science. For this purpose, sensory analysis can be combined with the use of biometric techniques (including facial expressions, heart rate, body temperature, skin conductance, and eye tracking). The integration of these novel techniques, nowadays used for economic and marketing studies, may provide deeper knowledge of human perception and preferences (Torrico et al., 2023).

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3. Research development

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

1) Bibliographic research.
2) Application of food technologies: flavoured oils are prepared using different ingredients and agri-food by-products treated using a sustainable non-thermal drying. The bioactive compounds, to be incorporated within the new formulations of flavoured oils, are extracted using distillation.
3) Compositional characterization of food products: 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), used as ingredients and flavouring agents.
4) Sensory analysis of flavoured oils: the flavoured oils are sensory tested to evaluate the liking and the intensity of some specific attributes; consumer tests are carried out to assess also neophobia and sustainability aspects.
5) Writing of research papers and final thesis.

Table 1. Gantt diagram regarding the PhD activities.

Activity	Month	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**	**22**	**24**	**26**	**28**	**30**	**32**	**34**	**36**
A1)	***Bibliographic research***																		
A2)	***Application of food technologies***																		
	1) Distillation																		

	2) Non-thermal drying							X	X	X	X								
A3)	***Compositional characterization of food products***			X	X	X	X	X	X	X	X	X	X						
	1) Characterization of by-products			X	X	X													
	2) Characterization of oils						X	X	X										
	3) Characterization of flavoured oils									X	X	X	X						
A4)	***Sensory analysis***							X	X	X	X	X	X	X	X	X	X		
	1) Sensory analysis of hemp-flavoured oils							X	X	X									
	2) Sensory analysis of flavoured olive oils										X	X	X						
	3) Joint sensory and biometric analysis										X	X	X	X	X	X	X		
A5)	***Writing of research papers and final thesis***	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

4. Main results

Firstly, this research is focused on the evaluation of how agri-food by-products can be included in the formulation of flavoured oils, improving sensory characteristics, composition and their stability.

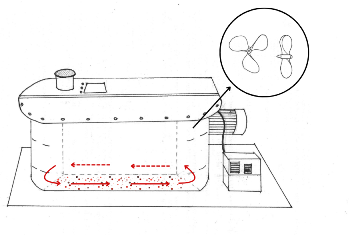

Figure 1*: Non-thermal drier prototype*

In particular, tomato pomace, made of seeds and peels, has been dried out using an innovative non-thermal drier to prolong its shelf-life allowing its use after the tomato campaign. It has been chosen to terminate the drying process, carried out with the prototype shown in Figure 1, of the tomato pomace after 3h of treatment since the a_w has reached levels of 0.41 ± 0.03. The levels reached are sufficient since around these values of a_w the growth of most microorganisms and moulds is very limited and under control (Labuza, 1980).
Lycopene content, measured both by HPLC-DAD and by spectrophotometric approach, the volatile profile and the microbial activity, have been tested for a time frame of 110 days, assessing its durability. Carotenoid content decreased over time by about 50% from T0 to T3 (110 days), with promising results compared to traditional drying techniques, which allow to obtain the same results immediately after treatment.
Concerning the production of hemp essential oils, a prototypal distillation system has been used to extract essential oils (EO) from green residues, inflorescences after CBD extraction, of *Cannabis sativa* L. Such a flavouring technique is now under evaluation for a possible patent. The obtained yield (0.19%) of essential oil is coherent with the literature (Zheljazkov et al., 2020). A commercial hemp seed oil has been flavoured using the previously mentioned essential oil extract, as well as a commercial extra virgin olive oil has been flavoured using orange pomace (the by-product obtained from juicing) and canned tomatoes, both by infusion.
The produced oils have been tested to evaluate their quality; several sensory and compositional parameters have been investigated (e.g. consumer tests with a focus on preferences, total phenols content, carotenoids content, tocopherols and volatile profile). Free acidity has been assessed for each vegetable oil sample, including the control samples, and each of them has shown values below the limits in the regulations and standard (Codex Stan 210-1999 and EU regulation 2022/2104), with values ranging from 0.34 ± 0.0 % to 0.45 ± 0.11 % of oleic acid for olive oils, and 1.72 ± 0.13mg KOH/g of hemp seed oil and 1.42 ± 0.13 mg KOH/g of hemp seed oils.

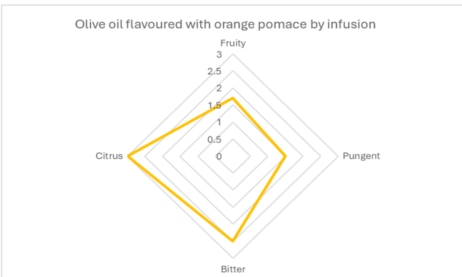

Figure 2 *Radar graph: intensity of the main and secondary positive attributes of a produced olive oil flavoured with orange pomace.*

Flavoured olive oils are not included in the commercial categories extra virgin, virgin or lampante, since they are "dressings based on olive oil", so they do not meet the definition stated in EU regulation 2022/2104; the levels of free acidity, in this case, provides anyway information regarding their quality.
The flavoured olive oils were characterized by peculiar sensory notes, assessed by descriptive analysis, derived from the added vegetable matrices (example in Figure 2), which have been related to the volatile compound composition determined through SPME-GC-MS).
Semi-quantitative analysis of the volatile compounds profiles has been carried out on hemp seed oil and flavoured hemp seed oil; it has been possible to highlight some differences related to the presence of the essential oils from hemp by-product. In particular, from the heat map shown in Figure 3, some terpenes (monoterpenes and sesquiterpenes) appear at higher concentrations in the flavoured hemp seed oil.
These compounds, such as β-myrcene, linalool, limonene, α-terpineol and β-caryophyllene are related to fruity, floral, sweet and sensory notes and anti-inflammatory and antioxidant properties have been found in the literature (Surendran et al., 2021; Tura et al., 2023).

In addition, from the results of a consumer tests (which has taken place at the DISTAL in Bologna and Cesena), there are no differences considering the liking of the aroma of the two hemp seed oils (HTQ control sample and HFL the flavoured one); while participants when asked to identify their favourite sample in a 2-AFC test, significantly resulted to prefer the flavoured one (n = 149, p = 0.007, α = 0.05). This finding, among others, will be verified with the further consumer tests data elaboration regarding the attributes of the samples (CATA and JAR tests).

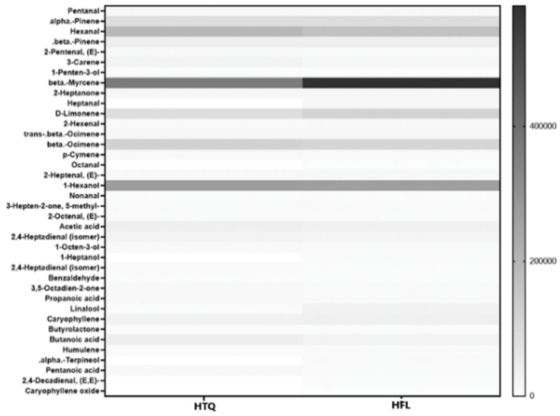

Figure 3 *Heat map comparing the content of the volatile compounds in the two hemp seed oils (HTQ, control sample of hemp seed oil, and HFL, flavoured hemp seed oil).*

Moreover, during my research period abroad, taken place thanks to the Erasmus + programme in Denmark at the Copenhagen Business School (CBS), the joint sensory and biometric test concerned all the prepared flavoured oils and their control samples (consumers, n=100). Among the three considered different biometric parameters analyzed during the entire sensory test (pupils movement and dilation with eye-tracker, galvanic skin response and conductance with Shimmer GSR and facial expression coding with AFFECTIVA AFFDEX), only the facial expression coding, able to detect the different emotions while tasting, was significant according to ANOVA analysis among samples. Indeed, two groups of consumers, one control group (assessing the oils blindly) and the other informed (receiving information about the samples, e.g. production and flavouring agents) were asked to taste the five different oils expressing liking and the intensity of some peculiar attributes. The informed group showed to pay particular attention to the description of the oils and their production processes (Figure 4). There were no significant differences considering the liking of every sample and between the two groups; in addition, the description of the oils has probably been the cause of the emotions flattening, which, instead, has been a significant, according to ANOVA analysis, biometric parameter for the control group. As it is possible to see in Figure 5, the control group showed to higher liking, from an olfactory point of view, the olive oil control sample (O65), the canned tomato flavoured olive oil (T48) and orange pomace flavoured olive oil (G19), the two hemp seed oils (P71 as the flavoured one and H32 as control sample) showed lower liking rates also for the informed group. For the control group among the detected emotions, engagement levels showed significant differences among the samples in accordance with the mean liking scores: higher levels of engagement correspond to the samples T48, G19 and O65, while the lowest were related to assessing of P71 and H32.

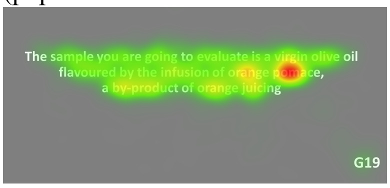

Figure 4 *Heatmap showing levels of attention and the time spent by the pupils on the screen. Red spots correspond to higher attention.*

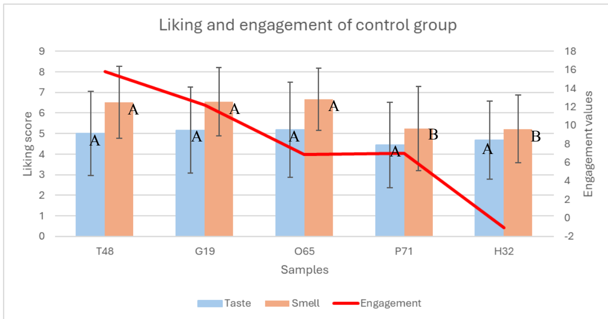

Figure 5 *Bar chart related to the liking and engagement levels of the control group. Capital letters refer to ANOVA analysis of liking (test HSD-Tukey, α=0,05)*

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

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

Cesare Ravagli (cesare.ravagli2@unibo.it)
Department of Agricultural and Food science, *Alma Mater Studiorum* – University of Bologna
PhD programme: agricultural, environmental and food science and technology
Research topic: Food Science and Biotechnology; PhD cycle: XXXVII; year: III
Tutor: Maria Fiorenza Caboni; Co-tutor: Federica Pasini, Silvia Marzocchi, Cristina di Cori

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. Another trend that strictly collides with the research of more sustainable fats and oils is represented by the necessity of developing innovative rapid, non-destructive analysis, to reduce the environmental impact of the laboratorial operations and to give to the operator quicker and simpler tasks in order to assess the quality of foods. In this context the use of Near Infrared (NIR) radiations is proving to be an effective instrument, given their high penetrability and the possibility of using them on minimally processed samples (Jr, Workman, and Weyer 2007).

However, to achieve this goals, 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.

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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 (Kiralan et al. 2019). 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 (Marzocchi et al. 2022) (Lajnef et al. 2017), 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. Following on the schedule a quick non-destructive approach for the vegetable oils to be implemented was developed mainly during the exchange period with the company designated for the PhD project (Bunge Italia s.r.l., Italy, Ravenna). For the purpose of having a rapid discriminating system, capable of assessing the quality of vegetable oils, a Near infrared (NIR) spectroscopic system was developed, in order to qualify the fatty acids, present in multiple oils within seconds. The system was developed using 120 samples of fresh vegetable oils of various types, namely: sunflower oil, both high oleic and low oleic, grapeseed oil, corn oil, soy oil, peanut oil and different blends of the aforesaid. The system developed various calibration curves, useful for the analysis of quality parameters, such as free acidity, red colour, and yellow colour, plus a partial discrimination of the fatty acids present, namely Stearic, Palmitic, Oleic, Linoleic, Linolenic, Eicosanoic, Arachic, Beenic and Lignoceric acids. The results were compromised in a series of calibration curves that demonstrated a determination coefficient (R^2) ranging from 0.920 to 0.999 based on the parameter, and a validation set ranging from 0.912 to 0.999. The results obtained indicated a reliable method capable of assessing the general quality of oils in rapid, simple, and more sustainable analysis, on which is possible to evaluate the quantity and quality of oil blends useful for the formulation of baked products.

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

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Pasini F, Marzocchi S, Ravagli C, Messia MC, Caboni MF (2022) Studio di shelf-life di biscotti con miscele lipidiche diverse - ELECTRONIC. - (2022), pp. 50-50. 12° Congress AISTEC "Cereali e Scienza: resilienza, sostenibilità e innovazione" Portici, Naples (Italy), 15-17 June 2022.

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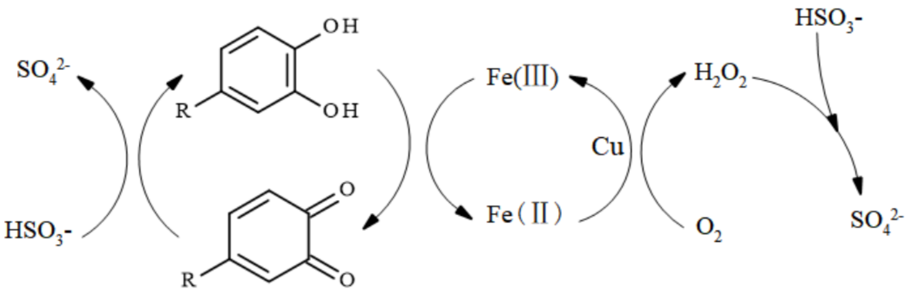
Exploring the influence of redox chemistry as driver in precision winemaking

Guanghao Wang (email: guanghao.wang2@unibo.it)
Dipartimento di Scienze e Tecnologie Agro-Alimentari, *Alma Mater Studiorum* - Università di Bologna
Corso di Dottorato: Scienze e Tecnologie Agrarie, Ambientali e Alimentari
Tematica: Scienze e Biotecnologie degli Alimenti; Ciclo di dottorato: XXXVII; Anno di frequenza: III
Tutor: Andrea Versari; Co-tutor: Arianna Ricci, Giuseppina P. Parpinello

1. Stato dell'arte

Wine oxidation affects the sensory characteristics of wine, such as color and aroma, which in turn affects consumer choice or acceptance of wine. Generally, controlled oxidation in red wines stabilizes the color, reduces the astringency and can improves the aroma profile, but for white wines, winemakers try to avoid the negative characteristics of oxidation, such as browning. The crucial importance of producing wines that are free of defects and have sensory characteristics that appeal to consumers is largely related to wine oxidation. More and more research is focusing on understanding wine oxidation to produce consumer-appealing wine products.
Wine oxidation can be classified into enzymatic and non-enzymatic oxidation (Li et al., 2008). In general, enzymatic oxidation may occur in the early stages of grape pressing and it is facilitated primarily by oxidoreductases. As the alcoholic fermentation advances and ethanol produced, the activity of oxidoreductases is gradually suppressed (Zhao et al., 2023). Subsequently, non-enzymatic oxidation, primarily manifesting during the aging and post-bottling phases, emerges as the predominant factor influencing the sensory characteristics of wine (Kilmartin, 2022), which is an essential factor influencing consumers' choice of wine.
Non-enzymatic oxidation occurs when polyphenol, oxygen, and transition metals as ligand are present at the same time (Danilewicz, 2003). Since they are prevalent in wine, this oxidation reaction is universally present in wine. SO_2 has been successfully used as an antioxidant to protect wines from oxidation. The above processes constitute the initial stages of non-enzymatic oxidation of wine as described in Scheme 1.



Scheme 1. Initial reaction mechanism of non-enzymatic oxidation of wine (Danilewicz, 2003)

The presence of oxygen significantly influences wine by generating H_2O_2 and quinone (formed by oxidizing polyphenols through the presence of iron), which can further oxidize other substrates or form color compounds, thereby impacting the color and aroma profiles of the wine (Zhao et al., 2023). The addition of SO_2 serves as a preventive measure against oxygen-induced wine oxidation by reacting with H_2O_2 and quinone. Quantitatively, 1 mol of SO_2 can react with 1 mol of H_2O_2 and 1 mol of quinone. Theoretically, a 2:1 ratio of SO_2 to O_2 is suggested, a finding substantiated in model wines (Danilewicz et al., 2008). However, in real wines, this ratio occasionally deviates from the anticipated 2:1, likely attributed to the inherent complexity of actual wine compositions (Danilewicz, 2016). SO_2 emerges as a potential predictor of wine shelf life, underscoring its significance in preserving wine quality.
The general change in the metal species during the oxidative and reductive aging of wine is not well understand and variation in results was observed in model wine and real wine. For example, Berg and Akiyoshi (1956) reported additive effects of iron and copper in white wine, while a more recent investigation by (Danilewicz, 2007) identified synergistic effects in model wine. Previous studies have confirmed that both iron and copper can expedite the production of xanthylium derivatives but their catalytic effects in autoxidation differ (Clark & Scollary, 2002). Clark and Scollary (2002) discovered that the browning rate was not strictly proportional to the concentration of Cu (II) in the model solution. It only exerted an impact when its concentration reached a certain level. In contrast to copper, iron exhibits higher efficacy in catalyzing the formation of xanthylium cations from (+)-catechin and tartaric acid (George et al., 2008).

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3. Sviluppo della ricerca

The overall objective of this PhD project is to investigate the effect of redox reactions on wine composition. 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 ageing, 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, etc.
A4) Experimental trials on real white wines to improve its 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.

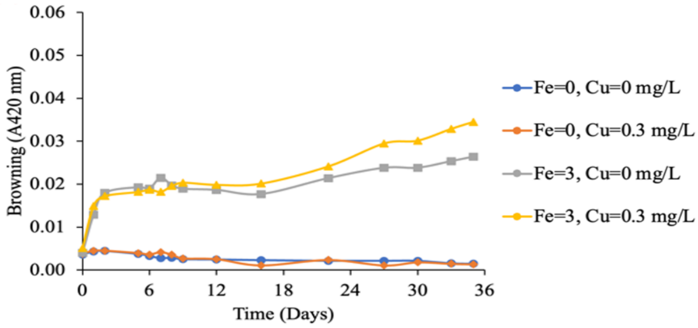
Table 1. Gantt Chart for the research activities in scope of doctoral study

Activities	Month	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. Principali risultati

Transition metals play an irreplaceable role in wine oxidation, and wine oxidation would hardly occur without transition metals such as iron (Fe) and copper (Cu). Different conclusions on the interaction of Fe and Cu have emerged from previous studies as described in State of Art. The interaction between Fe and Cu was explored in the model wine. In the presence of polyphenols, the browning (AU_{420nm}) trend was monitored by adding different amounts of Fe and Cu to simulate the catalytic effects of Fe, Cu, and both on wine oxidation, as shown in **Figure 1**. When no metal (Fe & Cu = 0) and only copper (Fe = 0, Cu = 0.3 mg/L) was added, no significant change in absorbance values was observed, indicating an absence of reaction over the 35-day period in each solution. The rate of browning was almost zero in the absence of transition metals. Subsequent experiments involving only 3 mg/L of iron exhibited lower absorbance values (0.025 AU_{420nm}) compared to solutions containing both Fe and Cu (0.035 AU_{420nm}). The presence of Fe overcomes the structural barrier that prevents polyphenols and oxygen from reacting (Pauli's exclusion principle), and the presence of Cu can facilitate this reaction.

Figure 1. Effect of the Fe and Cu on the 420 nm absorbance of the model white wine in the presence of SO_2 (45 mg/L) during storage at 35°C, in the dark.



An insightful study of Ferric Ion Reducing Antioxidant Power (FRAP), an antioxidant capacity assay, was carried out.

1. Time point of determination of FRAP.
   The time point for FRAP time measurement was determined. When antioxidants are added to the FRAP reagent, the absorbance increases with time. This is due to: (I) The reaction of active hydroxyl groups with Fe^{3+}-TPTZ, which is widely accepted as the mechanism of polyphenols oxidation in wine (as shown in Fig. 2). (II) polymerization may occur during the oxidation process, which may regenerate oxidizable phenolic hydroxyl groups. Considering the simulation of oxidation in the wine environment, fix-time measurement was adopted.

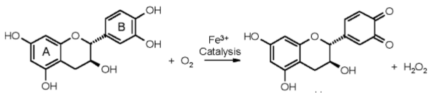


Figure 2 Oxidation of (+)-catechin in wine

   FRAP assay was carried out for typical phenolic compounds in wine (i.e. catechin, epicatechin, caffeic acid, gallic acid), where absorbance was read every minute and calculated to obtain stoichiometry. 6 min was utilized as the time of determination according to stoichiometry results.

2. Factors affecting FRAP assay in model wine.
   The components of model wine include ethanol, tartaric acid, polyphenols and SO_2. Preliminary experiments showed that ethanol has no effect on FRAP experiments. Subsequently, a 2^3 full factorial design was employed to explore the factors that have an impact on FRAP assay, including tartaric acid, polyphenols, and SO_2. The results of the experimental design are shown in **Table 2**.

Table 2. The results of 2^3 factorial design

N	Tartaric acid (g/L)	Catechin (mg/L)	SO_2 (mg/L)
1	0	0	0
2	2	0	0
3	0	30	0
4	2	30	0
5	0	0	20
6	2	0	20
7	0	30	20
8	2	30	20

   The analysis of variance (ANOVA) showed that the effect of the three components of the model wine on the FRAP results was significant ($p<0.05$). And there was an interaction between polyphenols and SO_2 ($p<0.05$).

3. Polyphenol and SO_2 in FRAP assay
   Polyphenols in wine can be categorized into reversible and irreversible polyphenols. They all react with the FRAP reagent and increase the absorbance at 593 nm. SO_2 can also react with FRAP reagent to form Fe^{2+}-TPTZ, increasing the absorbance (data not shown). For exploring the simultaneous presence of polyphenols and SO_2 in FRAP assay, SO_2 was added to the aqueous polyphenol solution in a molarity ratio (0, 0.5, 1, 1.5, 2, 3), where treatment with a ratio of 0 (i.e. polyphenol only) was considered control. The amount of Fe^{2+} produced by each treatment was calculated. SO_2 was able to increase the amount of Fe^{2+} produced, but this increase exceeded the amount of Fe^{2+} produced by SO_2 itself in the FRAP experiment compared to the control treatment. Further analysis revealed that the Fe^{2+} from addition of SO_2 was equimolar to the that of polyphenols. SO_2 is usually added as an antioxidant during

wine production, and its mechanism is currently thought to be the scavenging of H_2O_2 produced during wine oxidation and reaction with carbonyl compounds (e.g., quinone). In the context of the role of SO_2 as antioxidant, it can be hypothesized that SO_2 is able to, in FRAP, promote polyphenol redox, which enhances the production of Fe^{2+}. **Table 3** shows the interaction between catechin and SO_2 in the FRAP assay as a representative of reversible polyphenol. Irreversible polyphenols did not show the same interaction with SO_2.

Table 3. Interaction between catechin and SO_2

	concentration of polyphenol(μM)	SO_2 μM add	abs 593nm	Fe^{2+} produced (by calibration curve)	ratio of Fe^{2+} produced (compared to zero-SO_2 sample)	ratio of Fe^{2+} theoretically
catechin	100.77	0	0.144	146.38	-	-
	100.77	50	0.215	235.38	1.61	1.50
	100.77	100	0.279	315.13	2.15	1.99
	100.77	150	0.348	401.63	2.74	2.49
	100.77	200	0.403	470.38	3.21	2.98
	100.77	300	0.525	623.25	4.26	3.98

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

Wang G, Kumar Y (2024) Mechanisms of the initial stage of non-enzymatic oxidation of wine: A mini review, Journal of Food Science, 1–16.