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Application of novel technologies to elucidate new structural and shelf-life information in high protein bakery products (HighPBreads).

Thesis presented by

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For the degree of

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Declaration

I, Mariana Geraldes Maçãs, hereby declare that the work presented in this thesis is entirely my own, and has not been submitted, either in whole or in part, for any other degree or qualification at University College Cork, or any other institution. All references to other authors, sources of information, or previously published work have been clearly acknowledged and appropriately cited. I confirm that I have read and understood the regulations of University College Cork regarding plagiarism, research integrity, and intellectual property, and have complied with these standards throughout the course of this research.

Mariana Geraldes Maçãs

November 2025

Abstract

The transition towards more sustainable and nutritionally balanced food systems requires the valorisation of locally produced plant proteins. Peas are well adapted to the Irish climate and contribute to sustainable agriculture through their ability to fix atmospheric nitrogen and improve soil fertility, thereby reducing the need for synthetic fertilisers. They are also rich in protein, dietary fibre, and essential minerals. However, in Ireland, pea cultivation is primarily directed towards animal feed. Expanding their use in human food applications could improve local protein self-sufficiency and contribute to national climate and sustainability targets. Despite limited research on their functionality in bakery systems, Irish-grown peas represent a promising, locally sourced ingredient for the development of high-protein, fibre-rich, and environmentally sustainable bread products. This PhD research addressed these gaps by investigating the optimisation of the milling process and the improvement of flour functionality and flavour specifically for bread applications. In addition, the nutritional behaviour of the resulting breads was investigated through dynamic in vitro digestion studies.

A comprehensive review and meta-analysis initially established the scientific landscape on pulse flours in breadmaking, showing that processing methods such as milling, roasting, ultrasound and microwave can modify protein and starch structures, improve functionality and influence sensory quality. The review revealed that milling choices are often driven by equipment availability rather than functionality, and that while roasting, ultrasound and microwave are promising, their combined effects on bread quality and digestibility remain underexplored. These insights guided the experimental work of this thesis, focused on optimising the processing of Irish-grown peas gains and evaluating their flour techno-functional, structural and nutritional performance in bread systems.

Building on this foundation, experimental work was performed using Irish-grown peas to evaluate how milling techniques affect flour functionality, dough rheology and bread quality. Comparative studies on roller, hammer and cutting mills demonstrated that pea flour increased the protein content of bread, and considering the milling yield and bread characteristics, hammer milling proved to be the most effective method for breadmaking. Near-infrared spectroscopy (NIR) was employed as a rapid, non-destructive tool to characterise both flours and breads. It successfully distinguished formulations with similar compositions and classified breads according to storage time independent of their composition demonstrating strong potential for product standardisation, process-monitoring and staling assessment in bakery industry and retail.

Subsequent studies focused on advanced processing treatments, roasting (R), ultrasound (US) and microwave (MW), applied individually and in combination to Irish pea grains. These methods significantly altered flour microstructure, water absorption and carbohydrate-protein interactions on the bread matrix. Roasting modified starch gelatinisation and protein structure and enhanced flavour, whereas the combined US+MW+R treatment induced the most extensive microstructural breakdown , as evidenced by SEM. Incorporating these processed flours into bread formulations (35:65 pea:wheat) revealed that combined US+MW+R processing improved loaf volume, crumb softness and flavour complexity while maintaining high protein and fibre content, demonstrating the feasibility of producing nutritionally enriched breads with improved technological performance.

To understand the nutritional implications, the digestion behaviour of these breads was evaluated using a Dynamic In Vitro Human Stomach System (DIVHS-IV). Pea inclusion markedly reduced starch release and glycaemic potential relative to wheat bread, particularly for the bread containing pea flour treated by US+MW+R. The untreated Irish pea flour produced the highest protein hydrolysis. Digestion studies confirmed that the higher protein

and fibre contents of the pea-enriched breads enhanced protein bio accessibility while slowing starch release.

Overall, this research provides an integrated understanding of how processing and milling strategies influence the functional, structural and nutritional properties of pea-enriched breads. The findings demonstrate that Irish-grown peas can serve as a sustainable, high-quality ingredient for bakery applications, supporting local agriculture, reducing reliance on imported proteins, and promoting dietary transitions towards healthier, high in protein and lower-glycaemic foods. Furthermore, the use of advanced processing and rapid spectral tools presents scalable solutions for optimising legumes and bakery products within the circular agri-food economy. Together, these outcomes contribute to the scientific advancement of pulse-based ingredient technology and reinforce the strategic potential of Irish legumes in achieving sustainable food innovation.

Abbreviations

Abbreviation	Meaning
2D/3D	Two-dimensional/three-dimensional
AAA	Amino acid analysis
AACCI	American association of cereal chemists international
AMDIS	Automated mass spectral deconvolution and identification system
ATR	Attenuated total reflection
AUC	Area under the curve
BCA	Bicinchoninic acid
BSA	Bovine serum albumin
C-cell	Computerised cell structure analysis
CLSM	Confocal laser scanning microscopy
CM	Cutting mill
CP	Commercial pea flour
CP 35%	Commercial pea flour: wheat flour blend (35:65)
cRAP	Common repository of adventitious proteins
DIVHS-IV	Dynamic in vitro human stomach system
DNS	3,5-dinitrosalicylic acid method
GI	Glycaemic index
eGI	Estimated glycaemic index
ELISA	Enzyme-linked immunosorbent assay
FA	Free amino acids
FCF	Fast green (dye)
FDR	False discovery rate
FITC	Fluorescein isothiocyanate
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC-MS	Gas chromatography–mass spectrometry
GS1/GS2	Ion source gas 1 / ion source gas 2
HPP	High-pressure processing
HM	Hammer mill
HI	Hydrolysis index
HPLC	High-performance liquid chromatography
IP	Irish pea flour (untreated)
IP 35%	Irish pea flour: wheat flour blend (35:65)
IP+R	Irish pea flour + roasting
IP+R 35%	Irish pea flour + roasting: wheat flour (35:65)
IP+US+R	Irish pea flour + ultrasound + roasting
IP+US+R 35%	Irish pea flour + ultrasound + roasting: wheat flour (35:65)
IP+MW+R	Irish pea flour + microwave + roasting
IP+MW+R 35%	Irish pea flour + microwave + roasting: wheat flour (35:65)
IP+US+MW+R	Irish pea flour + ultrasound + microwave + roasting
IP+US+MW+R 35%	Irish pea flour + ultrasound + microwave + roasting: wheat flour (35:65)
LCA	Life cycle assessment

LC-MS/MS	Liquid chromatography–tandem Mass spectrometry
MRM	Multiple reaction monitoring
MIR	Middle Infrared
MS/MS	Tandem mass spectrometry
MW	Microwave
NIR	Near-infrared spectroscopy
OH	Ohmic heating
PCA	Principal component analysis
PC1, PC2, PC3	Principal components 1, 2 and 3
PCR	Polynomial chain reaction
PEF	Pulsed electric field
Q1 → Q3	Quadrupole 1 to quadrupole 3 (mass transitions in a triple-quadrupole MS)
R	Roasting
RVA	Rapid visco analyser
RM	Roller mill
SEM	Scanning electron microscopy
SNV	Standard normal variate
SNVD	Standard normal variate with detrending
TPA	Texture profile analysis
UHPLC-FLD	Ultra-high-performance liquid chromatography with fluorescence detection
UPLC	Ultraperformance liquid chromatography
US	Ultrasound
W	Wheat flour
WAI	Water absorption index
WSI	Water solubility index
WT	Wheat

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List of publications emerging from this thesis

Peer-reviewed manuscripts (published)

1. Mariana Maçãs, Alessandro Ferragina, Bárbara Biduski, Karen Hussey, Nooshin Vahedikia, Elke Arendt, and Eimear Gallagher. 2023. *A study of the milling process of Irish-grown peas: NIR spectroscopy, flour pasting properties and dough rheology.* Food Structure, 38: 100351. DOI: https://doi.org/10.1016/j.foostr.2023.100351
2. Mariana Maçãs, Bárbara Biduski, Alessandro Ferragina, Antonia AL dos Santos, Melanie Huet, Elke K Arendt, Eimear Gallagher. 2024. *Impact of conventional and emerging processing methods on alternative breads–a comprehensive review and meta-analysis*. Critical Reviews in Food Science and Nutrition. DOI: https://doi.org/10.1016/j.foostr.2023.100351
3. Mariana Maçãs, Barbara Biduski, Alessandro Ferragina, Antonia AL dos Santos, Jeremy C. Simpson, Margaritha M. Mysior, Mahesha M. Poojary, Elke K. Arendt and Eimear Gallagher. 2025. *Pea-wheat composite bread: Effects of milling methods and NIR spectral insights.* Innovative Food Science and Emerging Technologies. Doi: https://doi.org/10.1016/j.ifset.2025.104233

Manuscripts submitted to journals (under review)

1. Mariana Maçãs, Barbara Biduski, Alessandro Ferragina, Laura Macaraque, Elizabeth Codjoe Elke K. Arendt and Eimear Gallagher. *Physical modifications of Irish-grown peas through advanced processing technologies*. Submitted to: Food Research International.

2. Mariana Maçãs, Barbara Biduski, Laura Macaraque, Kieran N. Kilcawley, David T. Mannion, Paula M O'Connor, Elizabeth Codjoe Elke K. Arendt and Eimear Gallagher. *Multi-model processing strategies for protein-enhanced pea bread*. Submitted to: Food hydrocolloids.
3. Mariana Maçãs, Xinyu Tan, Sudipta Kumar Hazra, Xianglu Zhu, Barbara Biduski1, Liming Zhao, Elke K. Arendt and Eimear Gallagher. *Advanced Dynamic In Vitro Digestion of Protein-enhanced Bread*. Submitted to: Future Foods.
4. Mariana Maçãs, Xinyu Tan, Sudipta Kumar Hazra, Xianglu Zhu, Barbara Biduski, Liming Zhao, Elke K. Arendt and Eimear Gallagher. *Advanced Dynamic In Vitro Digestion of Pea–Wheat Composite Bread: Influence of Roasting, Ultrasound, and Microwave Processing on Pea Flour*. Submitted to: Nature Foods

List of presentations at national and international conferences

Oral presentations

1. Mariana Maçãs, Alessandro Ferragina, Barbara Biduski, Karen Hussey, Elke K. Arendt and Eimear Gallagher on "*A new food application of Irish-grown peas: Milling, dough rheology, bread profiling and NIR spectroscopy"* at IFSTI- 51st Annual Food Science and Technology Conference, Dublin, Ireland, 2023
2. Mariana Maçãs, Alessandro Ferragina, Barbara Biduski, Karen Hussey, Elke K. Arendt and Eimear Gallagher on *"Novel Food Research Approaches for Irish-Grown Peas: Milling, Product Profiling and NIR Spectroscopy"* at IUFOST- 22nd World Congress of Food Science and Technology, Rimini, Italy, 2024

3. Mariana Maçãs, Barbara Biduski, Laura G. Gomez-Mascaraque, Kieran N. Kilcawley, David T. Mannion, Paula M O'Connor, Elke K. Arendt and Eimear Gallagher on *"Advanced Processing Approaches for Protein-Fortified Bread: Influence on Bread Quality and Flavour Properties"* at ICFP6, International Conference on Food properties, Bangkok, Thailand

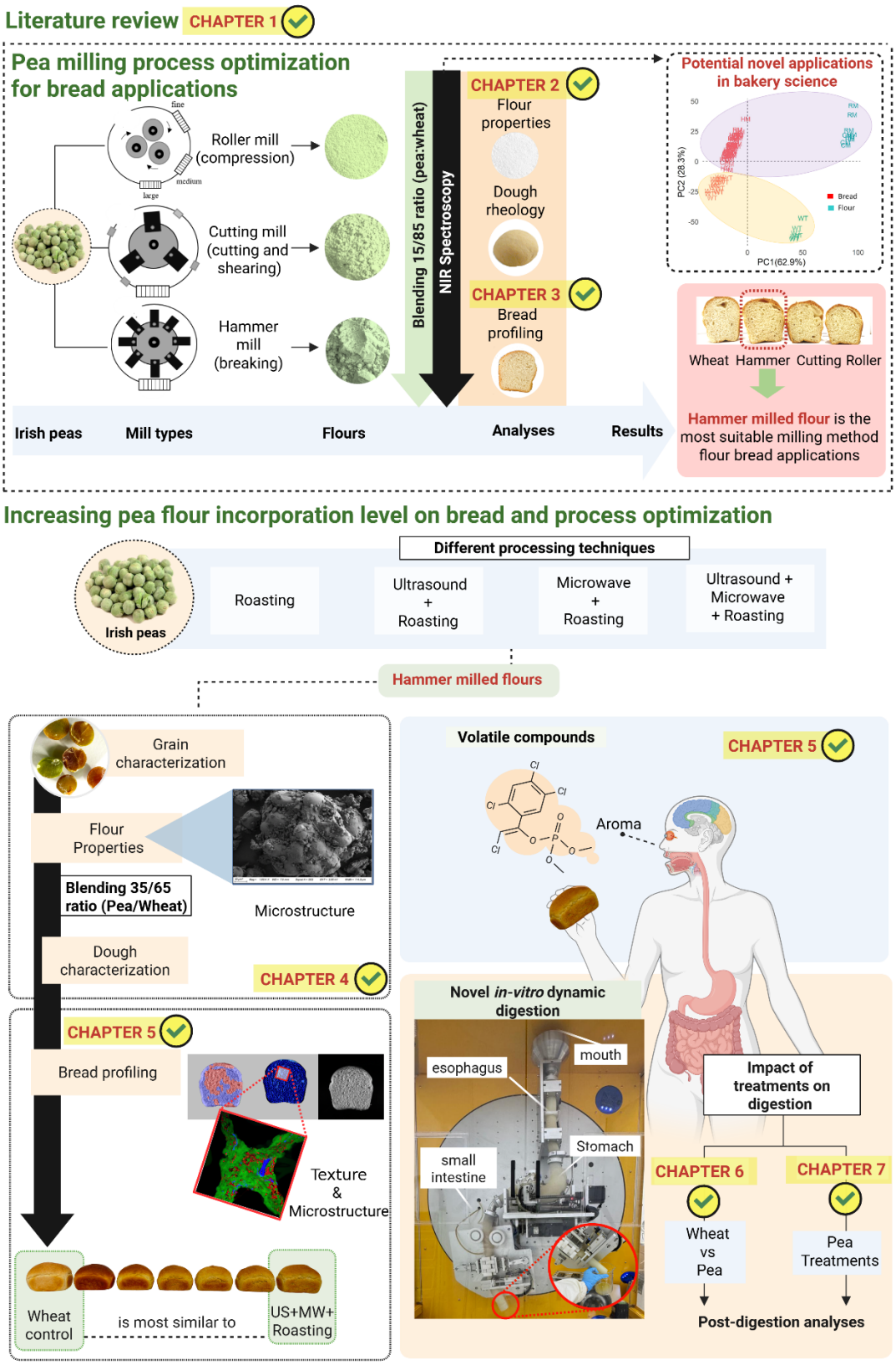
Poster presentations

4. Mariana Maçãs, Barbara Biduski, Mateo Grenier, Karen Hussey, Alessandro Ferragina, Elke Arendt and Eimear Gallagher, on *"Influence of milling processes of Irish-grown peas on the rheology and pasting properties of resulting bread doughs"* at 5th Food Structure and Functionality Symposium , Cork, Ireland, 2022.
5. Mariana Maçãs, Barbara Biduski, Mateo Grenier, Karen Hussey, Alessandro Ferragina, Elke Arendt and Eimear Gallagher, on "*A study of the milling process of Irish-grown peas for bread applications using rheology, texture profiling and NIR spectroscopy*" at 50th Annual Food Science and Technology Conference at University College, Cork, Ireland, 2022

Awards

1. Oral presentation award (2nd place) at national IFSTI- 51st Annual Food Science and Technology Conference, Dublin, Ireland, 2023
2. Walsh Scholar International Training Award, 2024
3. 1st Place in the Teagasc Food Research Program at the Walsh Scholars of the Year national competition, 2025
4. SCI Seligman APV Travel Bursary award to attend International Conference on Food properties, 2025

Schematic representation of thesis structure



1 Impact of conventional and emerging processing methods on alternative breads - a comprehensive review and meta-analysis

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Impact of conventional and emerging processing methods on alternative breads– a comprehensive review and meta-analysis

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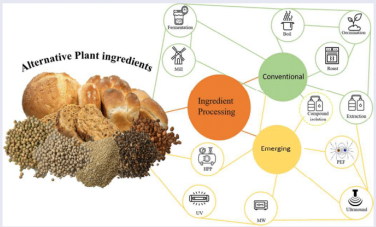
ABSTRACT

An increasing consumer demand for plant-based and high-protein options, motivated by health and sustainability, has resulted in a surge of food innovation in this area. Incorporating alternative plant sources, such as pulses and pseudocereals, has been proven to enhance the nutritional profile of baked products. However, these can also negatively impact the yeasted bread acceptability. In the bakery sector, it is crucial to consider how incorporating non-wheat ingredients influences product quality. Consequently, exploring effective treatments/processing methods becomes essential to minimize the impact of alternative plant ingredient additions. This review explores conventional and emerging processing approaches for alternative plant materials and discusses the nutritional value may be enhanced while maintaining high acceptability. A meta-analysis was undertaken to visualize the influence of plant processing technologies on product quality, specifically on loaf-specific volume and crumb texture. This review highlighted the importance of conventional processing methods when applied to bread. Additionally revealed the potential of emerging processing which can positively affect a loaf volume and texture when compared with non-processed plant ingredients. Such studies enabled the production of acceptable bakery products with higher levels of alternative ingredient incorporation. However, increased use of emerging technologies is dependent on further research and overcoming scaling-up difficulties.

KEYWORDS

Yeasted bread; alternative plant ingredient; bread processing; conventional and emerging processing; loaf specific volume; crumb texture

GRAPHICAL ABSTRACT



1. Introduction

Consumer demand for alternative plant-based ingredients, driven by health and sustainability concerns, is expanding innovation in protein and fiber enriched baked goods. (Biduski et al. 2024). Popular plant sources that are being widely studied are pulses (peas, beans, chickpeas, lupin, fava bean, cowpea) and pseudocereals (buckwheat, amaranth and quinoa) (Kahraman et al. 2022; Montemurro et al. 2019).

The use of unprocessed alternative plant sources in leavened bread can be constrained by low consumer acceptability (Wang and Jian 2022). Therefore, numerous studies have explored diverse processing technologies which may be applied to a broad array of plant ingredients, aiming to improve the textural and structural quality of the final bread product (Ouazib et al. 2016; Sopiwnyk et al. 2020; Sun et al. 2023). Conventional processing methods such as thermal processing, germination, fermentation and extraction have been shown to boost the

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1.1 Abstract

This chapter provides a comprehensive review of conventional and emerging processing approaches applied to alternative plant-based ingredients for yeasted breadmaking. It addresses the growing consumer demand for plant-based, high-protein bakery options driven by health and sustainability concerns and examines how the incorporation of pulses and pseudocereals, while nutritionally beneficial, can negatively impact bread quality and consumer acceptability. Special focus is placed on the effects of various treatments and processing technologies on loaf-specific volume and crumb texture. A meta-analysis was conducted to visualise the influence of plant ingredient processing on key bread quality attributes. The findings underscore the continued relevance of conventional processing methods and highlight the promising potential of emerging techniques to improve loaf volume and texture relative to non-processed alternatives. In addition, advanced analytical tools such as FTIR and NIR spectroscopy are discussed as rapid, non-destructive methods for monitoring structural, chemical, and quality changes in bakery products, thereby complementing processing innovations with precise quality control approaches. The insights derived provide a rationale and technical foundation for the experimental work detailed in the subsequent chapters of this thesis.

1.2 Introduction

Consumer demand for alternative plant-based ingredients, driven by health and sustainability concerns, is expanding innovation in products to produce protein and fibre enriched baked goods (Biduski et al., 2023). Popular plant sources that are being widely studied are pulses (peas, beans, chickpeas, lupin, fava bean, cowpea) and pseudocereals (buckwheat, amaranth and quinoa) (Kahraman et al., 2022; Montemurro et al., 2019).

The use of unprocessed alternative plant sources in leavened bread can be constrained by low consumer acceptability (Yaqin Wang & Ching Jian, 2022). Therefore, numerous studies have explored diverse processing technologies which may be applied to a broad array of plant ingredients, aiming to maximize their effectiveness in improving the textural and structural quality of the final bread product (M. Ouazib et al., 2016; Sopiwnyk et al., 2020; Sun et al., 2023). Conventional processing methods such as thermal processing, germination, fermentation and extraction have been shown to boost the nutritional profile, reduce the antinutrient content, improve taste and modify the functional properties of protein-enriched bread (Kahraman et al., 2022; Setia et al., 2020b; Villacrés et al., 2020; Xing et al., 2021)

Emerging green processing techniques, which typically involve less energy and water, such as ultrasound, microwave and pulse electric fields are expanding (Chemat et al., 2017). These technologies can have several positive effects, such as enhancing nutritional quality, extending microbial safety and modifying physicochemical properties (Amanda et al., 2022). However, despite the promising results obtained with these technologies, it is a dynamically developing area which still has limited industrial application due to machinery cost (Tiwari et al., 2021).

In bread making, the replacement of wheat flour with alternative plant ingredients requires a comprehensive knowledge of the techno-functional properties of flour, dough and bread due to the complexity of replacing the constituents of wheat flour. Although

incorporating high levels of nutritious ingredients is desirable for health reasons, partial replacement with alternative materials can usually be achieved only at lower levels to avoid compromising the bread quality characteristics, such as textural properties and taste (Hall, 2021b). The utilization of conventional and emerging processing technologies has opened new avenues of research for expanding the application of alternative plant sources in bread formulations, while maintaining the desired leavened bread crumb hardness and specific volume.

Alongside advances in processing strategies, reliable analytical methods are essential to understand the chemical, structural and functional changes that occur during breadmaking. Traditional analytical tools such as chromatography and immunoassays, while precise, often require destructive sampling and lengthy preparation (Wu & Sun, 2013). By contrast, rapid spectroscopic techniques—particularly Fourier Transform Infrared (FTIR) and Near-Infrared (NIR) spectroscopy—offer non-destructive, high-throughput ways to characterise dough and bread systems, monitor staling, detect contamination, and assess textural and nutritional attributes (Ringsted et al., 2017). These methods, often combined with chemometrics or machine learning, complement processing innovations by providing detailed insight into product quality and safety (Cebi, Bekiroglu, Erarslan, et al., 2023; Nielsen et al., 2001).

In this review, the term “alternative breads” refers to yeasted bread that incorporates partial or total substitution of wheat flour with non-wheat ingredients, including gluten-free options. The focus of this review is on conventional and emerging processing approaches for alternative plant ingredients that aim to enhance the nutritional value of bread while maintaining the high product quality standards expected by consumers. It reflects the current state of the art in bakery research strategies for incorporating higher levels of alternative plant ingredients. Particular emphasis is placed on volume and texture aspects, as they are critical parameters for consumer acceptance (Guiné, 2022).

1.3 Overview of bread as a food system

Leavened bread is a staple food which has been consumed in most cultures worldwide for thousands of years, with its main ingredient being wheat. Leavened bread is commonly composed of a mixture of flour, water, salt, yeast, and/or microorganisms that are combined to form a flexible and stretchy dough. This viscoelastic dough undergoes fermentation and is then baked (Goesaert et al., 2005). Important quality parameters for leavened bread include freshness, loaf specific volume, texture, taste, appearance, shelf life, nutritional content and product consistency (Clark & Aramouni, 2018; Dong & Karboune, 2021). Wheat bread typically has a soft elastic crumb with a crisp thin crust (Dong & Karboune, 2021). The aroma and taste should be well balanced and pleasant, with no undesirable off-flavours, and an attractive visual appearance (Longin et al., 2020).

1.3.1 Influence of wheat constituents

Wheat is the cereal crop most cultivated, consumed and traded in the world, most probably because it has several food applications and is adaptable to several environments and soils (Enghiad et al., 2017; Hoseney, 1994; Shewry, 2019). Wheat composition and functional properties will depend on factors such as the environment and variety (Rozbicki et al., 2015). Refined wheat flour consists of polysaccharides (70–75% starch and 2–3% dietary fibre), ≈14% water, 10–12% proteins, and ≈2% lipids (Goesaert et al., 2005).

Wheat starch granules have a bimodal size distribution, consisting of large (10–35 mm) lenticular and small (1–10 mm) spherical granules and the major constituents are amylose (25–28%) and amylopectin (72–75%) (Goesaert et al., 2009). During dough formation, the amylopectin semi-crystalline structure absorbs up to 46% of water acting as an inert filler within the continuous protein matrix of the dough, forming a network composed of both starch and protein (Goesaert et al., 2005). Throughout the baking process, the combination of heat,

moisture, and time leads to gelatinization of the starch, which plays a fundamental role in creating the structure of the bread (Hug-Iten et al., 1999). During the cooling process, amylose is a key factor in determining the initial firmness of the loaf, due to its rapid retrogradation (Goesaert et al., 2009). As storage proceeds, water migration and starch fraction transformations are the most important factors impacting bread freshness. This occurs mainly due to amylopectin retrogradation, in particular the formation of double helical structures and crystalline regions (Goesaert et al., 2005).

Wheat flour contains several proteins, in particular gliadins and glutenin, responsible for the development of the gluten network and the formation of an aerated crumb structure in products such as bread. These proteins have different structures and functionality and together they contribute to the unique viscoelastic and rheological properties of dough. Gliadin has shorter and more globular chains of low molecular weight and is soluble in 70% ethanol. Hydrated gliadin displays important flow properties and mainly confers extensible and stretchy properties to the dough. Glutenin is a long-chain protein aggregate with a high molecular weight, soluble in diluted acid and alkali solutions. Hydrated glutenin is extremely tough and primarily responsible for the elasticity of dough, providing the ability of the dough to return to its standard length after stretching (Buehler, 2006; Feng et al., 2020). The gluten network is primarily composed of hydrated glutenin and gliadin, connected through disulphide, hydrogen and hydrophobic bonds (Buehler, 2021). The strongest protein bonds are covalent disulphide (S—S) bonds and are responsible for the structural integrity of the gluten network during dough preparation playing an essential role in gluten formation and stability (Buehler, 2021; Preichardt & Gularte, 2013). The addition of other ingredients (e.g. salt, yeast, sugar, and butter), grain processing methods (e.g. milling, dehulling, germination, drying) and dough processing (e.g. mixing time, power and baking temperature) impact disulfide bonds which affect the gluten matrix (Both et al., 2021; X. Wang et al., 2022). During the baking process,

the applied heat converts the gluten from a viscoelastic state to a solid structure with open pores. As a result, its cohesion decreases a spongy texture is developed (Hug-Iten et al., 1999). The unique functional properties of gluten make it a vital component in aerated baked products (Biesiekierski, 2016; Sharma & Rallabhandi, 2015). Consequently, the gluten content quality of wheat plays a crucial role in bread textural properties. Wheat with a low gluten content is generally unsuitable for bread making and will produce weak dough, negatively affecting the loaf volume, whereas wheat with a higher gluten content will generally positively contribute to dough strength and loaf volume. (Geisslitz et al., 2018; Sharma & Rallabhandi, 2015). An optimal gluten content for yeasted bread flour is 14% as documented by Buehler (2021). In addition, Geisslitz et al. (2018) demonstrated that the differences in protein content and composition due to species and cultivars have an impact on baking quality.

1.3.2 Role of macronutrients and common ingredients in the bread matrix

Lipids, including wheat lipids, shortening, and surfactants, have various roles throughout the bread-making process. They act as gas cell interface stabilizers, influencing dough gas retention, which affects crumb structure, and bread firmness (Pareyt et al., 2011).

Salt, a common ingredient in wheat bread, is noteworthy because it slows down the fermentation process. Salt restricts water availability leading to yeast cell dehydration and reduced sugar uptake. In addition, the salt will also interact with the gluten, inducing changes in protein conformation. Salt increases dough strength by shielding gluten positive charges, which allows protein molecules to come together and tighten (Buehler, 2006).

The presence of fibre (whether from wheat or added sources) is also a determinant variable in bread making, affecting both dough processing and final product quality. During dough preparation, fibrous fragments can significantly impede protein association, which disrupts the viscoelastic network, producing weaker dough (Rosell et al., 2010). Additionally,

dietary fibre competes for water with the available starch. This impacts the water absorption and pasting properties of the dough (Rosell et al., 2010). Parameters such as bread yield, volume, moisture loss, texture, quality, colour taste and shelf life will be influenced by the presence of dietary fibre incorporation (Kurek, 2015). The influence of dietary fibre on the texture of bread is dependent on the origin of the fibre source. Its presence can lead to undesired outcomes such as increased crumb density and firmness, or conversely, it can enhance the overall stability and strength of the final product (Kurek, 2015). Regarding sensory attributes, dietary fibre has the potential to improve parameters such as creaminess and homogeneity. However, it can also have a negative impact on crumb acceptability (Kurek, 2015). It may be possible to increase the shelf life of the bread with the addition of dietary fibre by enhancing oil and water holding capacities consequently reducing syneresis (Kurek, 2015).

In addition to the key breadmaking ingredients, the breadmaking process also plays a crucial role. Processing methods, whether mechanical (kneading), thermal (freezing and cooking) or chemical (gluten network formation, enzymatic reactions, fermentation), will affect the final quality of the bread (Geisslitz et al., 2018; Sharma & Rallabhandi, 2015). According to Meerts et al. (2017), overmixing diminishes the strain-hardening behaviour of dough. During kneading, gluten proteins undergo extensive morphological changes until they reach optimal dough development, which occurs when the proteins form a continuous network. Over-mixing can lead to the breakage of disulphide bonds due to protein dispersion, thereby weakening the dough matrix. This will interfere with the fermentation process and the ability of the dough to retain gas cells, which compromises the final structure, such as the specific volume and crumb hardness of the bread (Meerts et al., 2017).

When formulating ‘alternative breads’, by introducing new ingredients into the formulation (such as pulses and pseudocereal) a deep understanding of the breadmaking

process and technological approaches are required to overcome the challenges of obtaining a nutritionally improved formulation that maintains the product's high-quality standards.

1.4 Irish-grown peas as a locally produced alternative food ingredient

Peas (*Pisum sativum L.*) are among the world's most widely produced legumes, accounting for approximately 26% of global legume production (Shen et al., 2022). They are nutritionally rich, containing 20–25% protein and substantial amounts of dietary fibre, making them suitable for enriching cereal-based products such as bread (Shen et al., 2022).

In Ireland, peas are cultivated on an estimated 800–1000 hectares of land each year, with production used for both animal feed and to a lesser extent, human consumption (Teagasc, 2025). Over 85% of the cereals and pulses produced in Ireland are directed towards animal feed (Merlo et al., 2025). Although current levels of human consumption are limited, Irish-grown peas represent a locally sourced protein and fibre rich ingredient with potential for food applications. Well adapted to the Irish climate and soils, peas contribute to sustainable agriculture by fixing atmospheric nitrogen and serving as a valuable break crop that enhances soil fertility and may increase yields of subsequent cereals (Merlo et al., 2025; Teagasc, 2025; Uebersax et al., 2023). This is particularly important, as increased local cultivation of protein crops can reduce reliance on imports, strengthen food system resilience, and contribute to achieving climate goals.

Although peas are a relatively new ingredient, they can be used to nutritionally enrich products, thereby creating value-added foods. They can have diverse applications across the food sector, including baked goods, meat and dairy alternatives, dressings, beverages and emulsified systems (Sengar et al., 2023). Pea flour, when incorporated into a bread matrix, exhibits different properties compared to wheat flour, thereby directly influencing dough rheology and final product quality. Nutritionally, breads enriched with pea flour tend to have a

higher protein content, a more balanced amino acid profile and a lower glycemic index (Uebersax et al., 2023). However, the lower levels of gluten present in pea-wheat breads often leads to reduced loaf volume and changes in texture and flavour (Young et al., 2019). Pre-processing treatments, such as roasting, fermentation and milling have shown to address these concerns, by improving dough handling and overall bread quality (Bourré et al., 2019; Guldiken et al., 2022; K. A. Millar et al., 2019; Nilufer-Erdil et al., 2012).

1.5 Alternative plant ingredients commonly used in bread

A growing percentage of consumers are adopting diets that are rich in plant sources, out of concern for health and well-being, sustainability, cost and ethics (e.g. animal welfare compassion). Popular nutritious plant sources that are currently being comprehensively researched are derived from pulses and pseudocereals. As already discussed, wheat is the most commonly used raw material to produce bread. This review covers a broad range of other plant ingredients that can be applied in conjunction with wheat or gluten-free applications in bread making. The review primarily focuses on pulses and pseudocereals, due to their high nutritional content and their prevalent use in current studies. These alternative plant sources can be incorporated in different forms, e.g. cooked, paste, flour, protein concentrates, protein isolates and fermented, into a wide range of food products (Belc et al., 2021; Bian et al., 2023; Kahraman et al., 2022; Sozer et al., 2019).

Pulses are defined by the Codex Alimentarius Commission as dry seeds of leguminous plants with low fat content (Commission, 2019). The consumption of pulses dates back to ancient times, and they continue to serve as a staple food in many countries worldwide. After cereal grains, pulses are the second most consumed food all over the world (Singh, 2017). Pulses represent green farming practices due to their ability to fix nitrogen in the soil and

prevent soil erosion, with potential yields of up to 200 kg N/ha per season (Calles et al., 2019; Ranjith & Sridevi, 2021).

Black beans, kidney beans, chickpeas, lentils, peas, soy, horse gram, cowpea, lupin and fava beans are frequently discussed as valuable sources of protein, due to their protein qualities/quantity, nutritional content and affordable price (Yvonne Maphosa & Victoria A. Jideani, 2017). They are also a good source of complex carbohydrates (± 60%, of which 5% to 37% are dietary fibre), protein (20% to 45%) with essential amino acids, minerals (e.g. iron and potassium) and B-complex vitamins. They are also low in fat (± 5%), apart from peanuts (± 45%), chickpeas (± 15%) and soy (± 47%) which are good sources of unsaturated fats. Therefore, pulses can be considered a suitable source for high-protein baked goods, cereal bars and crackers (Yvonne Maphosa & Victoria A. Jideani, 2017).

Pseudocereals are dicotyledons that produce seeds with a similar starch content and physical appearance to cereals (Graziano et al., 2022). The most common pseudocereals are quinoa (*Chenopodium quinoa Willd*), amaranth (*Amaranthus L. spp.*), and buckwheat (*Fagopyrum esculentum Moench and Fagopyrum tataricum (L.) Gaertn*) (Graziano et al., 2022). Pseudocereals have attracted attention due to their highly nutritious profile, lack of gluten, abundance of starch, and their ability, similar to cereals, to produce flour suitable for making pasta and baked goods (Graziano et al., 2022). These grains are significant sources of proteins, lipids, carbohydrates, vitamins, minerals and bioactive components (Alvarez-Jubete et al., 2009). Their protein content varies from 10 to 18 g/100 g, with a well-balanced presence of essential amino acids, such as lysine, threonine, methionine and tryptophan (Thakur et al., 2021). They also contain high levels of unsaturated fatty acids that can help prevent cardiovascular disease (Thakur et al., 2021).

The overall nutritional profile of pulses and pseudocereals is richer than conventional cereals such as wheat, maize and rice. However, despite the abundant nutritional benefits, these

grains also contain compounds with anti-nutritional properties (Derbyshire & Delange, 2021; Pratap et al., 2022). These compounds can disrupt protein digestion (e.g. trypsin and chymotrypsin inhibitors), decrease mineral absorption (e.g. lectins, phytates and oxalates), and inhibit starch digestion (i.e. amylase inhibitors and saponins) (Bessada et al., 2019; Patterson et al., 2017). The anti-nutrient level is dependent on genotype, environment and growth location (Singh et al., 2017). Breeding studies have been undertaken to reduce/eliminate anti-nutrients, however, grain processing is still the most efficient way to eliminate these compounds (Patterson et al., 2017). Conventional processing methods, including heat treatment, dehulling, popping, soaking, fermentation and germination, as well as emerging techniques such as microwave, micronization and irradiation, can be employed to decrease the levels of antinutritional compounds present in legumes and pseudocereals, thus enhancing their suitability for consumption (Amare et al., 2016; Patterson et al., 2017).

Techno-functional properties that pulses and pseudocereals exhibit include solubility, water and fat absorption capacity, foaming capacity and foam stability, emulsifying activity and gel-forming (Bessada et al., 2019; Langyan et al., 2023). These functional properties will differ among pulses and pseudocereals due to differences in protein molecular make-up (amino acid composition, molecular weight, structure, charge distribution), protein interaction with other molecules, processing method and environmental factors such as weather conditions and soil quality (Bessada et al., 2019; Langyan et al., 2023).

A significant body of ongoing research is investigating the acceptability of these pulses and pseudocereals in baked goods. Recent studies reported that the inclusion of broad beans, yellow peas, and green peas into a biscuit formulation increased their nutritional content, and the incorporation of pea flours in bread increased the protein content from 8.4% to ≈10.5% (Millar et al., 2017; Kim Adrienne Millar et al., 2019). Maia et al. (2021) investigated the inclusion of 21% of pulse flours (mangalo, cowpea and pigeon pea) in cereal bars. Sensory

analysis revealed satisfactory acceptability among panellists. According to a study by Wronkowska et al. (2019), significant differences were found in the intensity of the sensory properties of bread containing buckwheat flour (13%) versus a wheat bread control, including sweet odour, bread crust odour, crust and crumb colour, pore distribution, “sand-feeling” texture. However, such differences did not negatively impact consumer acceptance.

Alternative plant sources have also proven to be well accepted in gluten-free formulations, effectively enriching breads that typically lack protein and fibre, as gluten-free staples are often made using corn and rice. Alvarez-Jubete et al. (2009) demonstrated that pseudocereals could be incorporated into a gluten-free bread formulation without adversely affecting the sensory properties of the loaves. Aguiar et al. (2021) focused on the utilization of whole pseudocereal flours (amaranth, buckwheat and quinoa) to enhance the attributes of gluten-free bread. The interaction between pseudocereal and rice flours led to an improvement in dough consistency, bread volume, softness and overall acceptability. Blends of 50% amaranth, buckwheat and quinoa with 50% rice flour resulted in bread with high acceptability and an overall sensory score of 8 on a scale of 0-10.

1.6 Impact of unprocessed alternative plant ingredients on bread quality

The increasing use of plant ingredients such as pulses and pseudocereals provides health benefits, can be a way to increase the consumers protein and fibre intake and can also provide environmental advantages (Bresciani & Marti, 2019). Products containing cereals and pulses/pseudocereals can balance amino acid composition, lower glycemic index and improve the product's nutritional value (Melini et al., 2017).

The baking challenges that have been documented when applying pulses/pseudocereals to a wheat bread formulation are low volume, dry and friable crumb, rapid firming behaviour and unpleasant flavour, especially when attempting to incorporate substantial quantities

(Melini et al., 2017). Changes in leavened bread following the addition of alternative flours are mainly attributed to the presence of different proteins, dietary fibre, starch and lipids content and composition in these flours when compared to wheat flour. These constituents dilute the gluten, interact with the gluten proteins and promote water competition between the gluten and non-gluten material. Therefore the pulses/pseudocereals act as inhibitors to the development of the dough network, thus affecting the overall quality of the bread, in particular crumb hardness and specific volume (Prieto-Vázquez del Mercado et al., 2022).

Several studies have been published on the technological considerations of pulse flours in bread making. Differences in composition between cereals, legumes and pseudocereal flours result in different starch and protein structures in dough and textural properties of the bread. The studies below involve unprocessed or non-optimized production of these flours (such as the milling process) focusing on their impact on bread rather than maximizing their potential. The decrease in wheat flour percentage due to incorporating ingredients such as raw pulses can result in a change in the rheology, texture, shelf life and sensory acceptance (Liu et al., 2018; Rizzello et al., 2017; Y. Zhang et al., 2021). According to studies by Bhol and John Don Bosco (2014), replacing wheat flour with red bean flour in bread, at a ratio of 20 g/100 g, improved the nutritional characteristics, particularly dietary fibre, protein and mineral content. However, this substitution also decreased the softness of the bread, as well as the overall acceptability score.

Liu et al. (2018) described how including raw soybean, lupin and navy bean at levels of 10% in wheat bread reduced the volume by approximately 7%, 2%, and 10%, respectively, due to the weakening of the gluten network. Protein and fibre particles from these legumes likely became entrapped within the gluten network, disrupting the gluten network, leading to a decrease in dough elasticity and, consequently, a reduction in the volume of the bread. Despite this, sensory panellists found all breads to have good sensory acceptability.

According to the study by Nkurikiye et al. (2023), the incorporation of pulse flour into wheat bread resulted in a darker crust. Pulse flours such as peas, beans and chickpeas, are generally darker than wheat flour, and their higher protein content can induce Maillard reactions. The substitution of wheat flour for pulse flour also reduced the bread specific volume and increased crumb hardness. The reduction of gluten in the system decreased the ability of the dough to produce aerated bread. In addition, the increased hardness observed by Nkurikiye et al. (2023) is primarily due to the shrinking of the gas cells within the crumb, which condenses the structure, resulting in a firmer texture. Different performance between pulses was observed, with chickpeas showing better baking properties when compared to yellow peas and lentils (Nkurikiye et al., 2023).

1.7 Impact of conventional processing of alternative plant ingredients on bread quality

The antinutrient content, taste and functional properties of unprocessed alternative plant flours can limit their application in bread products. Conventional processing methods such as thermal processing, germination, fermentation and extraction can modify the functional properties of these flours, reduce/remove the antinutrients, improve nutrient digestibility and have a positive impact on bread characteristics (Kahraman et al., 2022; Setia et al., 2020b). **Table 1.1** gives a comprehensive overview of the effect of protein source and conventional processing/treatment on bread specific volume, crumb hardness and protein content.

Table 1.1. Effect of alternative plant ingredients and conventional processing/treatments on loaf volume, texture and protein content.

Plant ingredient	Sample	Processing/ treatment	Addition[1] (%)	Loaf Specific Volume (cm3/g)	Crumb hardness (N)	Protein (%)		Reference
						Bread	Flours	
Pea	Wheat flour	NP	0	3.74^{a}	3.25^{b}	8.4^{a}	NS	(Millar et al., 2019)
	Pea flour	NP	30	3.01^{ab}	8.19^{ab}	10.1^{b}	NS	
	Pea flour	Germination	30	2.3^{b}	11.98^{a}	10.8^{b}	NS	
	Pea flour	Toast	30	3.04^{ab}	8.12^{ab}	10.4^{b}	NS	
Chickpea	Rice flour	NP	0	2.63^{ab}	8.42	9.72^{b}	8.29	(Kahraman et al., 2022)
	Chickpea flour	NP	25	2.51^{b}	13.37	13.02^{a}	21–23.5	
	Chickpea flour	Roast	25	2.89^{a}	5.49	13.21^{a}	21–23.5	
	Chickpea flour	Dehull	25	2.75^{ab}	14.05	12.03^{a}	21–23.5	
Chickpea	Chickpea flour	NP	100	1.72^{c}	251^{c}	11.16^{c}	NS	(Ouazib et al., 2016)
	Chickpea flour	Germination	100	1.53^{b}	289^{d}	11.22^{c}	NS	
	Chickpea flour	Toast	100	1.79^{d}	167^{b}	10.14^{b}	NS	
	Chickpea flour	Boil	100	0.58^{a}	81^{a}	8.66^{a}	NS	
Chickpea	Wheat flour	NP	0	2.44^{b}	6.01^{a}	11.7^{a}	NS	(Xing et al., 2021)
	Chickpea protein	Extraction	20	2.10^{ab}	10.04^{b}	14.8^{b}	NS	
	Chickpea protein	Extraction	30	1.84^{a}	13.86^{c}	16.2^{c}	NS	
	Chickpea protein Sourdough	Fermentation	20	1.95^{a}	11.95^{bc}	15.2^{b}	NS	
	Chickpea protein Sourdough	Fermentation	30	1.78^{a}	16.92^{d}	16.6^{c}	NS	
Fava bean /Soy flour	Soy flour	NP	37	1.5	10.8	16	≈40.3	(Sozer et al., 2019)
	Faba bean flour	NP	50	2.1	5.7	16	≈35	
	Faba bean flour	Fermentation	50	2.4	8.0	16	≈35	
Pea	Wheat flour	NP	0	3.24^{a}	4	7.61^{f}	10.9	

	Pea protein concentrate	Extraction	5	2.22^{d}	≈6.5	9.34^{d}	77.96	(Belc et al., 2021)
	Pea protein concentrate	Extraction	10	1.74^{e}	≈11.2	11.49^{b}	77.96	
	Pea protein concentrate	Extraction	15	1.54^{f}	≈11	13.02^{a}	77.96	
Soy	Wheat flour	NP	0	3.24^{a}	≈4	7.61^{f}	10.9	
	Soy protein concentrate	Extraction	5	2.65^{b}	≈4.2	8.33^{e}	39.21	
	Soy protein concentrate	Extraction	10	2.47^{c}	≈5.2	9.28^{d}	39.21	
	Soy protein concentrate	Extraction	15	2.49^{c}	≈5.5	10.20^{c}	39.21	
Lupin	Wheat flour	NP	0	5.26^{a}	7.98^{d}	15.3^{d}	14.4	(Villacrés et al., 2020)
	Lupin flour	Debitter	10	5.29^{a}	7.86^{d}	18.5^{b}	54.6	
	Lupin flour	Debitter	15	4.95^{b}	9.59^{c}	20^{a}	54.6	
	Lupin flour	Debitter	20	4.66^{c}	28.28^{a}	20.40^{a}	54.6	
	Lupin flour	Fermentation	10	5.28^{a}	6.46^{e}	17.6^{c}	60.8	
	Lupin flour	Fermentation	15	4.61^{c}	9.38^{c}	19.9^{a}	60.8	
	Lupin flour	Fermentation	20	4.59^{c}	12.36^{b}	20.1^{a}	60.8	
Pea	Wheat flour	NP	0	7.3^{dC}	1.95^{aA}	NS	15.3^{aA}	(Setia et al., 2020)
	Pea flour	NP	10	6.9^{c}	2.34^{b}	NS	15.8^{b}	
	Pea flour	Germination	10	6.7^{bc}	2.57^{b}	NS	16.0^{b}	
	Pea flour	NP	20	6.4^{b}	2.80^{bc}	NS	16.7^{c}	
	Pea flour	Germination	20	6.0^{a}	3.39^{c}	NS	17.1^{d}	
Fava beans	Wheat flour	NP	0	7.3^{dC}	1.95^{aA}	NS	15.3^{aA}	
	Fava beans flour	NP	10	6.8^{B}	2.27^{B}	NS	16.3^{B}	
	Fava beans flour	Germination	10	6.8^{B}	2.34^{B}	NS	16.5^{B}	
	Fava beans flour	NP	20	6.5^{B}	2.48^{B}	NS	17.4^{C}	
	Fava beans flour	Germination	20	6.1^{A}	2.90^{B}	NS	17.9^{D}	
Chickpea	Wheat flour	NP	0	3.3^{b}	9.01^{a}	NS	12.3	

	Chickpea flour	Germination	5	3.4[c]	12.57[ab]	NS	21.1	(Atudorei et al., 2022)
	Chickpea flour	Germination	10	3.5[d]	16.38[bc]	NS	21.1	
	Chickpea flour	Germination	15	3.7[e]	19.20[cd]	NS	21.1	
	Chickpea flour	Germination	20	3.3[a]	20.04[d]	NS	21.1	
Pea	Wheat flour	NP	0	2.8[c]	≈26.95	NS	11.13	(Sun et al., 2023)
	De-hulled	NP	20	2.4[a]	≈62.72	NS	21.97	
	whole pea flour	NP	20	2.5[a]	≈78.4	NS	23.18	
	De-hulled	Roast	20	2.5[a]	≈53.9	NS	NS	
	whole pea flour	Roast	20	2.7[b]	≈71.54	NS	NS	
Pea	Wheat flour	NP	0	5.1	2.14	NS	11.9	(Sopiwnyk et al., 2020)
	Whole pea flour	NP	20	4.7	3.39[ab]	NS	22.3[a]	
	Whole pea flour	Heat 120°C, steam 0%	20	4.4	3.42[a]	NS	21.2[b]	
	Whole pea flour	Heat 120°C, steam 15%	20	4.5	3.20[abc]	NS	21.0[c]	
	Whole pea flour	Heat 140°C, steam 0%	20	4.5	3.20[abc]	NS	20.9[c]	
	Whole pea flour	Heat 140°C, steam 15%	20	4.4	3.18[abc]	NS	20.8[c]	
	Whole pea flour	Heat 160°C, steam 0%	20	4.4	2.95[c]	NS	21.2[b]	
	Whole pea flour	Heat 160°C, steam 15%	20	4.5	3.04[bc]	NS	21.2[b]	
	De-hulled pea flour	NP	20	4.6	3.58[ab]	NS	21.8[c]	
	De-hulled pea flour	Heat 120°C, steam 0%	20	4.5	3.65[ab]	NS	24.2[ab]	
	De-hulled pea flour	Heat 120°C, steam 15%	20	4.5	3.31[b]	NS	24.5[a]	
	De-hulled pea flour	Heat 140°C, steam 0%	20	4.4	3.61[ab]	NS	24.1[b]	
	De-hulled pea flour	Heat 140°C, steam 15%	20	4.5	3.31[b]	NS	24.1[b]	

	De-hulled pea flour	Heat 160°C, steam 0%	20	4.3	3.74[a]	NS	24.0[b]	
	De-hulled pea flour	Heat 160°C, steam 15%	20	4.4	3.34[b]	NS	24.1[b]	
Navy Beans	Wheat flour	NP	0	6.9	10*	NS	13.5	(Bourré et al., 2019b)
	Whole navy bean flour	NP	20	3.9	8*	NS	25.9	
	Whole navy bean flour	Heat 120°C/steam 0%	20	4	8*	NS	27.4[a]	
	Whole navy bean flour	Heat 120°C/steam 10%	20	4.5	9.5*	NS	25.9[b]	
	Whole navy bean flour	Heat 140°C/steam 0%	20	4.3	8.8*	NS	25.8[bcd]	
	Whole navy bean flour	Heat 140°C/steam 10%	20	4.3	9.5*	NS	25.9[bc]	
	Whole navy bean flour	Heat 160°C/steam 0%	20	4.2	9*	NS	25.7[cd]	
	Whole navy bean flour	Heat 160°C/steam 10%	20	4.2	8.8*	NS	25.6[d]	
	Whole navy bean flour	Heat 180°C/steam 0%	20	4.3	9*	NS	25.6[d]	
	Whole navy bean flour	Heat 180°C/steam 10%	20	4.2	10.3*	NS	25.8[bcd]	
Pea / Navy beans/ Lentil	Wheat control	NP	0	5.1	2.03	NS	12.1	(Bourré et al., 2019a)
	Split yellow pea fine flour	Milling	20	4.4	3.50[a]	NS	22.0[a]	
	Split yellow pea regular flour	Milling	20	4.5	3.27[b]	NS	21.5[b]	
	Whole navy bean fine flour	Milling	20	4.1	3.16[a]	NS	26.1[a]	
	Whole navy bean regular flour	Milling	20	4.1	2.87[b]	NS	25.9[b]	
	Decorticated red lentil fine flour	Milling	20	4.3	3.66[a]	NS	26.4[b]	

	Decorticated red lentil regular flour	Milling	20	4.4	3.31[b]	NS	27.0[a]	
Pea	Wheat flour	NP	0	2.81[c]	10.59[a]	8	11	(Kotsiou et al., 2021)
	yellow split pea	NP	10	2.67[bc]	11.91[ab]	9.1	NS	
	yellow split pea	Roast	10	2.92[c]	8.92[a]	9.1	NS	
	yellow split pea	Roast	15	2.41[ab]	14.88[bc]	9.5	NS	
	yellow split pea	Roast	20	2.30[a]	17.65[c]	10	NS	
Wheat/ Barley/ Chickpea/ Lentil/Quinoa	Wheat	NP	0	1.94[a]	41.97[d]	NS	17.46[d]	(Montemurro et al., 2019)
	Barley	NP	20	1.97[a]	48.35[c]	NS	14.02[e]	
	Chickpea	NP	20	1.93[a]	50.95[c]	NS	25.20[b]	
	Lentil	NP	20	1.98[a]	53.61[b]	NS	30.07[a]	
	Quinoa	NP	20	1.89[a]	58.65[e]	NS	20.64[c]	
	Wheat	Germination	0	1.99[a]	51.51[c]	NS	17.34[d]	
	Barley	Germination	20	1.92[a]	55.64[b]	NS	13.61[e]	
	Chickpea	Germination	20	1.94[a]	57.17[b]	NS	24.82[b]	
	Lentil	Germination	20	1.96[a]	54.95[c]	NS	31.57[a]	
	Quinoa	Germination	20	1.90[a]	62.92[a]	NS	18.25[c]	
Quinoa	Wheat flour	NP	0	2.45[c]	5.71[a]	8.35 c	NS	(Coțovanu et al., 2023)
	Quinoa flour large particle	Milling	9.13	3.22[a]	4.74[b]	9.01 bc	NS	
	Quinoa flour medium particle	Milling	10.57	2.81[c]	4.43[c]	9.96 a	NS	
	Quinoa flour fine particle	Milling	10.25	2.82[b]	4.39[d]	9.93 b	NS	
Quinoa/ Soy	Wheat flour	NP	0	2.55[a]	12.8[a]	NS	NS	(Bian et al., 2023)
	Quinoa flour	NP	100	1.67[f]	26.18[f]	NS	12.58	
	Quinoa flour+ soy protein isolate	Extraction	98% Quinoa + 2% Soy	1.79[e]	23.05[e]	NS	NS	

	Quinoa flour+ soy protein isolate	Extraction	96% Quinoa + 4% Soy	1.91^{d}	20.80^{d}	NS	NS	
	Quinoa flour+ soy protein isolate	Extraction	94% Quinoa + 6% Soy	2.17^{c}	18.576^{c}	NS	NS	
	Quinoa flour+ soy protein isolate	Extraction	92% Quinoa + 8% Soy	2.29^{b}	14.67^{ab}	NS	NS	
	Quinoa flour+ soy protein isolate	Extraction	90% Quinoa + 10% Soy	2.20^{bc}	15.76^{b}	NS	NS	
Quinoa	Quinoa flour	NP	15	2.71^{bc}	5.4^{a}	NS	15.53	(Lancetti et al., 2022)
	Quinoa acidified flour ChA	Acidification	15	2.62^{a}	7.9^{bc}	NS	NS	
	Quinoa sourdough 8014	Fermentation	15	2.74^{c}	5.2^{a}	NS	NS	
	Quinoa sourdough Q3	Fermentation	15	2.68^{abc}	5.2^{a}	NS	NS	
	Quinoa sourdough T5	Fermentation	15	2.66^{ab}	5.8^{ab}	NS	NS	
Buckwheat	Buckwheat flour	NP	15	2.70^{abc}	9.1^{cd}	NS	10.28	
	Buckwheat acidified flour ChA	Acidification	15	2.75^{c}	10.1^{d}	NS	NS	
	Buckwheat sourdough 8014	Fermentation	15	2.76^{c}	8.7^{cd}	NS	NS	
	Buckwheat sourdough Q3	Fermentation	15	2.61^{a}	8.2^{c}	NS	NS	
	Buckwheat sourdough T5	Fermentation	15	2.61^{a}	8.6^{cd}	NS	NS	
Chia/ Hemp/ Quinoa	Maize + rice flour	NP	0	2.04^{a}	17.49^{a}	NS	NS	(Jagelaviciute and
	Chia flour	NP	5	1.69^{ab}	21.34^{b}	NS	NS	
	Hemp flour	NP	5	1.88^{ab}	24.73^{c}	NS	NS	

	Quinoa flour	NP	5	1.86[a]	17.69[a]	NS	NS	Cizeikiene, 2021)
	Chia sourdough	Fermentation	5	1.53[b]	22.61[bc]	NS	NS	
	Hemp sourdough	Fermentation	5	1.76[ab]	21.61[b]	NS	NS	
	Quinoa sourdough	Fermentation	5	1.65[b]	16.68[a]	NS	NS	
Teff	whole teff flour	Roller milling	100	1.88[c]	9.23[a]	NS	9.88[a]	(Pulivarthi et al., 2022)
	whole teff flour	Pin milling	100	2.43[a]	5.99[c]	NS	9.97[a]	
	whole teff flour	Hammer milling	100	2.25[b]	7.49[b]	NS	9.69[a]	

NP: Non-processed, NS: Non-specified

[1]the percentage calculations account for the overall flour content

*crumb softness measured by finger compression -measured according to wheat bread which was given a score of 10 (higher the softness higher the value)

1.7.1 Thermal processing

Thermal processing has been widely employed in the food industry and encompasses a range of procedures from cooking and blanching to pasteurization and sterilization, all with distinct purposes. Optimizing the conditions of thermal processing involves achieving a balance between taking advantage of the positive impacts and mitigating the potential disadvantages in terms of palatability, conservation and nutritional attributes of the ingredient or product being processed. Thermal treatment can shift protein from native to denatured states, leading to degradation or interaction with other components (Quevedo et al., 2021). The water availability, temperature parameters and protein sources intricately shape the final product. The heat-moisture process modifies starch functionality, thus influencing its thermal stability and lowering the point of set-back, changing the crystallographic pattern of starch granules (Mathobo et al., 2021). Also, thermal heat processing can reduce antinutrient content, modify protein structure and improve starch digestibility. Rehman and Shah (2005) reported that processing legumes in an autoclave at 121°C and 128°C improved protein and starch digestibility and decreased antinutrient content compared to ordinary boiling. They explained that the higher protein digestibility after autoclaving may be due to increased susceptibility of the protein to enzymatic attack. The improvement in starch digestibility could be attributed to higher hydrolysis of starch, gelatinization, and the destruction of antinutrients. The pressure applied during autoclaving forces water into the seeds, increasing the extraction of antinutrients when compared to ordinary cooking.

1.7.2 Germination

Germination is a pre-milling treatment involving sprouting grains, activating hydrolytic and proteolytic enzymes that break down macronutrients (Setia, 2019). This process improves

protein digestibility, most probably due to phytates and tannins degradation, which increases mineral bioavailability (Garrido-Galand et al., 2021; Oghbaei & Prakash, 2016). Germination can be used to vary functional properties and improve the nutritional attributes of several flours (Setia et al., 2020b).

Several authors report that thermal treatments and germination of pulse grains can improve bread quality parameters such as specific volume and texture (Kim Adrienne Millar et al., 2019; Sun et al., 2023). The results of Kim Adrienne Millar et al. (2019), demonstrated promising results regarding bread volume and crumb hardness when substituting up to 30% of wheat flour with raw, germinated and toasted pea flour. Kahraman et al. (2022) studied the impact of incorporating raw, roasted, and dehulled chickpea flour into a rice based gluten-free bread. Both authors found that roasted pulse flour yielded the best outcomes in terms of volume and crumb hardness. This phenomenon is likely attributed to protein denaturation and to the starch degree of gelatinization of starch occurring during the roasting process, which facilitates gas retention and increases the bread crumb porosity, consequently improving the volume and textural features (Kahraman et al., 2022). M. Ouazib et al. (2016) demonstrated that bread with different types of chickpea flours (germinated, toasted, and cooked) showed variations in quality, with toasted chickpea flour resulting in the highest specific volume and cooked chickpea flour yielding the softest texture. Several authors verified that roasting is an efficient treatment to process pulses for maintaining optimal bread volume and soft crumb (Kahraman et al., 2022; Kim Adrienne Millar et al., 2019; Sun et al., 2023). In their studies, Setia et al. (2020b) replaced wheat flour with raw and germinated pea and fava bean flour at 10% and 20% levels resulting in bread with reduced volumes and increased crumb hardness. Atudorei et al. (2022) reported that incorporating germinated chickpea flour into a bread formulation enhanced the resulting bread quality in terms of specific volume and elasticity. However, crumb hardness increased with the increasing levels of germinated chickpea flour, to an optimal

level of 15%. Montemurro et al. (2019) compared bread composites containing raw and germinated pulses. It was concluded that the raw pulse flour inclusion resulted in higher loaf specific volume and crumb softness when compared with the resulting germinated pulse flour.

Atudorei et al. (2022) surmised that the germination process can increase protease activity. This hydrolyses the gluten proteins, leading to the weakening and disruption of the gluten network. However, germination is well known for enhancing the nutritional quality of whole grains, as it activates dormant enzymes and induces positive changes nutritional and physicochemical properties of the seed Montemurro et al. (2019). In addition, germination commonly leads to a pleasant sensory enhancement (Montemurro et al., 2019). The flavour enhancement in composite bread with germinated flour may result from the intensified Maillard reaction. Enzymes in germinated flour increase simple sugars and amino acids, contributing to this effect (Atudorei et al., 2022).

According to studies by Sopiwnyk et al. (2020), heat/steam treated pea flour inclusion resulted in bread loaves which exhibited a lower volume and firmer texture compared to wheat bread. No differences were found for specific volume between loaves made with untreated and heat/steam treated (whether whole or split).

1.7.3 Flour milling

Flour milling is the process of reducing the whole seed or cotyledons to flour through mechanical forces. The wheat milling process for bread applications has received significant attention and comprehensive research has been undertaken in the area. In contrast, there is a lack of comprehensive information regarding the pulse milling process (Hall, 2021b). The selection of an adequate milling process is an essential step in flour production, as it affects the final quality of the end product (Borsuk et al., 2012). The type of mill and the specific parameters employed can significantly impact the constituents and the functional properties of

the resulting flour. Parameters such as particle size, damaged starch and flour composition will vary, thus affecting the final quality of bread products (Borsuk et al., 2012; Maskus et al., 2016).

Bourré et al. (2019) determined that pulse flour particle size plays a significant role in the flour and bread properties of pulse composite bread. The incorporation of finer flour led to higher dough viscosity, increased water absorption capacity, higher starch damage and firmer texture; however, no significant differences were observed in bread volume. Coţovanu et al. (2023) observed the influence of particle sizes large (>300, <500 μm), medium (>180, <300 μm) and small (<180 μm) on bread and discussed how quinoa with medium particle size is the most suitable for consumer acceptance, resulting in breads of a higher specific volume, porosity and crumb elasticity compared to the control, while crumb hardness decreased. Also, flours of medium particle size had the highest overall sensory acceptability on the sensory evaluation. Pulivarthi et al. (2022) found that among three milling processes (roller, hammer and pin), the pin milling method was more suitable for milling brown teff grains for application in bread-making. Maçãs et al. (2023) **(Chapter 2)** studied the influence of different pea milling processes (roller, hammer and cutting) also for bread applications. The different milling processes affected pea flour particle size and composition, which may impact bread quality. Flours produced in the cutting mill had larger particles and higher damaged starch, reducing the viscosity profile (Rapid Visco analyzer) by limiting starch granule swelling. In contrast, roller-milled flours, with smaller particles, less fibre, and higher starch content, had a higher viscosity profile due to higher water absorption. In addition, roller-milled flour had the highest final viscosity, a parameter correlated with starch retrogradation and crumb hardness. As consumers prefer a softer crumb texture, this high final viscosity may impact the final bread quality Maçãs et al. (2023).

1.7.4 Fermentation

The fermentation process can be used to modify grains properties and/or dough food matrices through the addition of microorganisms and enzymes, which can enhance the nutrient bio accessibility and bioavailability and improve organoleptic properties of the resulting products (Nkhata et al., 2018).

In the initial fermentation phase, there is an increase in simple sugar levels; while after 24 hours of fermentation, a reduction in total carbohydrate content is expected. This decrease occurs as microorganisms utilize simpler sugars during the food fermentation process (Karovičová & Kohajdova, 2007; Osman, 2011). Regarding protein, fermentation breaks down complex storage proteins into shorter soluble forms which increase their digestibility (Chavan et al., 1988). Bakery science has applied fermentation as an ancient method that can enhance bread quality by increasing loaf volume, delaying staling, improving flavour, and influencing enzymatic activity (Jagelaviciute & Cizeikiene, 2021; Katina et al., 2006; Poutanen et al., 2009). The following paragraphs describe how fermentation can encompass both the fermentation of grains to create fermented flour for use in bread formulations and the fermentation processes involved in sourdough production.

Xing et al. (2021) investigated the enrichment of wheat bread by incorporating chickpea protein and sourdough chickpea protein. The sourdough chickpea incorporation notably lowered the levels of anti-nutritional factors (ANFs), as fermentation can reduce the content of oligosaccharides. However, specific volume and an increase in crumb hardness were also noted. A study by Sozer et al. (2019) demonstrated that replacing soy flour with raw fava bean flour and fava bean flour fermented with lactic acid bacteria in gluten-free bread formulations resulted in an increased loaf volume and softer crumb texture. In addition, the fermentation process improved the nutritional properties of fava bean flour by increasing in vitro protein digestibility of the faba bread. However, the incorporation of fermented fava bean flour resulted

in bread with lower specific volume and a firmer texture when compared to raw fava bean flour. In gluten-free dough, similar to cake batter, added proteins typically act as emulsifiers, preventing bread structural issues. The emulsifying ability of faba bean proteins was reduced by fermentation, which contributed to the less favourable textural properties in the gluten-free bread (Sozer et al., 2019).

From their research, Villacrés et al. (2020) described how using debittered and fermented lupin flour (with rhizopus oligosporus) strain up to a 10 % level of incorporation maintained similar characteristics to wheat bread, and enhanced the nutritional value in terms of crude fibre and protein. According to Jagelaviciute and Cizeikiene (2021) chia, hemp, and quinoa fermented (sourdough) and unfermented, inclusions were found to decrease the loaf volume and increase the crumb hardness of gluten-free bread, which they attributed to the increased content of fibres, resulting in lower gas retention. On a positive note, fermented chia, hemp, and quinoa applications in bread enhanced sensory acceptability for panellists. **Table 1.2** documents how fermentation time and flour type had a significant influence on sourdough properties. Lancetti et al. (2022) described how the inclusion of buckwheat and quinoa sourdough significantly influenced the characteristics of the bread, resulting in a slower staling rate and improved sensory acceptability. In addition, similar specific volumes and textures were found for the control and test breads. Softer bread texture as a result of the addition of fermented grains can be explained by dough acidification during fermentation. This acidity can alter starch and protein structure and activate endogenous enzymes such as proteases and amylases. Additionally, acid production during fermentation can extend shelf life (Lancetti et al., 2022). Yeşil and Levent (2022) incorporated buckwheat, quinoa and amaranth flour fermented by two different methods (spontaneous fermentation and yeast fermentation) and sourdough at 0, 15, 30 and 45% levels in a gluten-free bread formulation. The presence of the pseudocereals increased the quantity of ash, crude protein, phenolic content and antioxidant

activity of the bread. Compared to spontaneous fermentation, the flours treated undergoing yeast fermentation produced softer bread and improved sensory qualities, showing acceptability for bread up to 30% incorporation level of all pseudocereals. Buckwheat and amaranth containing products had more favourable appearance scores than quinoa, however, amaranth obtained a lower score in terms of taste and odour. Discrepancies among studies can be attributed to varying factors, including protein sources, additives, water levels, and variability caused by different microbial strains applied and baking methods.

1.7.5 Extraction methods

Protein, starch and fibre extracts are a result of an extraction process. This separation can be achieved either through a dry extraction method using air classification or, when aiming to obtain purer components, a wet extraction process (Wood & Malcolmson, 2021). In addition, researchers are also actively investigating the use of emerging technologies such as high pressure, pulse electric field and ultrasound, to influence chemical structure, enhance ingredient functionality and optimize the extraction process (Garcia-Vaquero & Tiwari, 2021; Pojić et al., 2018). The protein and fibre fractions are commonly incorporated into baked goods to fulfil nutritional claims such as source of protein, high protein, source of fibre and high fibre, while also providing favourable functional properties (Villarino et al., 2016). The application of emerging technologies is presented in the next section of the review.

Extracted protein isolates are also an option for incorporating high protein ingredients into food products, as they can be added in smaller quantities and achieve high protein levels (Belc et al., 2021; Kamal et al., 2021; Pojić et al., 2018). According to Bian et al. (2023), including 8% soy protein isolate in a quinoa bread formulation increased specific volume and decreased crumb firmness, bringing it close to the quality of wheat bread. Belc et al. (2021) highlighted the significant role of protein type and quantity of protein in bread fortification.

The incorporation of soy protein concentrate in bread exhibited more preferable results compared to pea protein bread in terms of bread specific volume and crumb hardness. Hoehnel et al. (2019) studied the application of alternative high protein plant sources in wheat bread formulations. Breads with 15% pea protein isolate, blue lupin protein isolate and fava bean flour showed tendencies towards lower specific volume and higher bread hardness compared to wheat bread. The use of the GlutoPeak, a rapid shear-based device that measures the aggregation behaviour of gluten (Wang et al., 2018), suggested the changes in bread parameters can be related to the secondary network formation between non-wheat proteins. Aider et al. (2012) characterized wheat bread as partially substituted by lentil, pea, and chickpea protein concentrates. Incorporating pulse protein concentrates at a 3% level led to a reduction in mass volume among pulse varieties, although in terms of hardness, at the 3% incorporation level, the enriched bread resembled that of the control wheat bread. However, when the inclusion level was increased to 9%, a significant increase in bread firmness was recorded. The authors concluded that pea protein concentrates exhibited the highest influence on hardness and mass volume and chickpea protein displayed the most favourable sensory scores showing potential as an ingredient for bread production.

1.8 Impact of emerging processing of alternative plant ingredients on bread quality

In recent years, the application of novel technologies such as microwave, ultrasound and pulse electric field have emerged, and have been used to explore the processing of raw materials and food ingredients. However, these emerging methods face fundamental scalability issues and, as a result, have not yet been widely implemented in the industry. Such technologies can be environmentally beneficial by decreasing the need for organic solvents and high temperatures during processing. These technologies also contribute to reducing energy

consumption and wastewater generation, as well as shorter processing times, consequently promoting the conservation of non-renewable resources (Amanda et al., 2022; Barba et al., 2016). Additionally, using lower temperatures contributes to lower nutrient loss compared with conventional thermal processes (Amanda et al., 2022). The use of emerging technologies can impart beneficial traits to the material being processed, including improved product nutritional profile and sensory properties, enhanced product quality and assured microbiological safety (Alvarenga et al., 2022).

Emerging technologies are divided into two groups: thermal and non-thermal technologies. Thermal technologies include ohmic heating (OH), thermosonication and microwave heating while non-thermal encompass ultrasound, high-pressure processing (HPP), pulsed electric fields (PEF), cold plasma, irradiation, pulsed light, and ultraviolet light (Santos Rocha et al., 2022). These can be employed as alternatives to or combined with conventional methods and have a wide range of applications in the food sector. They enable the optimization of multiple food processes such as blanching, pasteurization, sterilization, defrosting, fermentation, extraction, drying, homogenization, mixing dehydration and heating (Amanda et al., 2022; Barba et al., 2016; Gallo et al., 2018; Guzik P, 2022; Kamble et al., 2023).

The application of emerging technologies can modify macronutrient structure, promoting different interactions between food components (Li et al., 2021). In terms of bakery research, these technologies could consequently be key to increasing protein incorporation levels in bread, while maintaining their high quality and techno functional standards. It has been shown that these processing methods can also modify the functional properties of proteins, neutralize anti-nutritional elements and reduce cooking time (Amanda et al., 2022; Deng et al., 2022b). Although there is still quite limited published research in the area of bakery application, the following paragraphs summarise relevant work to date on bread properties, with specific

attention to loaf volume and crumb texture. **Table 1.2** presents a comprehensive overview of the influence of emerging technologies on quality parameters of baked goods.

Table 1.2. Effect of alternative plant ingredients and emerging processing/treatment on bread volume texture and protein content.

Plant ingredients	Sample	Processing/ treatment	Addition (%)	Processing Settings variations or Treatment configuration/ Parameters	Loaf Specific Volume (cm3/g)	Crumb hardness (N)	Protein (%)		Reference
							Bread	Flours/ Pulp	
Cassava	cassava starch + chickpea flour	NP	100	-	2.05±0.07^{ab}	40.17±1.23^{def}	NS	NS	(Garske, Mercali et al. 2023)
	cassava starch + chickpea flour	Microwave	50	20 % water; 4 min	1.80±0.42^{bc}	59.40±4.37^{a}	NS	NS	
	cassava starch + chickpea flour	Microwave	50	30 % water; 5 min	1.56±0.09^{cd}	53.51±1.06^{abc}	NS	NS	
	cassava starch + chickpea flour	Microwave	50	40 % water; 6 min	1.56±0.04^{cd}	49.79±2.98^{abcd}	NS	NS	
Chickpea	cassava starch + chickpea flour	NP	100	-	2.05±0.07^{ab}	40.17±1.23^{def}	NS	NS	
	cassava starch + chickpea flour	Microwave	50	20 % water; 3 min	1.64±0.08^{bcd}	43.03±1.43^{de}	NS	22.5	
	cassava starch + chickpea flour	Microwave	50	30 % water; 4 min	1.59±0.07^{cd}	41.55±2.64^{de}			
	cassava starch + chickpea flour	Microwave	50	40% water; 5 min	1.66±0.06^{bcd}	30.19±1.05^{gh}			
Rice	Wet-milled rice flour	NP	100	-	3.72±0.12^{a}	0.48±0.27^{b}	NS	8.21	(Qin, Xi et al. 2022)
	Semidry-milled rice flour	NP	100	-	3.14±0.10^{b}	0.50±0.047^{b}	NS	8.21	
	Semidry-milled rice flour	Ultrasound	15	20 kHz; 600W; 20 min	3.51±0.19^{a}	0.45±0.05^{b}	NS	8.21	
	Semidry-milled rice flour	Microwave	15	300 W; 5 min	3.63±0.08^{a}	0.41±0.04^{b}	NS	8.21	
Wheat	Wheat flour	High Pressure Processing	100	0 MPa; 2 min	4.2±0.0^{c}	2.48±0.10^{a}	NS	11.44	(Bárcenas, Altamirano-

	Wheat flour	High Pressure Processing	100	50 MPa; 2 min	2.8±0.0[b]	5.39±0.10[b]	NS	11.44	Fortoul et al. 2010)
	Wheat flour	High Pressure Processing	100	100 MPa; 2 min	2.7±0.0[b]	6.26±0.10[c]	NS	11.44	
	Wheat flour	High Pressure Processing	100	200 MPa; 2 min	2.0±0.0[a]	15.87±0.10[d]	NS	11.44	
Sorghum	Sorghum flour	High Pressure Processing	0	0.1 MPa	1.57±0.05[a]	8.46±1.04[a]	NS	NS	(Vallons, Ryan et al. 2010)
	Sorghum flour	High Pressure Processing	2	200 MPa; 10 min	1.57±0.11[a]	7.79±0.09[a]	NS	NS	
				600 MPa; 10 min	1.61±0.09[a]	7.64±0.89[a]	NS	NS	
	Sorghum flour	High Pressure Processing	10	200 MPa; 10 min	1.52±0.01[a]	8.51±0.43[a]	NS	NS	
				600 MPa; 10 min	1.43±0.02[b]	9.52±1.16[a]	NS	NS	
Potato	Wheat	NP	0	-	2.53 ± 0.01a	24.86[d]	NS	10.9	(Song, Jiang et al. 2023)
	Potato pulp	NP	30	-	2.11±0.01[c]	27.77[bc]	NS	1.9	
	Potato pulp	Ultrasound	30	200 W; 40 min	2.14±0.02[c]	29.5[ab]	NS	1.9	
	Potato pulp	Ultrasound	30	300 W; 40 min	2.23±010[b]	30.17[a]	NS	1.9	
	Potato pulp	Ultrasound	30	400 W; 40 min	2.51±0.01[a]	26.30[cd]	NS	1.9	
	Potato pulp	Ultrasound	30	500 W; 40 min	2.49±0.02[a]	24.54±[d]	NS	1.9	
Corn	Corn flour	Ultrasound	100	0% waves intensity; 3 min	2.1±0.1[e]	9.3±0.7[a]	NS	9.4	(Jalali, Sheikholeslami et al. 2020)
	Corn flour	Ultrasound	100	30% waves intensity; 3 min	3.5±0.2[e]	7.1±0.5[b]	NS	9.4	
	Corn flour	Ultrasound	100	70% waves intensity; 3 min	2.7±0.2[d]	7.1±0.2[b]	NS	9.4	
	Pre-gelatinized corn flour	Ultrasound	100	0% waves intensity; 3 min	4.1±0.1[b]	6.9±0.6[b]	NS	9.4	
	Pre-gelatinized corn flour	Ultrasound	100	30% waves intensity; 3 min	4.9±0.3[a]	4.4±0.3[e]	NS	9.4	
	Pre-gelatinized corn flour	Ultrasound	100	70% waves intensity; 3 min	3.9±0.1[a]	4.4±0.5[e]	NS	9.4	
	Wheat flour	NP	0	-	2.39±0.01[a]	20.46±0.60[cd]	NS	14.89	

Sweet potato	Sweet potato pulp	NP	50	-	2.01±0.02^{d}	24.20±0.30^{a}	NS	NS	(Zhang, Suo et al. 2022)
	Sweet potato pulp	Ultrasound	50	40kHz; 17.5 W/L; 40 min	2.10±0.02^{c}	23.37±0.68^{a}	NS	NS	
	Sweet potato pulp	Ultrasound	50	40 kHz; 20.0 W/L; 40 min	2.14±0.02^{c}	22.81±0.13^{ab}	NS	NS	
	Sweet potato pulp	Ultrasound	50	40 kHz; 22.5 W/L; 40 min	2.29±0.06^{b}	18.88±0.40^{d}	NS	NS	
	Sweet potato pulp	Ultrasound	50	40 kHz; 25.0 W/L; 40 min	2.17±0.01^{c}	21.15±0.44^{bc}	NS	NS	
	Sweet potato pulp	Ultrasound	50	40 kHz; 27.5 W/L; 40 min	2.13±0.02^{c}	19.97±0.45^{cd}	NS	NS	
Buckwheat	Buckwheat flour	Conventional oven	-	180 °C; 60 min	2.60±0.19^{a}	17.11±1.69^{ab}	NS	NS	(Bender, Gratz et al. 2019)
	Buckwheat flour	Ohmic heating without second heating step	-	First step : 2 kW; 15s + Third step : 0.3 kW; 5 min	2.98±0.06^{bcd}	16.08±1.17^{ab}	NS	NS	
	Buckwheat flour	Ohmic heating without second heating step	-	First step : 4 kW; 15s + Third step : 0.3 kW; 5 min	2.94±0.11^{bc}	25.65±1.45^{c}	NS	NS	
	Buckwheat flour	Ohmic heating without second heating step	-	First step : 5 kW; 15s + Third step : 0.3 kW; 5 min	3.29±0.11^{cde}	17.52±0.81^{ab}	NS	NS	
	Buckwheat flour	Ohmic heating without second heating step	-	First step : 6 kW; 15s + Third step : 0.3 kW; 3 min	3.20±0.32^{cde}	19.67±5.53^{b}	NS	NS	
	Buckwheat flour	Ohmic heating without second heating step	-	First step : 8 kW; 15s + Third step : 0.3 kW; 15 s	3.32±0.02^{de}	14.04±0.57^{a}	NS	NS	

	Buckwheat flour	Ohmic heating with second heating step	-	First step : 2 kW; 15s + Second step : 1 kW; 10s + Third step : 0.3 kW; 5 min	3.11±0.13^{bcd}	18.65±1.47^{ab}	NS	NS	
	Buckwheat flour	Ohmic heating with second heating step	-	First step : 4 kW; 15s + Second step : 1 kW; 10s + Third step : 0.3 kW; 5 min	3.02±2.4^{bcd}	26.11±5.03^{c}	NS	NS	
	Buckwheat flour	Ohmic heating with second heating step	-	First step : 5 kW; 15s + Second step : 1 kW; 10s + Third step : 0.3 kW; 5 min	2.86±0.18^{b}	34.43±4.40^{d}	NS	NS	
	Buckwheat flour	Ohmic heating with second heating step	-	First step : 6 kW; 15s + Second step : 1 kW; 10s + Third step : 0.3 kW; 3 min	3.44±0.04^{e}	19.72±1.05^{b}	NS	NS	
	Buckwheat flour	Ohmic heating with second heating step	-	First step : 8 kW; 15s + Second step : 1 kW; 10s + Third step : 0.3 kW; 15s	3.23±0.13^{cde}	19.97±1.85^{b}	NS	NS	
Lupin	Wheat flour	NP	0	-	4.27 ± 0.15^{a}	5.11 ± 0.53^{d}	12.07 ± 0.50^{d}	NS	(Yaver and Bilgiçli 2021)
	Lupin seeds	Soaking	100	25±2°C; 144h	2.72±0.99^{b}	11.73±5.11^{a}	15.29± 2.23^{b}	39.29	
	Lupin seeds	Ultrasound soaking	100	20 kHz; 200W; 25 min every 4h for 60h	3.12±0.83^{a}	9.68±4.65^{b}	15.41± 2.34^{a}	40.21	
Potato	Potato protein	Conventional	2	180 °C; 40 min	3.56±0.03^{C}	6.00±1.29^{b}	NS	86.68±0.54^{e}	(Waziiroh, Bender et al. 2023)
	Potato protein	Conventional	3	180 °C; 40 min	3.55±0.02^{b}	7.46±1.51^{c}	NS	86.68±0.54^{e}	
	Potato protein	Conventional	4	180 °C; 40 min	3.2±0.03^{a}	4.99±1.30^{a}	NS	86.68±0.54^{e}	
	Potato protein	Ohmic heating	2	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	4.26±0.04^{b}	3.50±0.35^{a}	NS	86.68±0.54^{e}	
	Potato protein	Ohmic heating	3	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	4.16±0.17^{a}	4.84±0.56^{b}	NS	86.68±0.54^{e}	

	Potato protein	Ohmic heating	4	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	4.11±0.05^{c}	3.23±0.50^{c}	NS	86.68±0.54^{e}
Rice	Rice protein	Conventional	2	180 °C; 40 min	2.56±0.06^{c}	5.91±1.84^{a}	NS	78.96±1.12^{a}
	Rice protein	Conventional	3	180 °C; 40 min	2.51±0.12^{b}	7.42±1.68^{c}	NS	78.96±1.12^{a}
	Rice protein	Conventional	4	180 °C; 40 min	2.03±0.00^{a}	7.89±0.69^{b}	NS	78.96±1.12^{a}
	Rice protein	Ohmic heating	2	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.60±0.12^{b}	3.61±0.55^{b}	NS	78.96±1.12^{a}
	Rice protein	Ohmic heating	3	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.87±0.02^{c}	4.97±0.19^{a}	NS	78.96±1.12^{a}
	Rice protein	Ohmic heating	4	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.33±0.04^{e}	3.50±0.08^{a}	NS	78.96±1.12^{a}
Soy	Soy protein isolate	Conventional	2	180 °C; 40 min	2.01±0.07^{b}	11.2±0.46^{a}	NS	84.56±0.10^{d}
	Soy protein isolate	Conventional	3	180 °C; 40 min	2.01±0.01^{b}	12.05±1.79^{b}	NS	84.56±0.10^{d}
	Soy protein isolate	Conventional	4	180 °C; 40 min	1.97±0.03^{a}	16.08±0.93^{c}	NS	84.56±0.10^{d}
	Soy protein isolate	Ohmic heating	2	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.05±0.16^{a}	3.71±0.13^{b}	NS	84.56±0.10^{d}
	Soy protein isolate	Ohmic heating	3	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.45±0.14^{c}	3.03±0.10^{a}	NS	84.56±0.10^{d}
	Soy protein isolate	Ohmic heating	4	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.43±0.06^{b}	4.34±0.15^{c}	NS	84.56±0.10^{d}

Lupin	Lupin protein isolate	Conventional	2	180 °C; 40 min	2.10±0.06^{b}	11.37±0.28^{a}	NS	92.28±0.25^{f}
	Lupin protein isolate	Conventional	3	180 °C; 40 min	2.05±0.14^{a}	17.54±2.18^{b}	NS	92.28±0.25^{f}
	Lupin protein isolate	Ohmic heating	4	180 °C; 40 min	2.10±0.01^{b}	22.27±0.10^{c}	NS	92.28±0.25^{f}
	Lupin protein isolate	Conventional	2	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.15±0.12^{b}	2.53±0.34^{a}	NS	92.28±0.25^{f}
	Lupin protein isolate	Ohmic heating	3	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.04±0.27^{a}	4.69±0.68^{c}	NS	92.28±0.25^{f}
	Lupin protein isolate	Ohmic heating	4	Three step heating : 5 kW for 15 s, 1 kW for 10 s and 0.3 kW for 80 s	3.20±0.12^{c}	4.00±0.09^{b}	NS	92.28±0.25^{f}

NP: Non-processed, NS: Non-specified

[1]the percentage calculations account for the overall flour content

1.8.1 Ohmic heating (OH)

Ohmic heating (OH) uses electrical current to produce heat within food ingredients through the Joule effect. This method requires shorter cooking times, ensuring nutrient retention and maintaining food quality (Amanda et al., 2022).

Waziiroh et al. (2023) have studied the effect of the baking method (conventional oven and OH) when incorporating several protein sources (potato protein, rice protein, soy protein isolate and lupin protein isolate) in different percentages in gluten-free bread. Breads baked using OH were found to have a greater specific volume and a softer texture compared to the conventional oven, most probably due to the higher protein solubility as a result of OH application. Moreover, the breads containing potato protein had the highest specific volume. Bender et al. (2019) studied the impact of different power levels of ohmic heating on the quality and texture of baked buckwheat bread. The application of ohmic heating was found to increase the specific volume of the bread when compared to a conventional oven. However, crumb hardness results were not consistent and the authors concluded that the texture is dependent on ohmic heating power, time and baking step application.

1.8.2 Microwave heating and ultrasound

Microwave heating uses non-ionizing electromagnetic waves to heat food by interacting with its chemical components through ionic and dipole mechanisms, where penetration depth and dielectric characteristics play a crucial role (Amanda et al., 2022). The use of microwave technology can enhance food nutritional content by modifying starch structure and digestibility, altering its pasting properties (Amanda et al., 2022; Deng et al., 2022b).

The application of ultrasound creates cavitation through the use of high-intensity, low-frequency sound waves (Amanda et al., 2022). This technology has a significant impact on

food, both the physicochemical properties (molecular weight, particle size and zeta potential) and functional properties (water solubility, foaming and emulsifying capacity). With regards to protein, ultrasound can alter the amino acid composition, digestibility and the presence of anti-nutrients. In addition, the structural properties of plant proteins (primary, secondary and tertiary) as well as their micro and nanostructures can be affected (Amanda et al., 2022).

Garske et al. (2023) treated cassava starch and chickpea flour with microwave heating with a view to replacing additives such as xanthan gum and carboxymethylcellulose in gluten-free bread. Different water contents (20%, 30% and 40%) were used to treat these flours. The resulting bread did not show significant differences regarding specific volume compared to the control bread, which could be caused by inadequate microwave settings or unbalanced formulation ratios (Garske et al., 2023). The authors also showed how the improved crumb hardness was dependent on the microwave settings (**Table 1.2**). W. Qin et al. (2022) found that semi-dry milled rice flour treated by microwave and ultrasound separately and incorporated into a bread formulation resulted in an increased bread specific volume compared to non-treated semi-dry milled rice flour. These results are due to changes in the particle size and structure of rice flour. Microwave heating weakened the hydrogen bonding force and changed protein structure from β-turn to β-sheet. Ultrasound reinforced the starch structure and the protein secondary structure decreased in β-turn and increased in α-helix. To improve the quality characteristics of steamed bread containing sweet potato pulp, M. Zhang et al. (2022) and Song et al. (2023), applied ultrasound with different power applications during dough fermentation. M. Zhang et al. (2022) demonstrated that ultrasonic-assisted dough fermentation reduced free sulfhydryl (SH) and increased wet gluten content improving dough extensibility, resulting in bread with a higher specific volume and lower crumb hardness. Song et al. (2023) documented that the conformation of gluten protein was changed by ultrasonic waves increasing β-sheet content and creating a continuous gluten network and stabilized crumb structure. This resulted

in an increased bread specific volume and improved texture by decreasing crumb hardness at levels between 400-500W (Table 1.2), compared to the control. Jalali et al. (2020) treated corn flour and pre-gelatinized corn flour doughs with a range of ultrasound intensities (Table 1.2). The authors found that the application of ultrasound in bread dough increased loaf specific volume with a corresponding decrease in crumb hardness compared to the untreated bread, attributing the results to better aeration of the dough. On a 5-point acceptance scale, samples treated at 30% ultrasonic intensity showed the highest overall acceptance (Jalali et al., 2020). According to Yaver and Bilgiçli (2021), ultrasound-treated lupin flour also resulted in greater loaf volumes and softer internal crumb texture compared the non-treated lupin flour. These may be due to the higher bread dough strengthening ability, an effect of hydro-peroxides as an oxidant formed during debittering process by ultrasound waves.

1.8.3 High-pressure processing (HPP)

High-pressure processing (HPP) applies hydrostatic pressure to a product under regulated temperature and time conditions. This technology modifies non-covalent bond structure, leading to denaturation, aggregation or gelation of food components. In cereals and legumes, HPP can alter protein functionality (foaming and viscosity characteristics) as well as protein digestibility, while also reducing anti-nutritional elements (Amanda et al., 2022).

Bárcenas et al. (2010) studied the impact of HPP of wheat flour in the range of 50-200 MPa on wheat bread characteristics. The resulting wheat breads showed distinct traits, including a brownish crumb appearance and an uneven distribution of gas cells. This modification of the crumb structure increased both the hardness and adhesiveness of the crumb texture and was discussed by the authors to be as a result of gluten network damage (Bárcenas et al., 2010). The authors employed a scale of 0 to 10 to rate the general sensory acceptability of the bread. Sensory analysis revealed that using HPP produced breads of a satisfactory and

equivalent score to untreated bread (score 7), except for the 200 MPa treated sample which was not found to be acceptable. The HPP-treated bread was described as having a soft and appealing layered crumb despite having a structure resembling croissants (Bárcenas et al., 2010). Furthermore, Vallons et al. (2010), studied the impact of HPP (200 MPa and 600 MPa) and different levels of sorghum flour incorporation (2% and 10%) on bread. No significant differences were found between the control and the treated bread, except at 600 MPa where the specific volume decreased.

1.8.4 Pulsed electric field (PEF)

Pulsed electric field (PEF) creates an electrical potential across cell membranes, increasing mass transfer. In food research, the application of PEF to ingredients offers advantages such as improved extraction yield, quicker processing time, reduced material degradation, lower energy costs and microorganism inactivation (Amanda et al., 2022). Duque et al. (2020) documented how the utilization of PEF will influence the physicochemical properties of fractionated oat flour. The application of PEF changed the gelatinization enthalpy of the flour and facilitated the release of beta-glucan into the suspension supernatant. Additionally, the intensity of PEF can impact the starch profile properties of the fractionated oat flour, resulting in lower gelatinization enthalpy and percent relative crystallinity (Duque et al., 2020).

1.9 Meta-analysis of conventional and emerging technologies for plant ingredients in bread

1.9.1 Methodology

A meta-analysis was undertaken to statistically investigate the patterns of how the selection of alternative plant ingredients, processing/treatment methods and percentage of addition impact bread quality, particularly loaf volume and crumb texture. Additionally, a meta-analysis was used to obtain an insight into the heterogeneity among the results. The authors aimed to address the following key questions through this meta-analysis:

- Identify the most commonly utilized alternative plant ingredients, processing/treatment methods and application levels in scientific bread research.
- Investigate the impact of increased alternative plant ingredient incorporation levels on bread quality.
- Assess whether, in general, these treatments have a positive or negative influence on the final product quality.

1.9.2 Data collection

Published studies were obtained from two primary online databases, ScienceDirect and Google Scholar. The collected data was organized into **Table 1.1** (conventional processing/treatment methods) and **Table 1.2** (emerging processing/treatment methods) with a total of 20 and 10 studies, respectively. Keywords used for the search included “emerging technologies”, “processing” “treatments”, “pseudocereals”, “pulses”, “bread” and “alternative plant ingredients”. Stringent selection criteria were applied, focusing on studies providing detailed information on plant sources, plant ingredient incorporation level, processing/treatment methods and values for loaf specific volume and crumb hardness. Only a

single study in Tables 1.1 and 1.2 lacks data on crumb hardness. This study was excluded from consideration when statistically analyzing the crumb hardness. Tables 1.1 and 1.2 also contain detailed information about source/treatment specifications and protein content. However, these were excluded from the meta-analysis due to challenges in classifying and organizing the data. This meta-analysis combines relevant numerical data from published studies regarding bread quality parameters, specifically loaf specific volume and crumb hardness. Additionally, non-numeric data, such as the alternative plant ingredients employed, percentage of addition and the methods of processing/treatment, were also included.

1.9.3 Data configuration

Graphs were generated using the R statistical packages 'ggplot2' and 'dplyr'(Team, 2022).

Frequency study

The frequency usage of alternative plant ingredients and ingredient processing/treatment methods were calculated based on their appearance in the referenced studies. When appearing multiple times within the same study were counted as a single occurrence (Tables 1.1 and 1.2).

Scatterplot processing/treatment effectiveness

Two scatterplots were generated to investigate the patterns in processing/treatment effectiveness and to assess their effects on loaf specific volume and crumb hardness for both conventional and emerging methods. The data from Tables 1.1 and 1.2 was used to calculate the percentage difference in specific volume and crumb hardness between the non-treated sample and the treated/processed sample in the same study.

1.9.4 Research findings

To visualize the impact of conventional and emerging technologies on bread quality parameters (represented in Tables 1.1 and 1.2) and their trends, distinct graphs were created.

Frequency study

Figure 1.1 represents the most utilized alternative plant ingredients and the conventional and emerging processing/ treatments applied to bread research. The figure shows that there are a larger number of published studies which focus on conventional processes when compared to emerging processes, which conveys that less knowledge has been generated in the application of emerging technologies bread research.

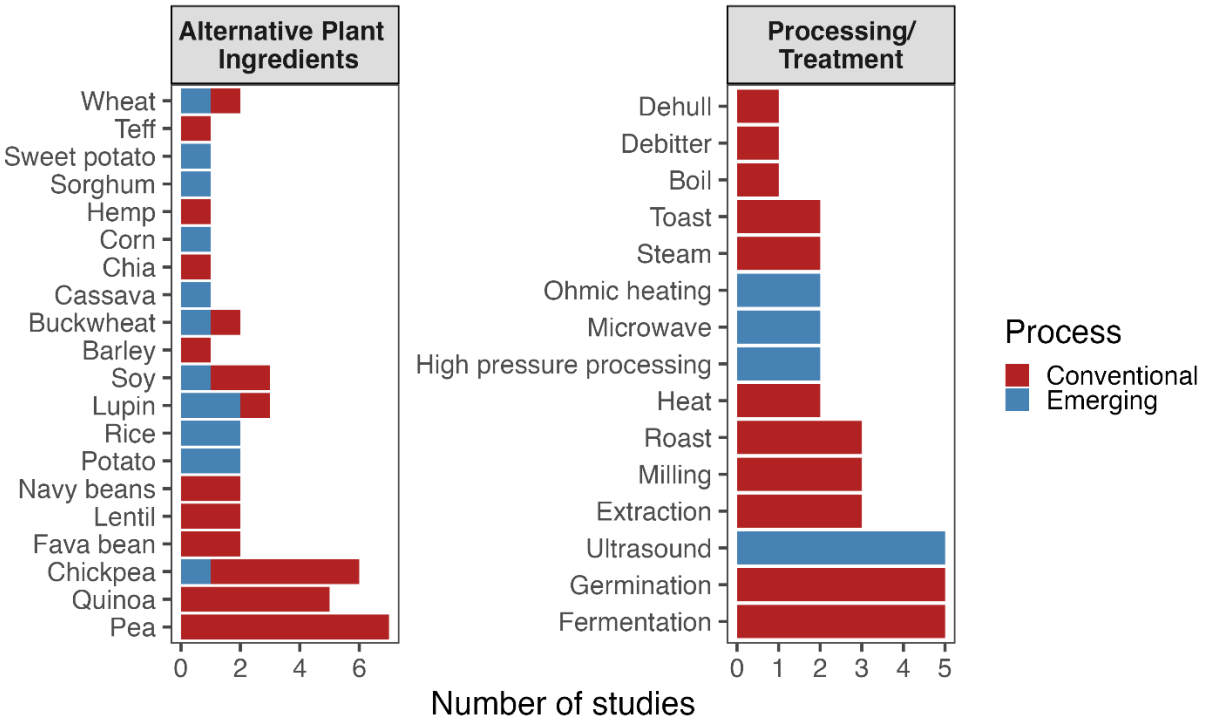

Figure 1.1. The frequency of plant ingredient/grain sources and processing/treatment utilized in the studies referenced in **Tables 1.1** and **1.2**, for both traditional and novel methods.

The frequency of the alternative plant ingredient sources which were processed/ treated by conventional and emerging methods shows that conventional methods have been used to predominantly study high protein plant ingredients such as pea, quinoa and chickpea (Figure

1.1). To date, comprehensive research has found that emerging processing methods have thus far focused on processing alternative plant ingredients with reduced protein content, such as potatoes, cassava, corn, wheat and rice. Emerging processing technologies have been used on gluten-free bread, where substitute ingredients and enhanced quality in gluten-free breads were the focus.

Frequency analysis of both, emerging and conventional processing/treatments reveals that the conventional methods, especially germination and fermentation, have been extensively studied. Among emerging approaches, the application of ultrasound has been the focus of a high proportion of studies.

Scatterplot processing/treatment effectiveness

Figure 1.2 A, analyses loaf specific volume difference (%) between non-treated and treated samples for each study (as outlined in **Tables 1.1** and **1.2**), comparing both emerging and conventional treatments. Data points below 0 indicate a decrease in specific volume (a negative impact), while values above 0 indicate an increase in volume (a positive impact). Additionally, the percentage difference quantifies how much the volume increased compared to a non-treated sample.

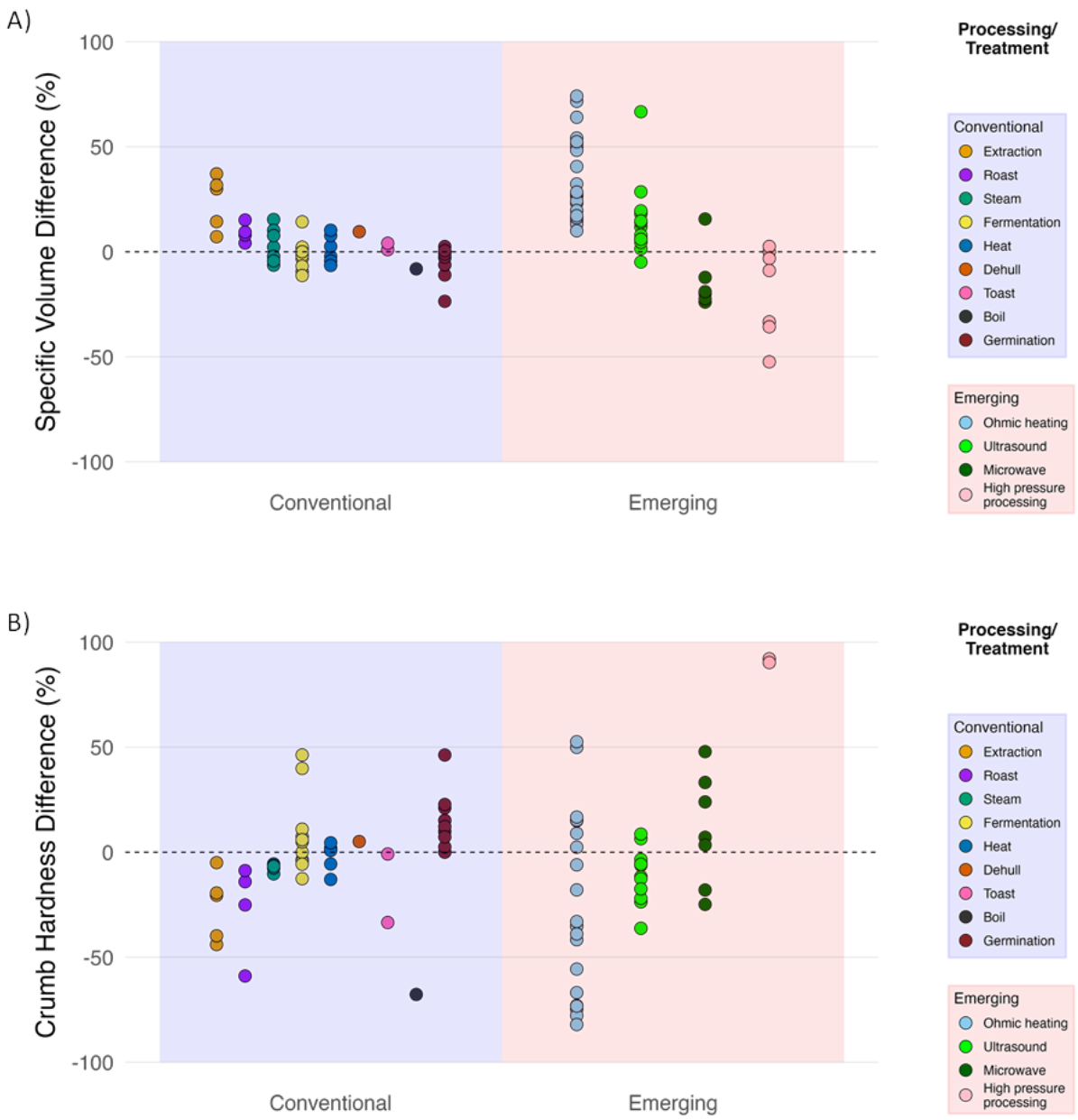

Figure 1.2. Scatterplots depicting the comparison between non-treated samples and treated samples in terms of specific volume and crumb hardness.

Conventional methods such as extraction, roasting, toasting and dehulling treatments had positive effects on the bread specific volume. Fermentation, heat and steam showed both a positive and negative impact, likely due to differences in treatment/processing settings and the source of plant ingredients. Conversely, germination and boiling treatments did not appear to be effective in improving the bread specific volume.

Focusing on the emerging methods, it is notable that both ohmic heating and ultrasound exhibit a positive impact on the bread specific volume. The results exhibit substantial variation, because the studies on ohmic heating and ultrasound, elucidated in **Table 1.2**, reveal a wide array of experimentation involving diverse ingredients and processing settings. These experiments also encompass varied levels of incorporated processed ingredients, thereby introducing significant variability into the results. However, these two treatments demonstrate great promise for the bakery industry, with potential improvements in specific volume reaching approximately 75% for ohmic heating and 65% for ultrasound when compared to non-treated samples. The results for microwave treatment were variable, showing both positive and negative impacts. Due to the limited number of studies in this area, reaching a definitive conclusion is challenging. Further studies need to be undertaken to fully assess the impact of microwave treatment. From the published studies, high-pressure processing has not so far proven to be an effective technology for improving bread-specific volume.

Figure 1.2 B illustrates the impact of different processing methods on crumb hardness (based on the studies in **Table 1.1**). To provide context, the common target is to reduce the hardening effect of bread associated with the addition of non-wheat ingredients. The data reveals that conventional techniques such as boiling, roasting, extraction, toasting and steaming effectively reduce crumb hardness. Fermentation and heat treatment exhibit both positive and negative influences, shaped by various factors, such as grain source and fermentation parameters, as mentioned earlier. Further investigation is needed to draw definitive conclusions regarding the ineffectiveness of dehulling, due to the limited number of studies. Germination is demonstrated to be inefficient in reducing crumb hardness. Among the emerging methods explored, all except high-pressure processing (HPP) demonstrate to be valuable methods to reduce the bread crumb hardness, with ohmic heating and ultrasound emerging as particularly noteworthy approaches. Both conventional and emerging methods achieve significant crumb

hardness reductions compared to untreated samples, approximately 70% and 80%, respectively.

Both **Figures 1.2 A** and **1.2 B** underscore the highly significant impact of processing/treatment on bread specific volume and crumb hardness parameters. Overall, among conventional methods, roasting stands out as an efficient and established technique. When the goal is to enhance nutritional content, extracted proteins have demonstrated effectiveness in maintaining bread quality standards. Also, ohmic heating and ultrasound highlight the considerable potential of these methods for bread processing.

1.10 In vitro digestion of value-added bakery products

As bakery foods are widely consumed staples, they represent a suitable vehicle for delivering nutrients with potential health benefits (Dossa & Riviş, 2024). Value-added bakery products are designed to provide additional nutritional or physiological benefits through the incorporation of ingredients such as dietary fibres, plant or animal proteins, antioxidants, prebiotics, and probiotics, but also through fortification with vitamins and minerals, reduction or replacement of sugar, salt and fats, or the use of alternative flours such as whole grain, legume and pseudocereals (Dossa & Riviş, 2024; Guiné & Florença, 2024). Conventional and emerging processing technologies also play a crucial role in digestibility, as they can modify starch, alter protein structure and aggregation, and thereby modify nutrient bioavailability. The synergy between enrichment strategies and innovative processing enables the development of high-quality, heathy bakery products (Guiné & Florença, 2024; Zhang et al., 2018).

Both the composition and processing methods for bakery products influence gastrointestinal digestion, as both determine the release and absorption of key nutrients and bioactive molecules, including starch, proteins, fibres, vitamins, minerals and phytochemicals, and thus play a key role in defining the physiological outcomes of their consumption

(Fernández-Tomé, 2024). Starch is the primary carbohydrate in bakery products, and its digestibility is influenced by type, crystallinity, matrix interaction and gelatinisation during baking, and retrogradation during storage (Udenigwe & Fogliano, 2017; Zhu, 2015b). Dietary fibres have been associated with a reduced risk of several diseases due to their physiological effects, including increasing faecal bulk, shortening intestinal transit time, lowering blood cholesterol and glycaemic responses, binding toxic compounds such as carcinogens, and promoting the growth of beneficial gut microbiota (Dhingra et al., 2012). Flores-Silva et al. (2017) showed that ultrasound treatment applied to gelatinized starch led to a marked increase in rapidly digestible starch, likely due to the formation of short-chain amylose molecules caused by the breaking of polymer chains under ultrasonic waves. In addition, J. Wang et al. (2022) compared white and brown rice digestion and demonstrated that the bran layer in brown rice delayed gastric emptying, maintained larger particle size and led to lower starch hydrolysis compared with white rice. The addition of sodium alginate to starch solutions was shown to decrease starch hydrolysis and glucose release, an effect attributed to increased viscosity and molecular interactions, that limited swelling and enzyme accessibility (Ramírez et al., 2015).

During digestion, dietary proteins are broken down by proteases and peptidases into amino acids, dipeptides, and tripeptides within the gastrointestinal tract. These smaller molecules are then either absorbed by the intestinal cells or used by gut bacteria in the small intestine (Wu, 2010). The amino acids and peptides generated from cereals, pseudocereals and legumes digestion provide health benefits such as antioxidant, antihypertensive, hypolipidaemic, antidiabetic and anticancer activities (Udenigwe & Fogliano, 2017). In addition, protein release varies according to the protein source and its structural conformation after processing (Ohanenye et al., 2022). Karaś et al. (2014) showed that heat treatment of yellow string bean proteins increased the antioxidant activities of their peptide-rich hydrolysates. According to Grundy et al. (2020) processing (toasting and puffing) impacted

protein and lipid digestibility in amaranth grains, with non-treated flour showing the highest digestibility properties. Furthermore, differences in composition and digestibility between grain sources demonstrated the influence of genotype and growing environment.

Polyphenols, often bound within the food matrix, can be released in the colon and contribute to microbial metabolism and health effects (Tagliazucchi et al., 2010). A study by Kan et al. (2020) showed that eating bread together with berry extracts (co-digestion) slowed starch digestion far more than adding the extracts into bread before baking. Co-digestion was more effective because polyphenols stayed free to interact with starch and enzymes, whereas baking trapped them in the bread matrix, reducing their activity

1.11 Elucidation of chemical compounds in food research

For the past number of decades, the food industry has been using analytical techniques. These include high performance liquid chromatography (HPLC), gas chromatography (GC), enzyme linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), gas chromatography mass spectrometry (GC-MS) and liquid chromatography mass spectrometry (LCMS-MS) to identify, quantify, and characterize nutrients, bioactive compounds and contaminants in food products (Hussain et al., 2019). However, these techniques have limitations, such as sample destruction, time consumption, the need for sample preparation, and some methods require the use of chemicals, which can cause environmental pollution (Wu & Sun, 2013).

Today, with the advancement of technology, both consumers and industry wish to be aware of what they are buying. The food industry has to ensure safety, quality and product standardization during processing operations. Due to the need for more efficient techniques and consumer demands, spectroscopic techniques such as raman, near-infrared (NIR), fourier transform infrared (FTIR) and hyperspectral imaging are steadily increasing (Aqeel et al.,

2025; Hussain et al., 2019). The following sections present an overview of FTIR and NIR spectral technologies, highlighting their principles and practical applications in the quality control of bakery products.

1.11.1 Fourier Transformer Infrared (FTIR) spectroscopy in bakery products

Infrared spectroscopy analyses how matter interacts with infrared (IR) radiation, by measuring the absorption of IR light as it passes through a sample. Each functional group in a molecule absorbs IR light at specific wavenumbers (cm^{-1}), creating a characteristic pattern that can be linked to its molecular structure (Cebi, Bekiroglu, Erarslan, et al., 2023). Fourier Transform Infrared (FTIR) spectroscopy is a reliable metabolic fingerprinting technique that provides a fast, accurate, green and non-destructive way to analyse samples within the mid-infrared region of the electromagnetic spectrum (Cebi, Bekiroglu, & Erarslan, 2023). The infrared spectrum of a molecule displays characteristic absorption bands of its functional groups within the 2500–25000 cm^{-1} region, providing information about its chemical structure (Cebi, Bekiroglu, Erarslan, et al., 2023). FTIR spectroscopy is already applied in research and industry for product quality and safety, however, many additional applications and sectors should be explored. As bakery products involve sensitive food systems, such as fermenting dough, that require rapid analysis, FTIR is especially valuable for its non-destructive nature and fast ability to deliver detailed chemical and structural information (Cebi, Bekiroglu, Erarslan, et al., 2023). **Table 1.3** provides a comprehensive overview of the applications and performance of FTIR spectroscopy

When combined with machine learning, FTIR has been shown to determine quality, authenticity and safety (Cebi, Bekiroglu, Erarslan, et al., 2023). A study by Osman et al. (2022) on wheat genotypes under heat stress combined FTIR spectroscopy with chemometric analyses and demonstrated that specific spectral regions could effectively discriminate between heat-

tolerant and susceptible varieties, highlighting the potential of FTIR as a tool for characterizing genotype-specific metabolic responses. According to, Abramovic et al. (2007) FTIR in attenuated total reflection (ATR) mode was applied to wheat samples contaminated with *Fusarium graminearum*, and the authors demonstrated that ATR spectra enabled 100% classification of infected versus control samples and allowed reliable estimation of deoxynivalenol (DON) content, highlighting the potential of FTIR as a rapid tool for detecting fungal contamination and mycotoxin presence in cereals. FTIR spectroscopy has also been applied to detect gluten contamination in gluten-free bakery products. Machine learning combined with FTIR was utilized to identify and quantify wheat flour in gluten-free corn bread (0.5–10%), achieving high accuracy with ensemble models and R^2 = 0.99 using KNN regression (Adedeji et al., 2023). In pita bread, FTIR has been applied to monitor bread staling and detected changes in saccharide (900–1150 cm^{-1}) and O–H (3000–3600 cm^{-1}) regions during storage, which is correlated with texture loss ($r = -0.97$ at 960 cm^{-1}). The study showed FTIR can effectively track staling and confirmed freezing as a way to slow quality deterioration (Al-Mahasneh et al., 2018). A recent study on sourdough bread prepared with scoby derived from kombucha investigated the effect of different dough hydration levels on bread quality. FTIR spectra provided evidence of how hydration altered protein and starch interactions, thereby supporting improved texture (Tanveer et al., 2025).

A principal application of FTIR in bakery science is the structural characterization of starch and proteins, as the technique enables real-time monitoring of order–disorder transitions in starch crystallinity alongside conformational changes in protein secondary structures (Pourfarzad et al., 2018)). Pourfarzad et al. (2015) explored how adding inulin (a dietary fibre) affects the secondary structure of gluten in model bread systems. FTIR was used to fit the amide I region (1600–1700 cm^{-1}), revealing that inulin shifts gluten structure from β-spiral (β-turns) toward β-sheet conformations. In addition, FTIR was used to monitor starch

retrogradation by the 1047/1022 cm^{-1} ratio, which increased linearly with storage time; results aligned with DSC and showed strengthened hydrogen bonding, confirming FTIR as a fast, reliable tool to assess starch crystallinity (Lu et al., 2021).

Table 1.3. Applications of FTIR spectroscopy in bakery products.

Category	Technique	Matrix/ Sample	Study and objective	Main Findings/ Application	Reference
Raw materials	FTIR and chemometrics (PCA, LDA)	Wheat	Heat stress in wheat genotypes-Characterize metabolomic responses under heat stress	FTIR and chemometrics effective for stress phenotyping	Osman et al. (2022)
Safety / contamination detection	ATR-FTIR	Wheat grains (control vs. infected with F. graminearum)	Detecting fungal contamination and mycotoxin presence	ATR spectra enabled 100% classification of infected samples; and reliable estimation of deoxynivalenol (DON) content	Abramovic et al. (2007)
Contamination	FTIR and Machine Learning	Gluten-free corn bread spiked with wheat flour (0–10%)	Detect and quantify gluten contamination in gluten-free corn bread	high accuracy detection with ensemble models and $R^2 = 0.99$ using KNN regression	(Adedeji et al., 2023)
Product quality	FTIR	Kombucha scoby sourdough bread	Assess hydration effect on bread quality	Hydration-driven changes in gluten and starch; 80% hydration provided the optimal quality	(Tanveer et al., 2025)
Product quality	FTIR and texture profile	Pita bread	Pita bread staling-Track structural and quality changes during storage	Saccharide (900–1150 cm^{-1}) and O–H (3000–3600 cm^{-1}) bands tracked staling; strong correlation with texture loss ($r = -0.97$ at 960 cm^{-1})	(Al-Mahasneh et al., 2018)
Protein characterization	FTIR (amide I deconvolution)	Bread containing gluten and inulin	Inulin–gluten interaction-Evaluate effect of functional fibre on protein structure	Inulin shifted gluten secondary structure from β-turns to β-sheets; demonstrated FTIR sensitivity to track conformational changes	Pourfarzad et al. (2015)
Starch characterization	FTIR (Starch crystallinity)	Rice starch	Analyse structural starch changes during starch retrogradation	FTIR was successfully used to determine degree of starch retrogradation	(Lu et al., 2021)

1.11.2 Near-Infrared (NIR) spectroscopy in bakery products

Near-infrared (NIR) spectroscopy applies light in the 700–2500 nm range to analyse the chemical composition and physical characteristics of materials without causing any modification to them. This instrument are widely used in bakery technology as a rapid, accurate, non-destructive analytical method to identify and quantify quality parameters such as protein, moisture, particle size, ash, colour, starch, damaged starch, total sugar, fat and water absorption (Amigo et al., 2019; Dowell et al., 2006; Jirsa et al., 2008; Ozaki Y., 2007). The cereal industry and baking sector were one of the earliest adopters of NIRS technology due to its convenience for analysing grains samples (ground, flour, bread) and the need for rapid quality control in wheat and final products (Christy, 2007). NIR has been applied in the baking industry at different stages along the production line (raw ingredients, intermediate processes and final products) (Donald et al., 2008).

In recent years, spectroscopy techniques have significantly evolved to detect fraud and adulteration. According to Üçüncüoğlu et al. (2013), shortening and butter in cake products can successfully be distinguished by NIRS. Spectroscopy instruments coupled with DNA markers and phenotypic assessment have also been applied successfully to differentiate spelt and bread wheat grains to prevent adulteration (Curzon et al., 2019). Ellen Almeida et al. (2021) demonstrated that NIR spectroscopy, digital imaging and chemometric analytical systems were good tools for determining and authenticating cassava starch content in wheat flour. Wang et al. (2019) determined the potato flour content in Chinese steamed bread using NIR spectroscopy.

Researchers have successfully utilised NIR for assessing product quality and have also achieved satisfactory predictive accuracy for quality-related parameters. Sylvio Barbon et al. (2019) used NIR spectroscopy to predict specific quality traits of wheat flour using Multi-target

Regressor Stacking, obtaining an improvement of up to 7% compared to Single-target approaches. The quality characteristics of sourdough bread were evaluated using NIR, electronic nose and machine learning models, yielding highly accurate outcomes (Claudia Gonzalez et al., 2022). These technological advancements can facilitate the adoption of AI technology for quality assessment in bread-making. Azizinia et al. (2023) used NIR to investigate the selection of end-product quality traits in wheat breeding and achieved genomic prediction accuracy at a level that enables selection for end-product quality traits early in the breeding cycle. Near-infrared spectroscopy has also been used to monitor the baking process, proving that NIR is a valuable tool for determining the optimum dough properties. Li Vigni and Cocchi (2013) employed NIR spectroscopy to monitor dough leavening in wheat flour and bread properties (e.g. volume) could be predicted from its behaviour during leavening. Chang et al. (2021) focused on monitoring the dough fermentation process in Chinese steamed bread. They demonstrated that NIR spectroscopy, combined with multivariate models, can replace traditional evaluations and enable rapid, automated monitoring of dough fermentation. The chemical processes that occur during bread staling, such as starch retrogradation and water loss, also can be followed with near-infrared spectroscopy (Ringsted et al., 2017). Amigo et al. (2019) utilised NIR hyperspectral imaging to examine the staling aspect of white bread crumb, revealing that NIR in combination with chemometrics can predict the hardness development of the control bread. This efficient monitoring of bread staling could help the food industry to increase product shelf life (Ringsted et al., 2017). At last, Yin et al. (2021) demonstrated high accuracy in detecting foreign contaminants in bread, highlighting the advantages of using NIR as a tool for assessing bread safety. **Table 1.4** provides a comprehensive overview of the applications, techniques, evaluated parameters, and performance of NIR spectroscopy in assessing essential aspects of bread quality and safety.

Table 1.4. Applications of Near-Infrared (NIR) spectroscopy in the analysis of bakery products.

Matrix/ sample	Technique	Objective	Calibration set and analysis	Main findings	Reference
Wheat flour	NIR spectroscopy	Prediction of quality parameters of bread wheat flour	Hectolitre weight Falling Number Protein content Alveograph indexes Farinograph stability	Improvement of up to 7% on the predictive performance of quality parameters compared to Single-target approaches.	(Sylvio Barbon et al., 2019)
Chinese steamed bread dough	NIR spectroscopy	Monitoring of dough fermentation	150 samples measured over 120 min at a 5 min Interval	NIR spectroscopy coupled with multivariate calibration models can achieve rapid and online monitoring of dough fermentation.	(Chang et al., 2021)
Wheat dough	NIR spectroscopy	Monitoring of dough leavening	Bread dimensions Bread volume Bread weight	The relationship between NIR spectra and leavening time was validated. NIR also provide information on bread properties.	(Li Vigni & Cocchi, 2013)
White bread	NIR hyperspectral imaging	Monitoring staling and impact of maltogenic α-amylases	Bread texture	It was demonstrated that the constituents of stored bread evolve differently depending on maltogenic α-amylase. Also, was verified which parts of the crumb are primarily exposed to changes.	(Amigo et al., 2019)
Bread	NIR and MIR spectroscopy	Monitoring of bread staling	Bread texture	High Correlation between NIR and MIR spectra with bread hardness. Staling processes can be followed with both near- and mid-infrared spectroscopy.	(Ringsted et al., 2017)
Chinese steamed bread	NIR	Predict the potato flour	132 samples with different proportions of potato flour	NIR determined the potato flour content with a Coefficient of	(Wang et al., 2019)

		content in Chinese steamed bread		determination ranging from 0.794 to 0.896	
Spelt and bread wheat grains	NIR spectroscopy	Differentiation between spelt and bread wheat	Protein content Phenotypic assessment DNA markers for γ-gliadin- , γ-gliadin-B, and the Q-gene	Highly accurate distinction between spelt and bread wheat based on Q-gene genotyping and three-waveband NIR model	(Curzon et al., 2019)
Wheat Flour and Cassava starch	NIR spectroscopy and Digital Images	Determination and authentication of cassava starch in wheat flour	200 sample mixtures of different proportions of cassava starch in wheat flour	NIR, Digital Images and chemometric multivariate analysis were demonstrated to be a good tool for the determination and authentication of cassava starch on wheat bread	(Ellen Almeida et al., 2021)
Bread	NIR spectroscopy and Computer vision	Identification of foreign contaminants in bread	Metallic Iron Polypropylene Plastic Hair	NIR spectroscopy and Computer vision are both operational ways to detect foreign contaminants in bread.	(Yin et al., 2021)
Sourdough Bread	NIR spectroscopy and electronic nose	Quality assessment of sourdough bread and wheat type classification	Volatile aromatic compounds Bread dimensions Bread texture Bread colour salt PH	Highly accurate machine learning model predictions were obtained to assess the type of wheat used for traceability and to evaluate 16 volatile aromatic compounds.	(Claudia Gonzalez et al., 2022)
Wheat Bread	NIR spectroscopy	Early-stage testing and selection of end-product quality traits in wheat breeding	Crumb yellowness Water absorption Bread texture Flour yield Grain protein Flour swelling volume	Genomic prediction accuracy was achieved at a level that enables selection for end-product quality traits early in the breeding cycle	(Azizinia et al., 2023)

1.12 Concluding remarks

Increased focus on, and use of, alternative plant ingredients has become a significant movement in the food industry, driven by factors which include general health benefits, the rise of personalized nutrition to meet individual dietary needs, environmental concerns, cultural and ethical considerations. Due to growing consumer awareness and the continued focus on health and environment, the demand for plant-based ingredients and foods is most likely to continue to increase.

Incorporating alternative plant sources, despite their nutritional benefits, can often negatively affect the texture, flavour, and overall acceptability of yeasted baked products. In the bakery industry, both researchers and producers must be aware of these potential changes when introducing such ingredients. In particular, changes in the gluten/starch matrix, dough viscoelastic properties and dough proofing capacity may lead to the production of less optimal bread loaves, with inferior crumb structure and textural attributes and ultimately reduced consumer acceptability. Based on the previously referenced studies in this review, both the grain processing method and level of incorporation significantly impact bread quality. Given the inherent challenge of preserving structure, texture and sensory attributes, extensive and rigorous research in high protein plant-based bakery applications is essential and must continue.

The production of bread which contains alternative plant ingredients begins with the selection of the plant source, followed by careful monitoring of the variability introduced through the processing techniques employed. Initial processing of the particular plant ingredient can be the key to producing a high-protein bread dough with a unified protein-starch matrix, rather than weakening gluten bonds. A meta-analytical study has underscored the importance of conventional methods and highlighted the promise of innovative plant ingredient processing techniques in enhancing bread hardness and boosting loaf specific volume when

contrasted with non-processed plant ingredients. It has been shown by several authors that applying particular emerging processing technologies to plant ingredients may offer a pathway to maximize nutritional value (overall product nutritional enrichment, increased nutrient digestibility) while simultaneously upholding optimal consumer acceptance. Conventional methods which include roasting, toasting, extraction, and fermentation are efficient and can enhance overall bread quality. Emerging technological advancements, particularly ohmic heating and ultrasound, demonstrate their strong capacity to improve bread quality. Technologies such as pulsed electric field have the potential to conserve energy and deactivate pathogens. However, the utilization of these technologies remains largely confined to laboratory or pilot scales. The cost of machinery remains a limiting factor for their widespread adoption within the food processing sector (Ahmed, 2021). Beyond cost considerations, further research is also necessary to evaluate the effects on the environment, economy, and society, taking heed of factors such as maintaining energy equilibrium, minimizing waste and enhancing living standards (Picart-Palmade et al., 2018).

Broader use of FTIR and NIR in bakery science requires improved calibration, standardization, and integration with machine learning.

1.13 Research gaps identified

a. Limited results on the optimal milling strategies for pulses in bakery applications, as most research has focused on wheat, leaving a knowledge gap in how different milling methods affect flour functionality, dough rheology, and bread quality.
b. Limited studies on emerging green processing techniques to improve functionality (e.g. ultrasound, microwave, pulsed electric fields, high-pressure processing) applied specifically to pulse and pseudocereal enriched breads.
c. Unexplored synergies of combined treatments, such as fermentation plus ultrasound, or germination plus extrusion, which could enhance both nutritional and techno-functional properties.
d. Insufficient understanding of the impact of emerging treatments on human digestion
e. Scarcity of studies evaluating the technical and economic feasibility for scale-up of applying novel treatments at industrial level.
f. Limited environmental and life cycle assessment data for emerging treatments when used as an extra process to improve ingredient functionality, making it difficult to evaluate their true sustainability compared to conventional processes.
g. Need for broader application and calibration of FTIR and NIR spectroscopy in bakery systems. More robust calibration models, standardized protocols, and adaptations for routine industrial and academic use are required to fully exploit FTIR as a rapid, non-destructive quality control tool.

1.14 Research objectives

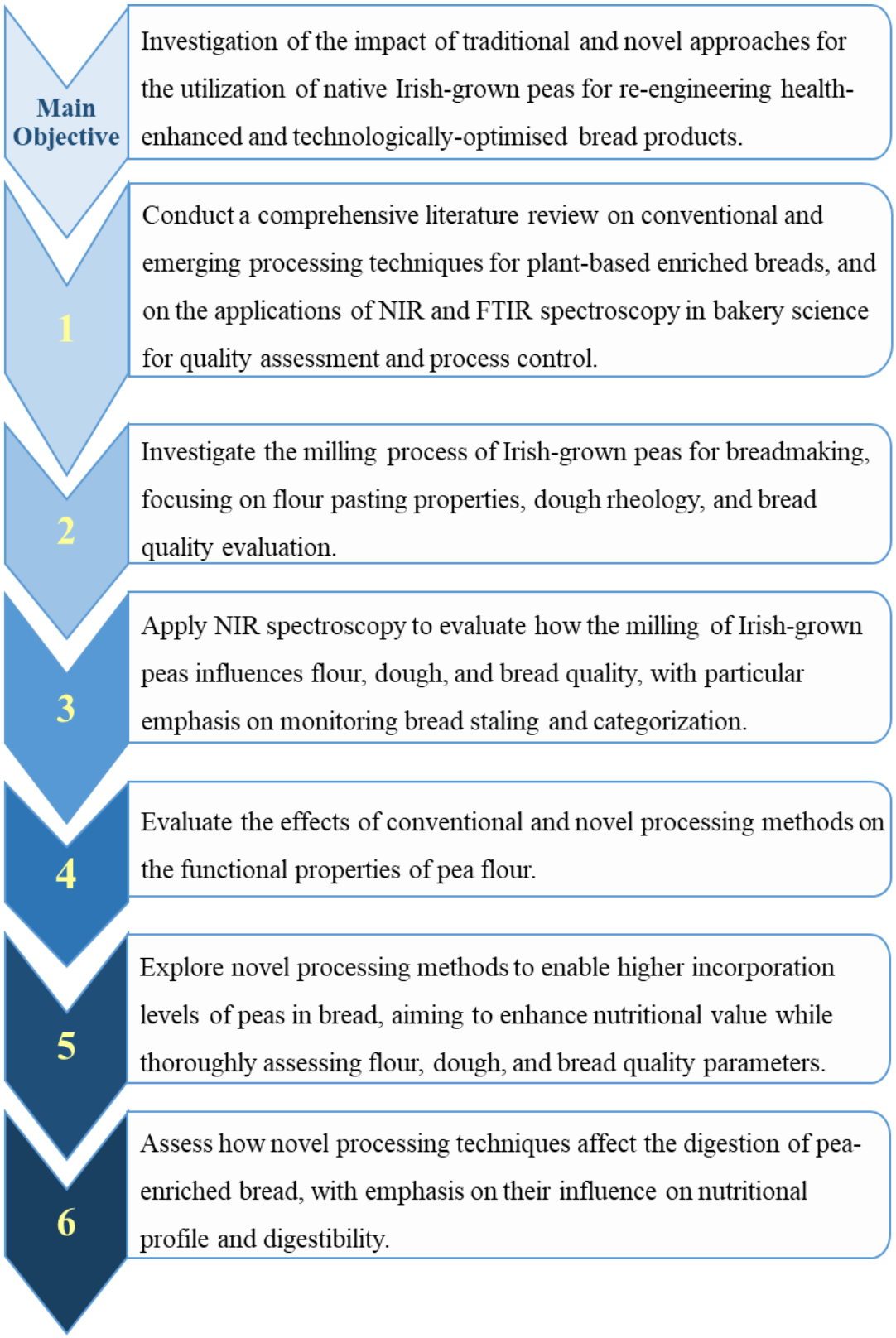

Figure 1.3. Primary objectives of thesis.

2 A study of the milling process of Irish-grown peas: NIR spectroscopy, flour pasting properties and dough rheology

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A study of the milling process of Irish-grown peas: NIR spectroscopy, flour pasting properties and dough rheology

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NIR

ABSTRACT

Peas contain valuable macronutrients, such as protein and dietary fibre, associated with health benefits. Blending pea flour (PF) with wheat flour (WT) can improve the nutritional profile of bakery products. In addition, the use of locally grown peas for food innovation activities can benefit the environment and the local economy. However, the pea milling process is of paramount importance and can affect the final flour quality. This study aims to evaluate the influence of different milling processes of Irish-grown peas on flour composition, spectral profile (Near Infrared - NIR), pasting properties and dough rheology. Three mills were used: roller (RM), hammer (HM) and cutting (CM) producing RM, HM and CM flours, respectively. A commercial strong wheat flour was used as the base flour. For producing doughs, wheat flour was blended with each pea flour at a 15:85 (pea: wheat). Flour composition, particle size, Scanning Electron Microscopy (SEM), dough mixing properties, viscosity profile, gel texture, dough extensibility and rheology were assessed for the control, all pea flours and flour blends. The hammer mill produced the highest yield of pea flour (93.9 %). Near Infrared Spectroscopy reflected the same results for proximate composition, and the PCA and was able to discriminate the main differences between flour samples. SEM demonstrated that higher particle sizes obtained from the CM tended to have larger starch and protein matrix aggregates. Pea flour from the RM presented the highest viscosity profile and hardness, most likely due to the higher starch and lower total dietary fibre content. The lower pasting viscosity profile of pea flour obtained from CM is most likely due to a high level of damaged starch present in this flour. There were no significant differences found among WT and flour blends for gel hardness. This may imply that the incorporation of 15 % HM and CM flours perhaps will not impact the staling process of the final bread products. Further research is being undertaken to examine the influence of the pea milling process on bread-making performance.

1. Introduction

We are experiencing a true plant-based revolution, with a greater percentage of people adopting diets that are rich in plant sources (Henri, 2019). According to Hall (2021), pulses contain approximately twice the amount of protein found in cereal grains. In addition, pulses are abundant in lysine, an amino acid that is not present in cereals. Thus, combining pulses with cereals results in a complete protein source. High-protein bakery innovation is driven by consumer demand, however, pulses in baking systems are still an area under development (Hall, 2021).

In Ireland, meat and meat-based products have the highest contribution (37 %) to the mean daily protein intake (Harrington et al., 2001). Increasing the production and application of high-protein crops would bring several environmental advantages, including promoting soil health and a low carbon footprint (Uebersax et al., 2023). Moreover, pulses are nutritious and uncomplicated to grow, and they are available at an affordable price (Maphosa & Jideani, 2017; Uebersax et al., 2023).

Substituting wheat with pea in bread requires significant reformulation knowledge of the flour and dough functional and rheological properties due to the differences in the overall makeup of the flour composite. To not compromise bread structure and acceptability, low

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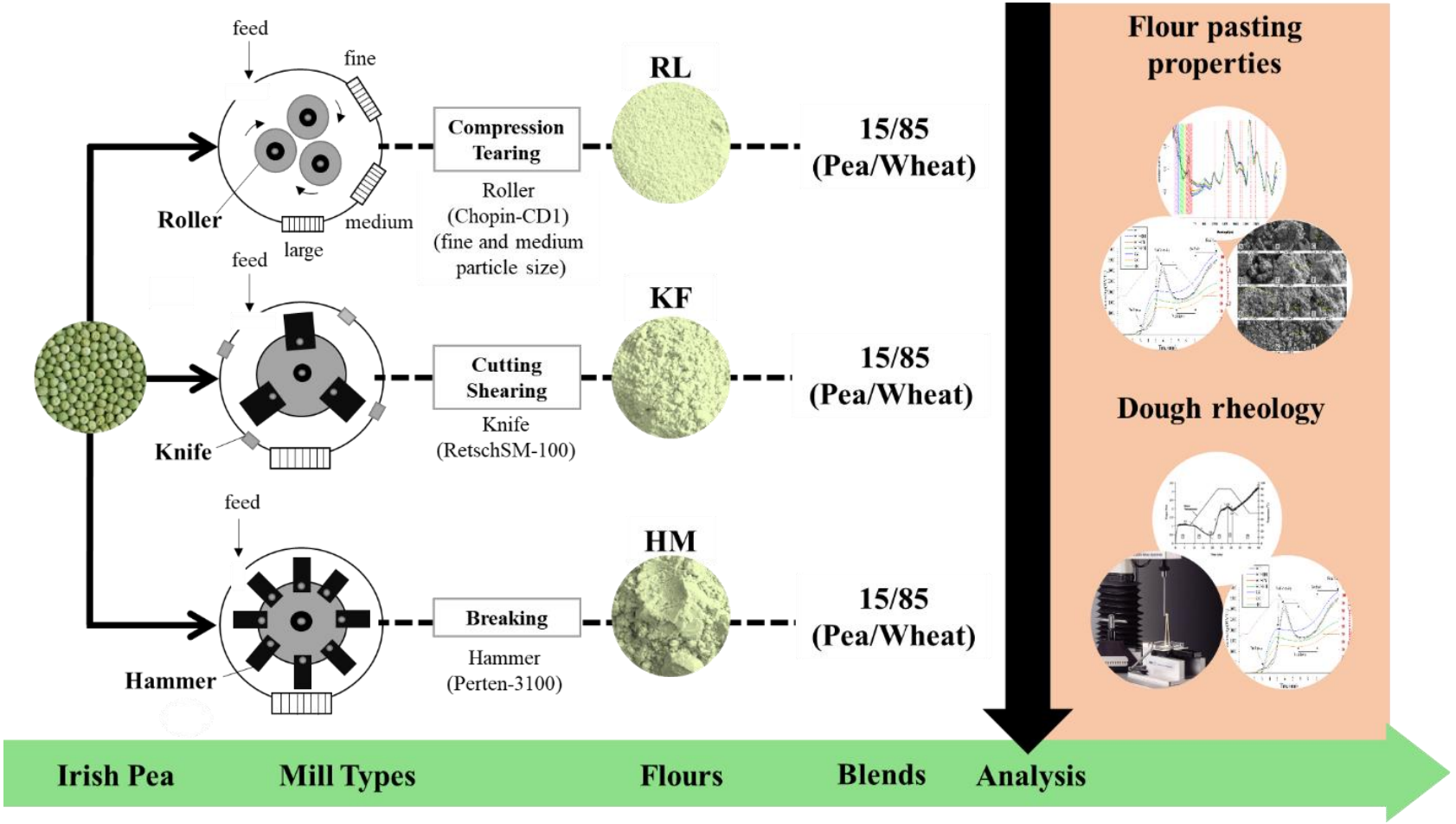
2.1 Graphical abstract



2.2 Abstract

Peas contain valuable macronutrients, such as protein and dietary fibre, associated with health benefits. Blending pea flour (PF) with wheat flour (WT) can improve the nutritional profile of bakery products. In addition, the use of locally grown peas for food innovation activities can benefit the environment and the local economy. However, the pea milling process is of paramount importance and can affect the final flour quality. This study aims to evaluate the influence of different milling processes of Irish-grown peas on flour composition, spectral profile (Near Infrared - NIR), pasting properties and dough rheology. Three mills were used: roller (RM), hammer (HM) and cutting (CM) producing RM, HM and CM flours, respectively. A commercial strong wheat flour was used as the base flour. For producing doughs, wheat flour was blended with each pea flour at a 15:85 (pea:wheat). Flour composition, particle size, Scanning Electron Microscopy (SEM), dough mixing properties, viscosity profile, gel texture, dough extensibility and rheology were assessed for the control, all pea flours and flour blends. The hammer mill produced the highest yield of pea flour (93.9 %). Near Infrared Spectroscopy reflected the same results for proximate composition, and the PCA and was able to discriminate the main differences between flour samples. SEM demonstrated that higher particle sizes obtained from the CM tended to have larger starch and protein matrix aggregates. Pea flour from the RM presented the highest viscosity profile and hardness, most likely due to the higher starch and lower total dietary fibre content. The lower pasting viscosity profile of pea flour obtained from CM might be due to a high level of damaged starch present in this flour. There were no significant differences found among WT and flour blends for gel hardness. This may imply that the incorporation of 15% HM and CM flours perhaps will not impact the staling process of the final bread products. Further research is being undertaken to examine the influence of the pea milling process on bread-making performance.

2.3 Introduction

We are experiencing a true plant-based revolution, with a greater percentage of people adopting diets that are rich in plant sources (Henri, 2019). According to Hall (2021), pulses contain approximately twice the amount of protein found in cereal grains. In addition, pulses are abundant in lysine, an amino acid that is not present in cereals. Thus, combining pulses with cereals results in a complete protein source. High-protein bakery innovation is driven by consumer demand, however, pulses in baking systems are still an area under development (Hall, 2021a).

In Ireland, meat and meat-based products have the highest contribution (37%) to the mean daily protein intake (Harrington et al., 2001). Increasing the production and application of high-protein crops would bring several environmental advantages, including promoting soil health and a low carbon footprint (Uebersax et al., 2023). Moreover, pulses are nutritious and uncomplicated to grow, and they are available at an affordable price (Yvonne Maphosa & Victoria Adaora Jideani, 2017; Uebersax et al., 2023).

Substituting wheat with pea in bread requires significant reformulation knowledge of the flour and dough functional and rheological properties due to the differences in the overall makeup of the flour composite. To not compromise bread structure and acceptability, low levels of pea flour have previously been added to wheat in bread (Hall, 2021a). Incorporation of higher levels would be nutritionally more attractive, but to achieve this, the techno-functional properties of the pea flours must be comprehensively characterized. The influence of parameters such as particle size, damaged starch and flour composition on pea flour properties have been verified by several authors (Borsuk et al., 2012; Maskus et al., 2016; Zucco et al., 2011). Milling technologies have been shown to impact these parameters, and consequently affect the final quality of the end products (Borsuk et al., 2012). In addition, grain genotype is a critical factor that influences the milling performance (Scanlon et al., 2018).

Therefore, to increase the pea level of incorporation in food/bread formulations, it is crucial to initially determine the optimal pea milling process.

Three common feed-mills that can be used for pulse processing are roller, cutting and hammer. They are straightforward to control and contain a continuous feeder, making them attractive to producers (Hoyle B, 1968). Roller milling (RM) is usually more expensive to operate than hammer and cutting due to its robust manufacture. This type of mill is being used by most commercial wheat flour companies and is recommended for pulse milling (Arce & Kratky, 2022). The principal mechanisms responsible for the reduction in the particle size are compression and tearing (Arce & Kratky, 2022). The roller mill works with a fractionation system producing large, medium and fine particle sizes. This mechanism is useful as it can break and separate the outer coat of the grains and consequently mill the internal section more easily. Wheat passes through a series of opposing cylinders and flour is sifted between stages (Cappelli et al., 2020). Cutting and shearing are the forces applied by the cutting mill. The resulting particles fall through a screen sieve to a storage tank (Arce & Kratky, 2022). The hammer mill is currently the predominant pulse milling technique for commercial settings (Price et al., 2021). When using the hammer mill, the grains are subjected to a dynamic effect of pressure force and spin toward a breaker plate. Therefore, the primary reduction mechanisms responsible for size reduction of the grains is breaking. The grains are broken until the appropriate size is achieved to pass through the sieve (Arce & Kratky, 2022).

Near-infrared (NIR) spectroscopy instruments are widely used in cereal and bakery science to measure quality parameters such as protein, moisture, particle size, ash, damaged starch, and water absorption (Amigo et al., 2019; Dowell et al., 2006; Jirsa et al., 2008; Ozaki Y., 2007). The baking industry was one of the earliest to invest in NIR spectroscopy, due to the ease of preparing a ground sample (e.g. from grains, flour and bread), and the capability for fast quality control of the product (Christy, 2007). According to Donald et al. (2008), NIR can be applied to raw ingredients, intermediate processes and final products. Near-infrared

spectroscopy has been used for monitoring the baking process, where the possibility of a chemical interpretation of the samples has been proven (Aït Kaddour et al., 2008).

The present study aimed to evaluate the roller, cutting and hammer milling performance of Irish-grown peas for bread making. The objective of the study was not to emulate the properties of wheat flour. However, wheat flour was used as a comparative system to set a standard for typical flour blending and dough technological properties for onward bread applications. A detailed analysis of the pea flour composition, pasting properties and dough rheology was conducted on the resulting flours from each milling process. In this study, the inclusion of NIR spectroscopy enables a comprehensive view of the milling process of Irish-grown peas and strengthens conclusions of the quality parameters and chemical composition. In addition, emphasizes its potential for future flour characterization, thus highlighting its practical applications in the field.

2.4 Materials and methods

2.4.1 Flour analyses

2.4.1.1 Raw material and milling process

Strong wheat spring flour was purchased from Nelstrops Co., Ltd., UK. Spring green peas harvested in 2022, were supplied by Teagasc Oak Park, Ireland.

The peas were initially broken with a food processor (23 - Robot Coupe, Blixer®, USA) for 2 min at speed 2 to facilitate the subsequent milling process. This preliminary step is fundamental for ensuring the smooth and uniform feeding of grains during the milling process. The peas were then processed through three different mills (cutting, hammer and roller). The cutting mill (SM300, Retsch®, Germany) was set to 1500 rpm and minimum sieve (0.75 mm). The hammer mill available (Labmill 3100, Perten®, Sweden) had a 0.8 mm sieve at 16,800 rpm with an adjustable vacuum feed control to regulate the sample feed rate, feed rate 2 was

selected. The roller mill (CD1, Chopin®, France) only the break stage was performed, and fractionated the flour into small (break), medium (middling) and larger (bran) particle sizes using the method 26-70.01 (Reddy et al., 2015), using only the medium and small fractions.

Based on preliminary trials, we selected an optimal flour blend ratio to accurately assess the rheological properties of dough blends from the different milling processes. Wheat flour was blended with pea flour to obtain flour blends 15:85 (pea:wheat). A control sample (100% wheat flour) was also used. Each milling trial was carried out three times and subsequent analyses were carried out in triplicate.

2.4.1.2 Flour composition

Total proximate composition for pea and wheat flours was determined. Moisture content was evaluated using method 44-15.02 (AACC, 2010b). Total nitrogen was determined by the combustion method based on the Dumas principle using LECO equipment (FP-628, LECO Corp., USA) (Nx5.7), according to method 968.06 (AOAC, 2000b). Fat content was determined using the ORACLE Rapid NMR Fat Analyser (CEM Corp., USA) (AOAC, 1991). The total dietary fibre (TDF), including insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), was determined by applying an enzymatic-gravimetric method using Ankom automated dietary fibre analyser (Ankom, USA) using the method 991.43 (AOAC, 1994). Ash content was determined using a Gallenkamp Muffle Furnace, pre-drying overnight at 550 °C by the method 08-01.01 (Gallenkamp, UK)(AACC, 2010a).

The total carbohydrate content (%) was determined by subtracting the sum of protein, fat, ash, and moisture from 100%. The total starch content was calculated by subtracting the TDF from the total carbohydrate content (Charrondiere et al., 2004).

Damaged starch was performed utilising assay kits (Megazyme International Ltd.) following the method 76-13.01, (AACC, 2000).

2.4.1.3 Visible and near-infrared (Vis/NIR) spectroscopy

The spectral acquisition of the flours was performed using the Vis/NIR spectrometer (Hi-Res ASD Labspec® 4, Malvern Panalytical, UK). The Labspec spectral range is 350–2500 nm, with 1 nm data point interval. Labspec is equipped with 3 detectors. The visible range detector is a reflective holographic diode array and ranges from 350 to 1000 nm (Cayuela-Sánchez et al., 2019). For the NIR range are used two different detectors, a holographic fast scanner InGaAs from 1000 to 1800 nm and a high-order blocking filter from 1800 to 2500 nm (Cayuela-Sánchez et al., 2019). Three replicates per sample were recorded. Before spectra analysis, the three replicates were averaged and Standard Normal Variate and Detrend (SNVD) was used as a spectral treatment to reduce the scattering effect and correct the baseline. The spectra editing was performed under Software R Studio (R Studio Team 2022) using the "detrend" function from "prospectr" package (Ramirez-Lopez, 2022; Team, 2022). Principal components analysis (PCA) was used to discriminate all flour samples. The PCA was performed using the "pca3d" R package on centred and scaled spectra considering the first 10 principal components (Weiner, 2020). The spectra of Wheat starch-Native, Hydrolysed wheat protein CWS (≥84%), Soluble wheat protein W (≥84%), Isolate pea protein Nutralys S85XF (≥84%) provided by Roquette, France and Vitessense Isolate pea protein- Pulse 1803 (≥85%) provided by Ingredion, US were recorded and used as standards to identify informative peaks and make accurate comparisons with the flours samples analysed.

2.4.1.4 Particle size

The particle size of pea and wheat flours was measured by laser scattering using a Mastersizer (3000, Malvern Instruments Ltd, UK). Hydro EV Flexible volume wet dispersion with refractive index (1.53) and absorption index (0.01). Samples (g) were diluted into reversed osmosis water until they reached the acceptable obscuration limits (10–20%). Particle size was reported at 10% (d10), 50% (d50), and 90% (d90) of the volume distribution.

2.4.1.5 Scanning electron microscopy (SEM) of flours

Pea and wheat flours were fixed to the sample holders using double-face carbon tape and coated with a gold layer using a sputter coater (Emitech K575X, Quorum Technologies, UK). The samples were imaged using a SEM (Supra 40VP, ZEISS, Germany) equipped with a secondary electron detector at 2 kV accelerating voltage.

2.4.1.6 Flour pasting properties

The pasting properties of the pea flours, wheat flour, and flour blends 15:85 (pea:wheat) were determined using the Standard Profile 1 of the Rapid Visco Analyser (RVA-4, Newport Scientific Pty. Ltd., Australia). The flour (4 g) was added to distilled water (25 mg) with moisture correction equations to 14% according to method 76-21.02 (AACC, 2010d). The pasting properties were determined by peak viscosity, breakdown, setback viscosity, final viscosity and pasting temperature.

2.4.1.7 Texture profile analysis (TPA) of the RVA gel

The pastes formed in the RVA canister were covered with film to avoid weight loss and kept for 24 h at room temperature. Gel texture was assessed by a texture analyser (TA-XT2i, Stable Microsystems, UK). Texture profile analysis (TPA) was performed using a 20 mm cylindrical probe with a deformation of 40% and a test speed of 1 mm/sec.

2.4.1.8 Dough mixing properties

Flour blends 15:85 (pea:wheat) were analysed using Mixolab (Mixolab-2, Chopin, France) using the standard Chopin+Protocol. Moisture content of the flour was previously measured (AACC, 2001) and used to determine flours weight. The resulting mixing curve provided information on water absorption (required to reach the consistency at 1.1±0.05 Nm); dough stability (time the torque was kept at 1.1 Nm); dough development time, C2 (protein

quality, Nm), C3 (starch gelatinisation), C4 (starch gel stability, Nm) and C5 (starch retrogradation, Nm) following the method 54-60.01 (AACC, 2010c).

2.4.2 Dough analysis

2.4.2.1 Sample preparation

The dough was prepared in a Mixolab (Mixolab-2, Chopin, France) using wheat and flour blends, salt (2% flour basis), unsalted butter (1% flour basis) (both purchased locally, Dublin, Ireland) and improver (1% flour basis) (Lesaffre UK & Ireland Ltd., Worcester, UK). Water was added according to the water absorption results obtained previously when using the Mixolab (section 2.1.7). The percentage of each ingredient was calculated based on the flour content (ingredients/flour). Consequently, to obtain a torque of 1.1 Nm, the water absorption was reduced by 4%.

2.4.2.2 Fundamental oscillatory rheology

Rheological tests were conducted using an Anton Paar MCR301 controlled stress rheometer (Anton Paar GmbH, Austria) with a 25 mm serrated parallel probe and base plate. Excess sample was trimmed and the exposed edges were covered with petroleum jelly. Before performing the test, dough samples were allowed to rest for 45 min at 30°C.

2.4.2.3 Amplitude sweep

A 2 g dough piece was placed on the base plate using a 1 mm gap. The dough was allowed to rest for 5 min to allow relaxation of residual stresses. The test was carried out with a temperature setting of 25 °C under a Peltier hood. The linear viscoelastic region of the dough was analysed over a strain range of 10-3 to 102% (γ), and the dough displayed a linear region between 0.001% and 0.05% strain.

2.4.2.4 Frequency sweep

The dough piece (2 g) was placed on the base plate with a 1 mm gap. After a 5 min rest, the frequency was gradually increased from 0.1 to 10 Hz with a constant strain of 0.01% (previously determined during the amplitude sweep). The values of the storage modulus (G′) and loss modulus (G″) were recorded at 25 °C.

2.4.2.5 Dough extensibility

A texture analyser (TA-XT2i Stable Microsystems, UK) was used to evaluate the dough extensibility and resistance to extension. The device was equipped with a Kieffer dough and gluten extensibility rig. A 10 g dough ball was placed on the Teflon mould (previously brushed with petroleum jelly), pressed into strips, and left to rest for 45 min at 30°C. The Kieffer measurement was performed using the following settings: test speed of 3 mm/s, distance of 75 mm and trigger force of 5g.

2.4.2.6 Statistical analysis

Flour and dough results were assessed by analysis of variance (ANOVA) using Minitab software (version 17.0). ANOVA was performed to compare and evaluate the existence of significant differences in the analysed parameters. Tukey's test for the comparison of average was used for a statistical significance level of 95% ($p < 0.05$). Samples were analysed in triplicate and results are presented as average ± standard deviation. To perform ANOVA the samples were divided into two groups, 100% pea flour plus wheat flour (RM, CM, HM and WT) and flour blends plus wheat (WT+RM, WT+CM, WT+HM and WT). A correlation study (Pearson’s correlation coefficients) was performed using Software R Studio (Team, 2022). Correlation between flour analyses was performed using R statistical package ‘corrplot’. This is a heatmap where the blue colour indicates positive correlations and the red colour indicates

negative correlations (Simko, 2021). The colour intensity varies from high to low and determines the level of correlation.

2.5 Results and discussion

2.6 Flour properties

2.6.1.1 Flour composition

Table 2.1 presents the yield and composition of WT, RM, CM and HM flours. Among the pea flours, significant differences were not found for protein, fat, ash, SDF and carbohydrate content. However, the TDF was significantly lower ($p < 0.05$) for the flour obtained by the roller mill. The roller mill fractionates the pea into small, medium and large particle sizes. The larger fraction, which is made up of the outer layer of the pea, containing the majority of the IDF, is discarded. Consequently, a decrease in the content of total dietary fibre in this flour was observed and associated with a slight increase of other constituents (i.e. protein and starch) as a consequence of the reduced dilution provided by the fibre. When comparing the total fibre among all the flour samples, it was found that all pea flours (RM, CM and HM) had significantly higher levels in comparison to the wheat flour ($p < 0.05$).

Table 2.1. Proximate composition of wheat flour and pea flours.

Composition (g/100g)*	WT	Pea flours		
		RM	CM	HM
Yield	-	55.64±1.72^{b}	39.15±5.68^{c}	93.89±4.76^{a}
Protein	12.77±0.04^{b}	19.97 ±1.13^{a}	18.38±0.25^{a}	18.61±0.65^{a}
Fat	0.75±0.16^{a}	0.96±0.05^{a}	1.09±0.19^{a}	1.07±0.22^{a}
Ash	0.84±0.02^{b}	2.47± 0.07^{a}	2.32±0.13^{a}	2.71±0.20^{a}
Total dietary fibre	4.13±1.10^{c}	12.13±0.06^{b}	18.00±1.18^{a}	16.66±0.06^{a}
Soluble dietary fibre	2.70±0.26^{b}	5.16±0.25^{ab}	5.80±1.97^{a}	5.10±0.26^{ab}
Insoluble dietary fibre	1.90±0.78^{c}	6.96±0.25^{b}	12.20±1.08^{a}	11.53±0.29^{a}

Carbohydrates	85.62±0.19[a]	76.57±0.24[b]	78.96±01.42[b]	77.60±0.58[b]
Starch	81.38±1.27[a]	64.43±0.27[b]	60.96±2.55[c]	60.93±0.59[c]
Damaged Starch	7.87±0.36[a]	2.52±1.62[c]	4.92±0.07[b]	2.60±1.22[c]
Moisture	12.26±0.10[a]	12.49±0.10[a]	12.17±0.05[a]	11.8±0.25[b]

*WT: Wheat flour; RM: Roller Mill pea flour; CM: Cutting Mill pea flour; HM: Hammer Mill pea flour. Data presented as means ± standard deviation. Values with a different letter (a-d) are significantly different ($p < 0.05$).

The damaged starch content was significantly higher ($p < 0.05$) for the CM pea flour. The cutting mill is a continuous process and consists in cutting and shearing the grains with sharp rotating knives (Puckett & Daum, 1968). During the milling process, the CM mill had to be opened multiple times to remove coarser particle size fractions that did not pass through the sieve. Consequently, cutting milling took longer to process the grains. Thus, the continuous force applied to the grains, over a longer time, may have increased the damaged starch content. Furthermore, due to the amount of flour that was unable to pass through the 0.75 mm sieve and was discarded due to the fraction being extremely coarse, the CM yield was significantly lower than flours obtained from the roller and hammer mill. The reduced yield of the cutting mill can likely be attributed to its suitability for processing soft, medium-hard, elastic, and fibrous materials. In the case of dried peas, their hardness might pose challenges for this mill, resulting in difficulties when attempting to achieve flour particle sizes smaller than 0.75 mm (scientific, 2021).

Hammer mills use high-speed rotating hammers to break the grains, while roller mills use compression and tearing forces (Arce & Kratky, 2022). The hammer and roller mills resulted in smoothly milled peas. The yield of RM was also significantly lower ($p < 0.05$) than the HM, which is a consequence of a larger fraction of the sample from RM being discarded, in accordance with the operating procedure. The HM greater flour yield ($p < 0.05$) can lead to lower production costs and less waste, as more flour can be produced using the same amount of raw materials, thus reducing the overall environmental impact of the production process.

Damaged starch content of all pea flours was significantly lower than wheat flour, most probably due to the completely different botanical source of pea flour compared to wheat.

Bourré et al., (2019) observed the same trend, demonstrating that the grain differences affected the level of physical starch damage.

2.6.1.2 VIS-NIR spectroscopy

SNVD Vis-NIR spectra of flours are shown in Figure 2.1. NIR standards of pea and wheat isolate components are represented in Figure 2.2. Figure 2.3 shows the 3D score plot (A) and loadings (B) of flour samples for the first three principal components.

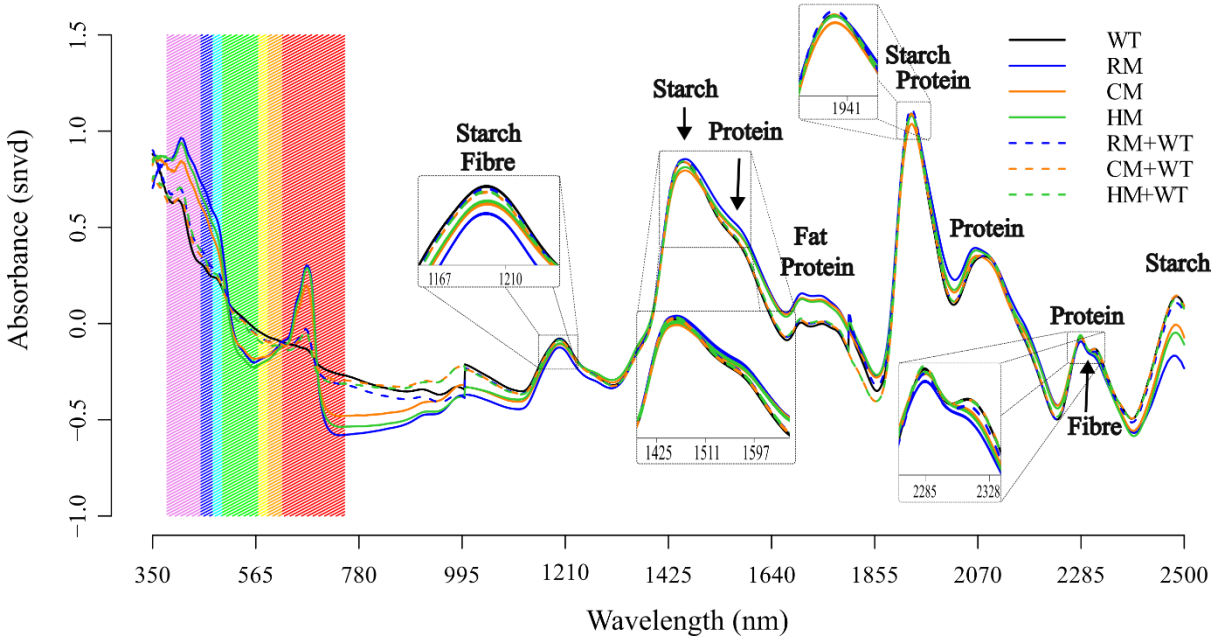


Figure 2.1. SNVD Vis-NIR spectra of flours samples.

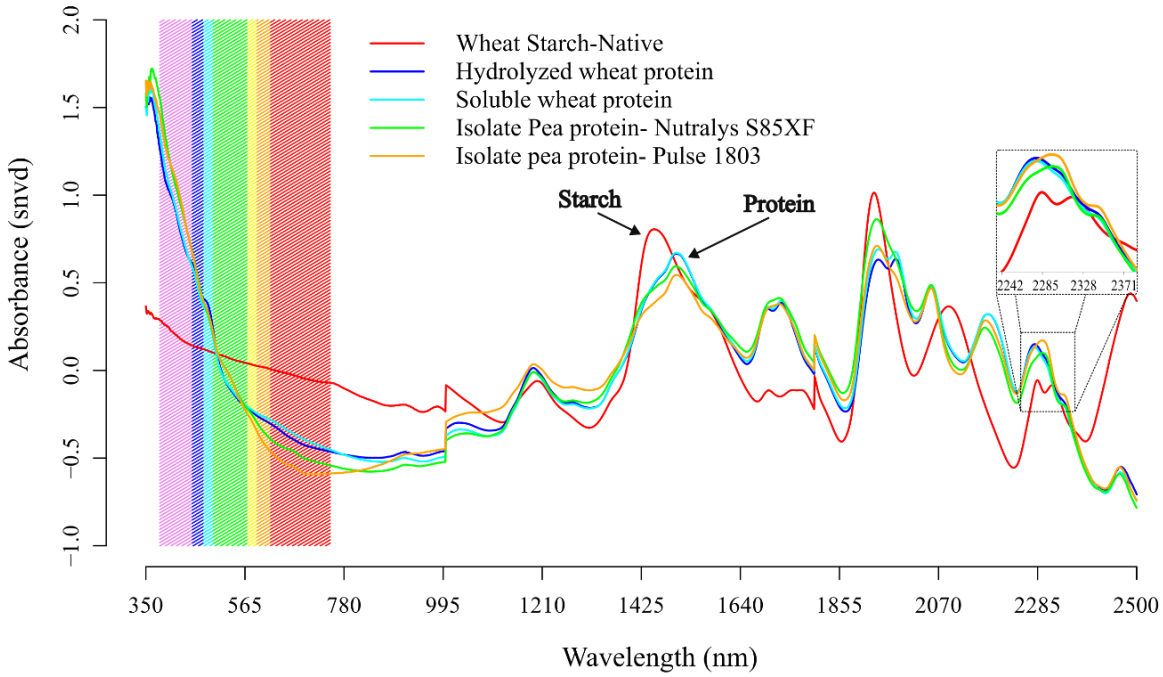


Figure 2.2. SNVD Vis-NIR spectra of standard flours.

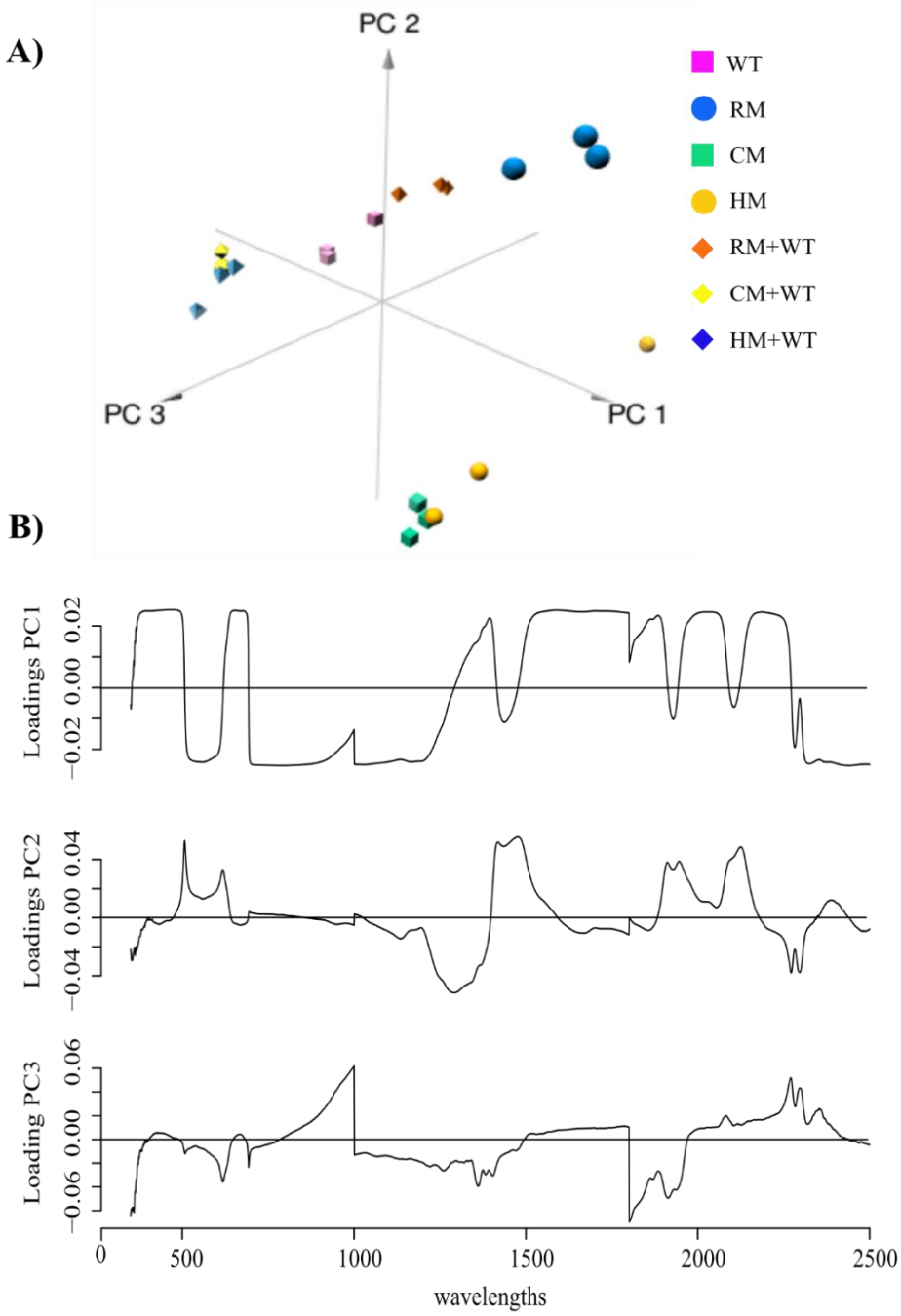


Figure 2.3. (A) 3D Score plots of PC1, PC2 and PC3, explaining 72%, 14% and 8% of total variance, respectively.

The main peaks in the visible region (350-750 nm) (Figure 2.1) were identified in the violet (380–450 nm) and red areas (620-750 nm), respectively. Also, important differences among flour types were found in the green region (496-570 nm). Pea flours showed a higher absorption of violet and red light and a higher reflection of green light compared to wheat and flour blends. Among the pea flours in the green region (Figure 2.1), HM exhibited the highest reflection and, consequently, the greenest colour, followed by RM and CM, respectively. This

indicates that colour distinctions are quantitatively and visibly possible among the flour samples. In terms of specific values at 550 nm (**Figure 2.1**; SNVD spectra), where the main peaks are shown in the green area, the reflection for HM was -0.213, RM was -0.181 and CM was -0.165.

These differences in the visible region could be justified by the chlorophyll present in the pea flours which is responsible for their green pigmentation (Koca et al., 2007). Moreover, colour is an important parameter when assessing flours products as will influence the end product quality (Black & Panozzo, 2004).

The most relevant differences among flours in the NIR region were observed around the following spectral ranges: 1160-1240, 1400-1620, 1650-1800, 1900-2150, 2250-2350, and 2450-2500 nm.

The spectral range from 1160 to 1240 nm showed higher absorption for wheat flours and blends, RM flour showed the lowest absorption, confirming what reported by Badaró et al. (2019), that 1206 nm is related to fibre absorption, more specifically the C-H stretching vibration in the first overtone. According to Peiris et al. (2021) peaks absorption at 1160 and 1240 nm are associated to starch groups absorbing in the second overtone of C–H. In addition, 1210 nm is also associated with carbohydrates, C–H stretching vibrations (Beć et al., 2020). The standards confirm the association with starch molecules. In this area, the lower water absorption of the RM flour may be due to its lower fibre content, while the higher absorption of the wheat flour is most likely related to its higher starch content.

Within the range of 1400 to 1620 nm two main peaks were identified around 1450 and 1550 nm. At 1450 nm the wheat showed higher absorption than pea flours, while at 1550 nm, pea flour showed higher absorption than wheat. The literature associates the peaks 1480 nm and 1565 nm with carbohydrates (C-H stretching vibrations) and protein group (N-H stretching vibrations), respectively (Beć et al., 2020). These results are in line with the composition described in **Table 2.1.** Nonetheless, RM stood out as it exhibited higher absorption along the

entire area. This can be attributed to its high content of starch and protein. The same peaks were clearly identified for starch and protein standards.

Between 1650-1800 nm, there is an evident differentiation when comparing pea flours with wheat flours and blends. Vines et al. (2005) observed that at 1728 nm, C-H first overtone asymmetric stretch occurs, and at 1760 nm, symmetric stretch is linked with fat content. Moreover, Ingle et al. (2019) demonstrated that absorption at 1743 (C-H) increased as the protein content increased. In addition, the standards revealed high absorption of protein for this range. Pea flour has a higher fat and protein content than wheat flour justifying the higher absorption rates for pea flour for these specific peaks.

In the 1900-2150 nm range, two main peaks are identified, wheat flour and blends displayed higher absorption around 1930 nm and pea flours around 2050 nm. Absorption at 1955 nm corresponds to a carbohydrates group, C-H stretching vibrations (Beć et al., 2020). At 1980 and 2050 nm, peaks are associated with specific protein groups and correspond to the N–H asymmetric stretch and amide II and N–H stretch and amide II, respectively (Kays et al., 2000). The standard, for this specific range, showed high starch absorption at 1930 nm and high protein absorption at 2050 nm. Our spectral profile is in line with the literature, standards and proximate composition.

RM showed the lowest absorption in the range 2250-2350 nm that can be attributed to its lower fibre content in accordance with Badaró et al. (2019), who associated this area with the fibre content (C-H stretching vibration in the second overtone). Differences and shifting of NIR absorption shown in **Figure 2.2** in this area can be justified by the difference in structure and conformation of protein (Bolje and Gobec, 2021).

At 2450-2500 nm, three main spectral groups can be identified, higher absorption is shown for wheat and blends, lower for RM and KF and HM in the middle. Vitelli et al. (2021), found relevant absorption for starch samples in the same range which is supporting the flours samples and standard results.

Studying **Figure 2.3**, four main clusters can be clearly identified based on the first three PCs (RM; CM and HM; CM+WT and HM+WT; WT and RM+WT).

PC1 (72% variability) clearly discriminates two main clusters, pea flours (positive values) from blends and wheat flours (negative values). The difference in these two clusters can be associated with both colour (visible region) and overall chemical composition (NIR range) (Manley, 2014), as suggested also by the loading of PC1 that shows relevant peaks in the visible and NIR in correspondence of absorptions for chlorophyll, starch, protein and fibre which represent the main composition-related differences among flours.

The second principal component (14% variability), discriminates among three main clusters, RM flour with positive values, wheat and blends close to the centre and HM and CM flours in the negative area. The main peaks identified in PC2 loading were 510, 620, 1420, 1480, 1920, 1955, 2120 and 2400 nm on the positive area and 1140, 1300, 2280 and 2300 nm on the negative area. These results suggest that in the visible region, the main difference between RM and the other flours is given by green pigments (as shown also in **Figure 2.1**) and in the NIR region by starch and protein (positive) and fibre (negative) content. Thus, PC2 explains the variability caused by the milling process on pea flour samples, which has a direct effect on the fibre and its dilution effect.

The third principal component (8% variability), discriminates the WT and RM+WT (negative values) from the remaining samples (positive values) in two clusters. Positive peaks at 2280 and 2300 nm represented on PC3 loading are associated with fibre and protein groups, while in the negative area peaks at 1360, and 1955 nm are mainly related to starch groups. Hence, the PC3 elucidates the differentiation of RM+WT and WT due to their higher starch content and lower protein and fibre content.

2.6.1.3 Particle size

Table 2.2 summarises the particle size of pea flour processed with different mills (roller, cutting and hammer) and wheat flour. The flour particle size is an important parameter as it can impact important functional properties such as water absorption capacity and staling and sensory characteristics of the final product (Hall, 2021a).

Table 2.2. Distribution of particle sizes in wheat flour and pea flours based on percentiles.

Flours*	Percentiles of distributions		
	d10	d50	d90
WT	17.90±1.0^{a}	82.87±4.9^{b}	187.67±14.2^{c}
RM	19.70±0.8^{a}	181.00±16.5^{a}	423.67±13.6^{b}
CM	11.30±1.5^{b}	64.87±15.0^{bc}	686.33±51.2^{a}
HM	11.28±1.4^{b}	45.40±2.7^{c}	347.33±43.5^{b}

*WT: Wheat flour; RM: Roller Mill pea flour; CM: Cutting Mill pea flour; HM: Hammer Mill pea flour. Data presented as means ± standard deviation. Values with a different letter (a-c) are significantly different ($p < 0.05$). At d10, the 10th percentile, 10% of the volume of flour particles are the indicated size (µm) or smaller; at d50, the 50th percentile, 50% of the volume of flour particles are the indicated size (µm) or smaller; and at d90, the 90th percentile, 90% of the volume of flour particles are the indicated size (µm) or smaller.

In Table 2.2, is visible that there is a variation in the Percentiles of distributions and respective particle sizes. Moreover, Figure 2.4 represents the volume density (%) per size classes (logarithmic scale). This graph allowed the visualization of the occupied space for a specific particle size. Wheat flour particle size is represented for a single peak and pea flour for two peaks each.

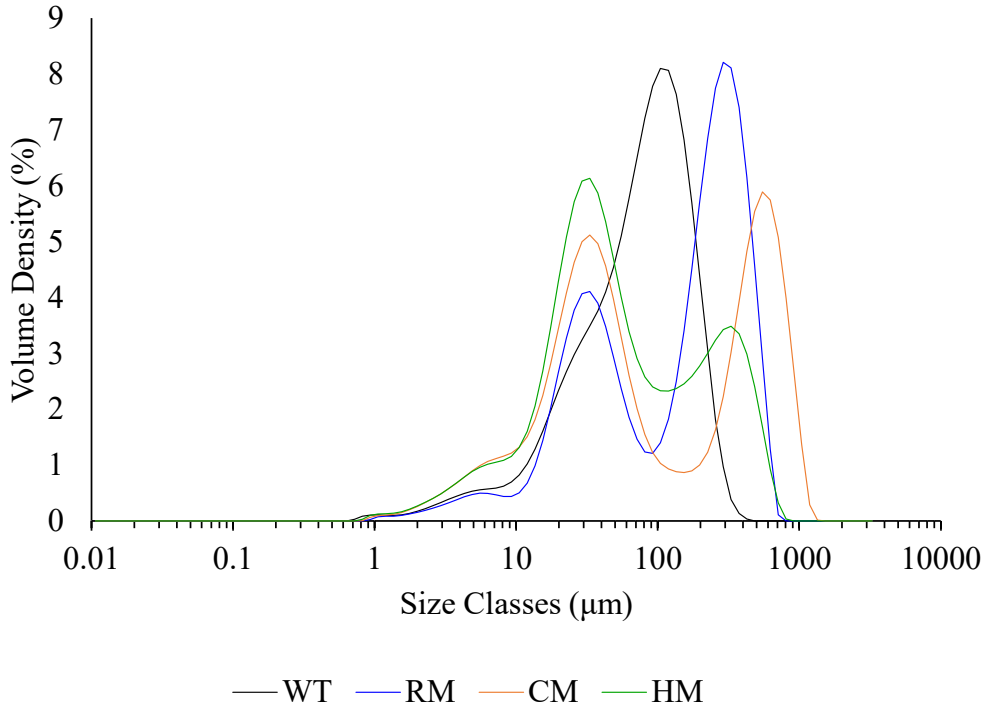


Figure 2.4. Particle size distribution curves of wheat (WT) and pea flours (RM, CM, HM).

The millability property is affected by the grain size and hardness, i.e., harder and bigger grains are more difficult to mill (Thakur et al., 2019). As reported by Monnet et al., (2019) flour particle size and grain hardness are correlated, which means that softer grains tend to have higher damaged starch and small particle size. Wheat grains have a high millability and, consequently, produce finer flour of a lower particle size and higher damaged starch.

The milling process significantly ($p < 0.05$) impacted the particle size and percentiles of distributions of pea flours. The flour obtained from the roller mill had the highest ($p < 0.05$) particle size for the d50 ($p < 0.05$). This difference may be caused by the distinct engine of the roller mill. The roller mill fractionates the raw material into small, medium and large particle sizes. In this study, the large fraction (mainly composed of the outer layer of the cotyledon) was discarded. Observing volumes density (%) represented in **Figure 2.4**, the RM flour picks represent the small and medium fractions. Therefore, the d50 represents the flour fraction with a smaller particle size and part of the medium fraction.

The RM and HM flours had lower ranges particle sizes (19.70-423.67 µm and 11.28-347.33 µm, respectively) compared to the CM (11.30±1.50-686.33±51.19). The CM produced

the flour with the highest d90. RM, HM and CM use different milling mechanisms and sieves, therefore affecting the particle size of the resulting flour. Furthermore, the RM uses a fractionation system, yielding different particle sizes and distributions. Moreover, in their studies, Martinez-Flores et al. (1998), concluded that, due to the mill mechanism and sieve sizes, the knife mill produced corn flour with greater particle size than the hammer mill.

Although samples of a smaller particle size can result in higher damaged starch content, the CM milling mechanism had higher starch damage and higher d90 particle size. The level of starch damage is affected by several parameters during milling, such as pressure, gap size, speed ratio, and milling time. As explained in section 3.1.1 on flour composition, the cutting mill required more time to complete the milling process, which could have influenced the level of damaged starch. Moreover, the cutting and shearing mechanism of the cutting mill might cut the starch granules which can lead to a higher level of damaged starch. In contrast, the hammer and roller mills use breaking, compression and tearing mechanisms, which are less likely to cause starch damage (Arce & Kratky, 2022).

2.6.1.4 Scanning electron microscopy (SEM) of flours

Figure 2.5 shows the scanning electron micrographs of WT, RM, CM and HM flours. SEM is a valuable tool to visualize and understand flours properties. As widely reported, wheat flour has starch granules of different sizes, spherical small granules and oval large granules surrounded by a protein matrix (Ahmed, 2021). Wheat starches can present in the flour large granules (type A) of approximately 25 µm and small granules (type B) of approximately 6 µm (Stoddard, 2005). Pea starches are characterised as thick disks with a “mark” and an indentation around the centre of the granule and can present a granule size between 2.72 and 31.8 µm (Aggarwal et al., 2004; Jane et al., 1994).

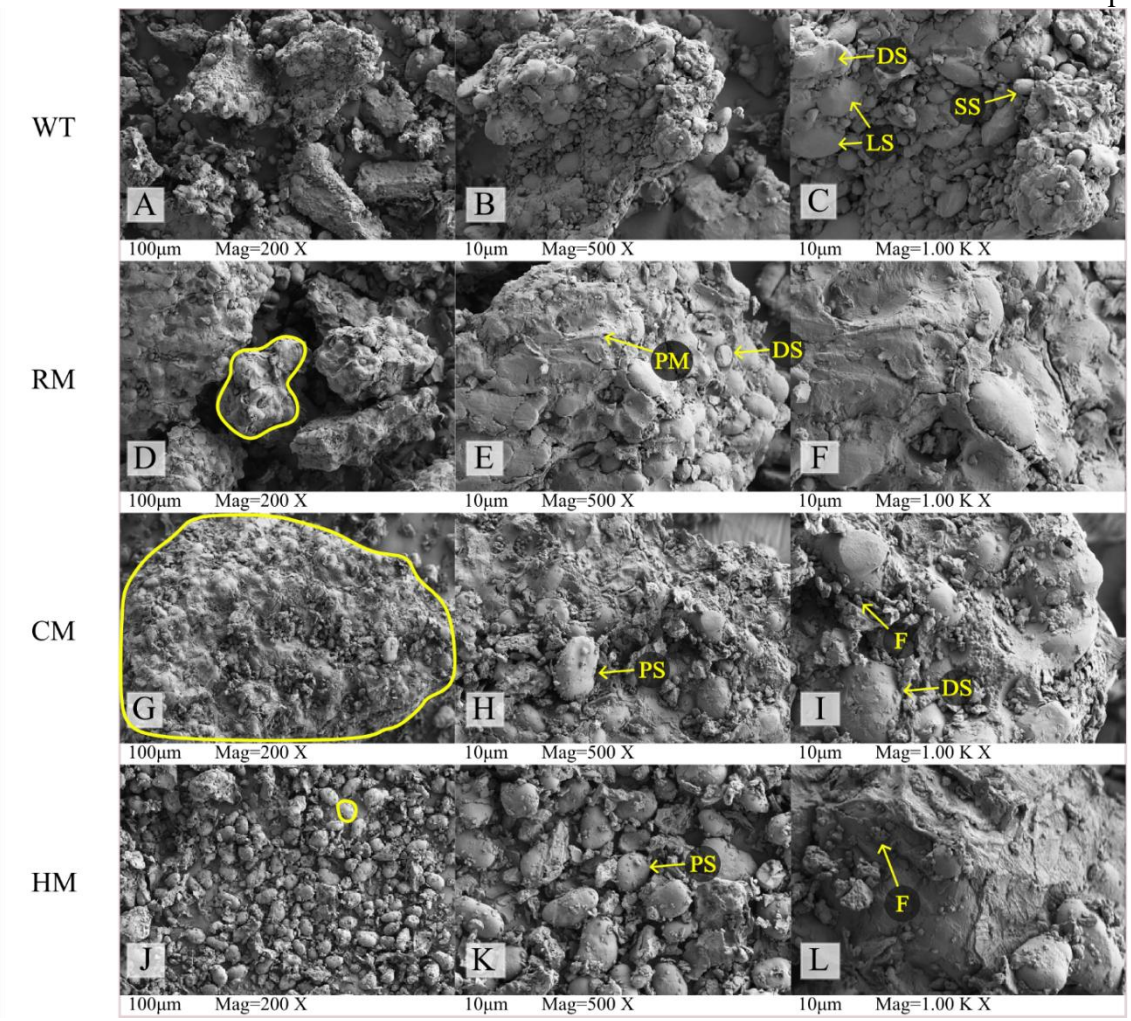


Figure 2.5. Scanning electron micrographs.

Both small and larger granules are represented in the micrographs of each flour (Figure 2.5 C). It can be seen that some starch grains were slightly deformed mechanically on their surface (Figure 2.5 C; E; I). This is a consequence of the milling process and represents the damaged starch. Aggregates of starch and protein matrix are represented on the flours (Figure 2.5 E). However, it is evident that the protein and starch aggregates from the CM process are large (Figure 2.5 G), likely due to the greater particle size (Table 2.2). Higher particle sized flours tend to have larger starch and protein matrix aggregates which can restrict the grains swelling and influence pasting properties (Bala et al., 2020). Our results mirrored these findings. Sakhare et al., (2014) showed that the micrograph of coarse wheat flour had higher aggregates of starch and protein matrix than a fine flour. On the other hand, for the hammer milled flours, the starch granules were more individualized. The impact force on the hammer

mill may influence the disaggregation between the protein matrix and starch granules. Images of flours from the CM and HM processes show visible fibre fragments of the disintegrated portions of the outer layers of the pea grains (Figure 2.5 I; L). SEM allowed a closer examination of the physical attributes of the samples, providing insights into their structural characteristics.

2.6.1.5 Flour pasting properties

Pasting behaviour of the starch is a crucial property of flour and will affect the dough and bread characteristics. The pasting properties are influenced by starch granule structure, amylose and amylopectin ratio, flour particle size, damaged starch, protein, fibre and lipid content (Ahmed, 2021; Balet et al., 2019). The milling process influences several of these parameters. Figure 2.6 presents the pasting curve for each flour and Table 2.3 provides the corresponding data.

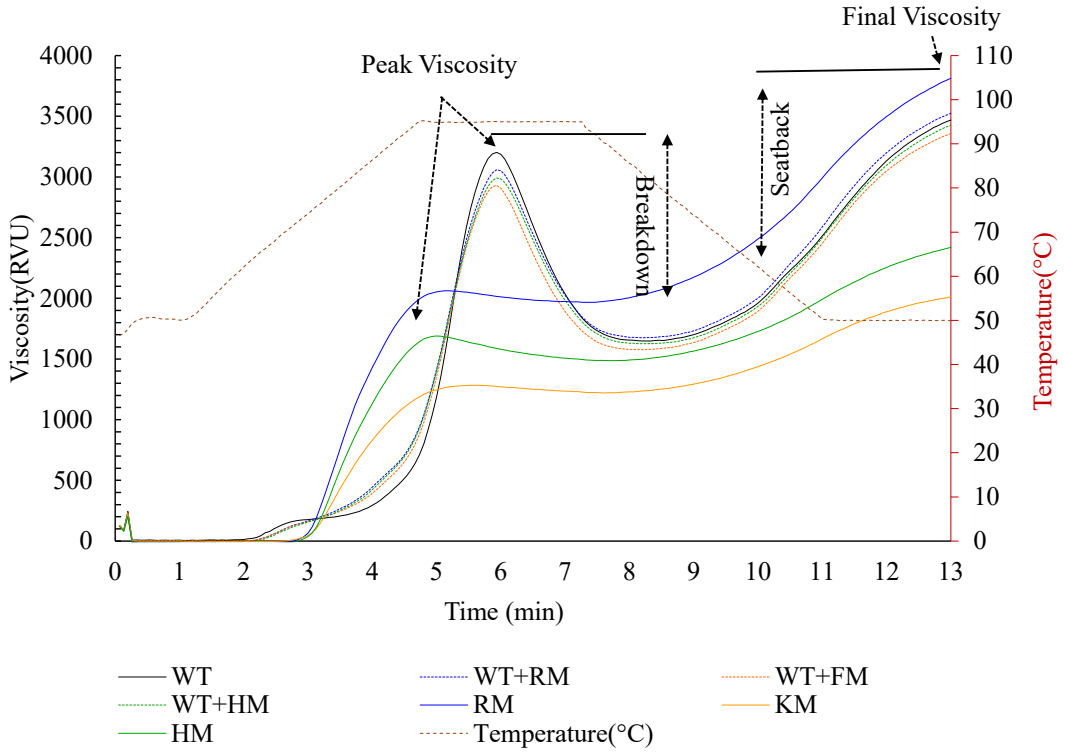


Figure 2.6. RVA starch pasting curves of wheat flour (WT), pea flours (RM, CM, HM) and flour blends 15:85 (pea:wheat).

Table 2.3. RVA of wheat flour, pea flours and flour blends 15:85 (pea:wheat).

Flours*	Peak viscosity (cP)	Breakdown (cP)	Setback (cP)	Final viscosity (cP)	Pasting temperature (°C)
WT	3208 ± 3.9^{Aa}	1419 ± 13.2^{Aa}	1822 ± 15.2^{Aab}	3468 ± 16.3^{Ba}	65 ± 0.15^{Bb}
RM	2174 ± 261.6^{B}	237 ± 287.5^{B}	1854 ± 11.5^{A}	3792 ± 28.6^{A}	72 ± 1.27^{A}
CM	1286 ± 76.4^{C}	68 ± 19.7^{B}	794 ± 22.8^{C}	2013 ± 79.9^{D}	73 ± 0.27^{A}
HM	1693 ± 106.4^{B}	207 ± 45.0^{B}	944 ± 44.3^{B}	2430 ± 92.8^{C}	72 ± 2.00^{A}
WT+RM	3119 ± 29.7^{ab}	1419 ± 14.9^{b}	1880 ± 7.8^{a}	3580 ± 17.7^{a}	67 ± 0.70^{a}
WT+CM	2932 ± 37.6^{c}	1357 ± 3.2^{c}	1782 ± 14.4^{b}	3498 ± 283.8^{a}	67 ± 0.23^{a}
WT+HM	3005 ± 76.3^{bc}	1376 ± 29.73^{bc}	1802 ± 38.3^{b}	3430 ± 84.0^{a}	67 ± 0.65^{a}

*WT: Wheat flour; RM: Roller Mill pea flour; CM: Cutting Mill pea flour; HM: Hammer Mill pea flour. WT+RM, WT+CM and WT+HM are flour blends 15:85 (pea:wheat) from the different mills. Data presented as means ± standard deviation. For significant differences, the samples were divided into two groups. A-D: 100% pea flours plus wheat flour (WT, RM, CM and HM). a-d: flour blends plus wheat (WT, WT+RM, WT+CM and WT+HM). A-D and a-d values are significantly different ($p < 0.05$) along the column. Note: cP: centipoise.

Roller-milled (RM) flours presented a higher pasting profile ($p < 0.05$) than CM and HM, most likely due to the higher starch content as discussed in section 1.4.1. Additionally, this flour had lower fibre content than other flours. The fibre will bind with the water, making less water available for starch, and reducing granule swelling (Boita et al., 2016). This would also clarify the lower peak viscosity, seatback and final viscosity obtained for all the CM and HM compared with RM. Similar results were found by Mojisola, (2020) where the viscosity profile of wheat flour decreased with the addition of cashew apple fibre.

Cutting-milled flour (CM) had the lowest viscosity profile ($p < 0.05$) most probably due to the higher damaged starch content. Damaged starch can lead to the destruction of the granular and crystal starch structure, resulting in a swelling reduction (Chen et al., 2011). Additionally, the particle size can restrict the grains swelling. Higher particle size leads to larger agglomerates of matter as shown in SEM micrographs of the CM flour (**Figure 2.5**). Pea starch granules are surrounded by a rigid protein framework which difficult the water access to starch granules (Bala et al., 2020). The milling mechanism of the cutting mill contributes to both the higher damaged starch and higher particle size thus explaining the viscosity results in **Table 2.3**.

All pea flours had a lower peak viscosity and breakdown ($p < 0.05$) when compared to wheat flour. A number of authors have discussed how less disintegration of the swollen starch granules due to the higher amylose content of pea flours (24-65%) compared to wheat flours (18-35%) justifies the lower peak viscosity and indicates an improved thermostability during shear stress (Labuschagne et al., 2007; Singh et al., 2010). In general, pea starch has been reported to have high thermostability resulting in a low breakdown viscosity (Kaiser, 2019). The WT+RM blend did not demonstrate a significant difference compared to wheat flour for the RVA properties, apart from the breakdown parameter. These findings are due to the higher pasting profile of the roller-milled flours.

Roller-milled flours (RL) had the highest final viscosity ($p < 0.05$), which can be attributed to several factors, such as the pea starch content and starch structure (which differs from the wheat) (Ahmed, 2021). Additionally, the lower fibre content of the RM flour, when compared with the other pea flours allows the re-association between starch molecules during the cooling process, resulting in a higher final viscosity compared with CM and HM flours (Boita et al., 2016). Final viscosity is linked to starch retrogradation and crumb hardness (Buehler, 2021). As consumers prefer a softer crumb texture the high final viscosity of the RM flours could possibly have an onward impact on the final bread quality of products containing pea flour (Buehler, 2021).

The type of milling process applied to the peas did not influence the pasting temperature ($p > 0.05$). However, the pasting temperature of the pea flours was significantly higher ($p < 0.05$) compared to that of wheat flour (WT). The pasting temperature represents the moment when the viscosity of the starch begins to rise. The higher pasting temperature for the pea flours can be related to reduced hydration of amorphous areas in starch granules. Higher pasting temperatures were also found for the WT+Pea blends ($p < 0.05$). Ocheme et al., (2018) discussed how the higher protein and fibre content restrict water binding and consequently starch gelatinization. Similar results were found by Wu et al., (2021), where interactions

between the rice bran fibre and starch molecules increased the flour pasting temperature. Pasting temperature indicates the minimum temperature necessary to cook the product. (Balet et al., (2019) found that a higher temperature was required to cook pea flour in comparison with wheat flour.

2.6.2 Gel texture profile analysis (TPA)

The textural properties of the RVA gel can provide valuable insight of the retrogradation of starch paste over a time period (24 h) after RVA is completed. Retrogradation is the main cause of bread staling. Moreover, is desirable lower retrogradation rates when working with bread making (Denardin and Silva, 2009). Gel textural parameters for each gel are summarised in Table 2.4.

Table 2.4. Gel texture analyses (TPA) of wheat flour, pea flours and flour blends 15:85 (pea:wheat).

Flours*	Hardness (g)	Adhesiveness (g sec)	Springiness	Cohesiveness	Gumminess
WT	219 ± 7.2^{Cb}	-261 ± 17.6^{Aa}	0.92 ± 0.02^{Aa}	0.58 ± 0.02^{Aa}	129 ± 5.9^{Ca}
RM	874 ± 107.3^{A}	-448 ± 183.3^{A}	0.98 ± 0.07^{A}	0.50 ± 0.01^{B}	443 ± 52.8^{a}
CM	722 ± 7.8^{B}	-226 ± 24.5^{A}	0.99 ± 0.01^{A}	0.49 ± 0.01^{B}	359 ± 12.2^{b}
HM	766 ± 19.9^{AB}	-200 ± 68.1^{A}	0.95 ± 0.06^{A}	0.50 ± 0.01^{B}	389 ± 15.6^{ab}
WT+RM	246 ± 4.7^{a}	-336 ± 23.5^{a}	0.90 ± 0.02^{ab}	0.57 ± 0.02^{a}	141 ± 8.5^{a}
WT+CM	229 ± 9.2^{b}	-348 ± 54.0^{a}	0.89 ± 0.01^{a}	0.55 ± 0.01^{a}	125 ± 5.1^{a}
WT+HM	232 ± 5.0^{ab}	-332 ± 56.2^{a}	0.89 ± 0.02^{a}	0.56 ± 0.01^{a}	130 ± 4.1^{a}

*WT: Wheat flour; RM: Roller Mill pea flour; CM: Cutting Mill pea flour; HM: Hammer Mill pea flour. WT+RM, WT+CM and WT+HM are flour blends 15:85 (pea:wheat) from the different mills. Data presented as means ± standard deviation. For significant differences, the samples were divided into two groups. A-D: 100% pea flours plus wheat flour (WT, RM, CM and HM). a-d: flour blends plus wheat (WT, WT+RM, WT+CM and WT+HM). A-D and a-d values are significantly different ($p < 0.05$) along the column.

The hardness of all pea gels was higher ($p < 0.05$) than that of the wheat flour gel. This parameter is influenced by the final viscosity and retrogradation of starch granules. However, WT final viscosity was higher compared with HM and RM. The RVA measures the trend of the retrogradation of the cooling process being related to amylose retrogradation. There is a kinetic difference between the retrogradation of amylose and amylopectin. Amylose

retrogrades rapidly and amylopectin retrogrades at a slower rate over an extended period of time (Denardin and Silva, 2009). Pulses have higher amylose content and the molecular structure of pulse starches causes them to retrograde more extensively compared to cereal starches (Ahmed, 2021). These results justify the lower hardness of the WT control. The higher hardness observed for roller-milled pea flour is due to the higher starch content, and lower IDF content caused by the use of distinct milling technologies. This was previously mentioned in this study and has also been discussed by Ahmed, (2021). Except for the RM pea flour, the wheat flour substitution for 15% of pea flour did not significantly affect the hardness of the gel ($p > 0.05$), implying that perhaps will the staling process of a final bread product may not be impacted, when incorporated at these levels. The retrogradation process impacts bread quality in several ways, such as texture, acceptability, digestibility, and ageing (Denardin and Silva, 2009).

With regard to the gumminess parameter of the pea gels, results were significantly higher ($p < 0.05$) compared with WT, exhibiting a comparable pattern to those seen for hardness, due to the high relative weight of hardness when calculating the gumminess parameter. Cohesiveness measures the attractive forces within the same material to keep it together (Peleg, 2019). The pea flours have lower ($p < 0.05$) cohesiveness than WT. **Table 2.4** indicates that there were no significant differences ($p > 0.05$) in adhesiveness, cohesiveness and gumminess for the flour blends, meaning that these parameters were not significantly influenced by the milling process.

Gel TPA results for each of the flour blends revealed no significant differences for each parameter, compared with the gel TPA properties of WT with one exception: the hardness of the WT-RM was significantly higher than that of the wheat gel ($p < 0.05$).

2.6.2.1 Mixolab

Table 2.5 and Figure 2.7 present the dough mixing properties of the flours assessed by Mixolab. Dough hydration is a critical factor for gluten network development and is affected by water absorption (Alfaris et al., 2022). The water absorption of the WT+CM flour blend was not significantly different compared the water absorption for wheat flour (WT). The WT+RM and WT+HM blends revealed a significant decrease in water absorption in comparison to WT flour. However, all three wheat+pea blends had numerically similar results.

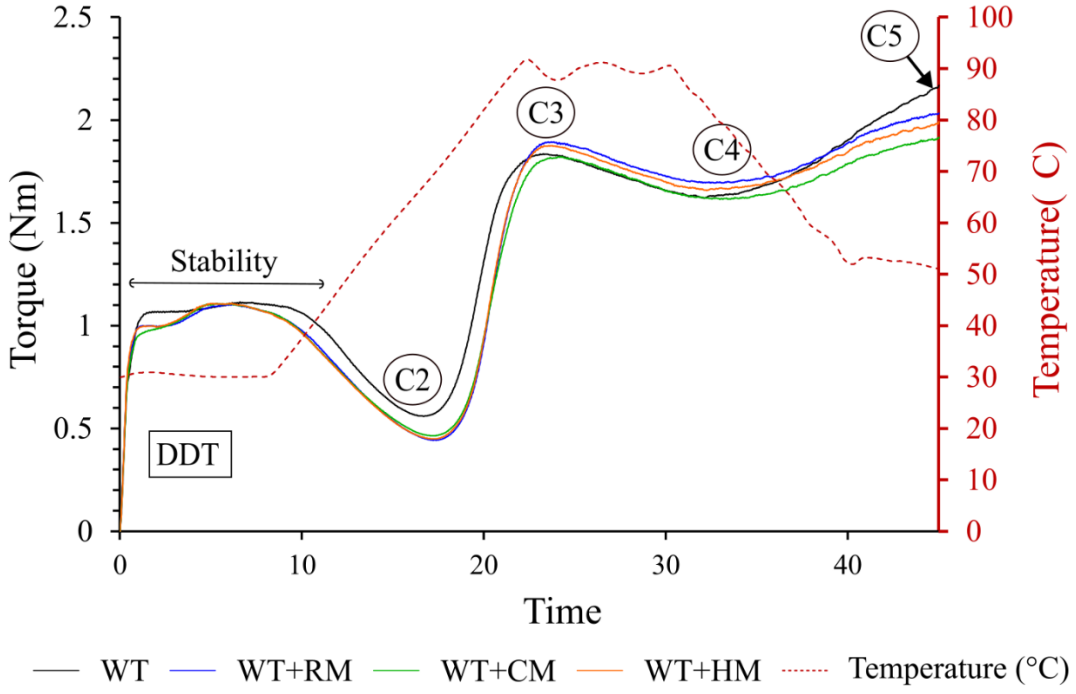


Figure 2.7. Mixolab dough curves of wheat flour and wheat plus pea flour blends 15:85 (pea:wheat), using Chopin + test. DDT: Dough Development Time (min); C2: protein weakening (Nm); C3: starch gelatinisation (Nm); C4: gel stability (Nm); C5: starch retrogradation (Nm).

Table 2.5. Dough properties Mixolab parameters of wheat flour and flour blends 15:85 (pea:wheat).

Parameters*	WT	WT+RM	WT+CM	WT+HM
Water absorption (%)	63.26±0.20^{a}	63.01±0.10^{b}	63.45±0.10^{a}	62.96±0.05^{b}
Stability (min)	10.47±0.17^{a}	9.38±0.43^{b}	8.87±0.23^{b}	9.15±0.11^{b}
DDT (min)	6.81±0.18^{a}	5.76±0.42^{ab}	5.45±0.25^{b}	5.41±0.337^{b}
C2 (Nm)	0.56±0.02^{a}	0.44±0.02^{b}	0.45±0.01^{b}	0.45±0.02^{b}
C3 (Nm)	1.84±0.03^{a}	1.90±0.04^{a}	1.84±0.01^{a}	1.89±0.03^{a}
C4 (Nm)	1.62±0.05^{a}	1.68±0.02^{a}	1.60±0.02^{a}	1.65±0.03^{a}
C5 (Nm)	2.17±0.01^{a}	2.04±0.04^{b}	1.92±0.02^{b}	2.00±0.03^{b}
Torque (Nm)	1.12±0.01^{a}	1.11±0.04^{a}	1.12±0.02^{a}	1.12±0.03^{a}

*WT: Wheat flour; WT+RM, WT+CM and WT+HM are WT+pea flour blends 15:85 (pea:wheat) from the different mills. DDT: Dough Development Time (min); C2: protein weakening (Nm); C3: starch gelatinisation (Nm); C4: gel stability (Nm); C5: starch retrogradation (Nm). Data presented as means ± standard deviation. Values with different letters (a-d) are significantly different ($p < 0.05$) along the row.

Dough Stability, dough development time (DDT) and C2 (protein weakening) are critical parameters for assessing bread dough resistance to mechanical mixing. The flour blends exhibited significantly lower stability, DDT and C2 ($p < 0.05$) when compared to WT. The incorporation of pea flour results in the formation of a less strong gluten network due to gluten dilution, consequently, there was a reduction in dough consistency. Moreover, pea flours contain a high fibre content, which can interfere with the starch-gluten matrix (Sadowska et al., 2003). Bojňanská et al., (2021) reported that the addition of legume flour, due to the fibre content, could disrupt the dough viscoelastic matrix, resulting in a weaker dough.

The C3 values reflect starch gelatinization, the C4 values are associated with starch gel stability during heating and the C5 values reflect retrogradation. Significant differences ($p > 0.05$) were not found between the wheat flour and flour blends for the C3 and C4 parameters. However, the starch retrogradation properties (C5) of blended flours was lower than for the wheat control, with no significant differences ($p > 0.05$) among blended flours. These findings paralled the results obtained for final viscosity (RVA parameter) where there were no significant differences ($p > 0.05$) in the blended flours. This suggests that adding 15% pea flour to bread formulations could be unlikely to significantly affect the starch retrogradation of bread during the cooling process.

2.6.3 Dough properties

2.6.3.1 Extensibility

Table 2.6 provides measurements of the resistance to extension and extensibility of the dough. The fracture of dough membranes during fermentation and handling characteristics of the dough affects the bread quality. Resistance to extension and extensibility of the dough prior

to fermentation are crucial parameters in pre-determining the bread rise qualities and structure (Gómez et al., 2003).

Table 2.6. Dough extensibility and resistance to extension of wheat dough and pea dough blends 15:85 (pea:wheat).

Dough*	Resistance to Extension (g)	Extensibility (mm)
WT	60.42±2.04^{a}	30.68±1.82^{b}
WT+RM	48.03±4.74^{b}	24.29±1.55^{a}
WT+CM	49.64±2.50^{b}	27.21±1.63^{ab}
WT+HM	49.43±3.10^{b}	26.38±1.15^{a}

*WT: Wheat flour; WT+RM, WT+CM and WT+HM are WT+pea flour blends 15:85 (pea:wheat) from the different mills. Data presented as means ± standard deviation. Values with different letters (a-d) are significantly different ($p < 0.05$) along the column.

Wheat substitution with 15% of pea flour significantly decreased the resistance to extension, meaning that less amount of strength is required for dough deformation ($p < 0.05$). The highest extensibility was obtained for the wheat dough (WR) ($p < 0.05$). The type of pea milling process did not significantly affect resistance to extension and extensibility of the dough. The reduced resistance to extension and extensibility observed in the flour blends may be attributed to the gluten dilution caused by the higher non-gluten protein and higher fibre content of the pea flour. Glutenin is responsible for dough elasticity, which is a key contributor to extensibility (García-Segovia et al., 2020). Similar results were obtained by several authors. Wang et al. (2002) showed that adding 3% pea hulls (mainly composed of fibre) to wheat flour decreased dough extensibility, resulting in reduced loaf volume. Mohammed et al. (2012) demonstrated that adding raw chickpea flour to wheat flour at levels up to 30% decreased the resistance to extension of the resulting doughs. Mohammed et al. (2012) justify similar results in their study as a result of the presence of constituents that interact with gluten proteins, inhibiting the development of the dough network, and thus yielding doughs with reduced resistance to extension.

2.6.3.2 Fundamental oscillatory rheology

Figure 2.8 represents the rheological properties of the dough that are a critical factor to determine the bread quality, such as specific volume and textural attributes. Bread dough must have enough elasticity for rising without breaking the formed network during proofing and baking (Chin & Martin, 2014). The storage modulus (G') represents the deformation energy stored in the material after removing the oscillation and measures the material elasticity. The loss modulus (G") provides dough energy lost during oscillation and is an indicator of the material viscous behaviour (Sullivan et al., 2011).

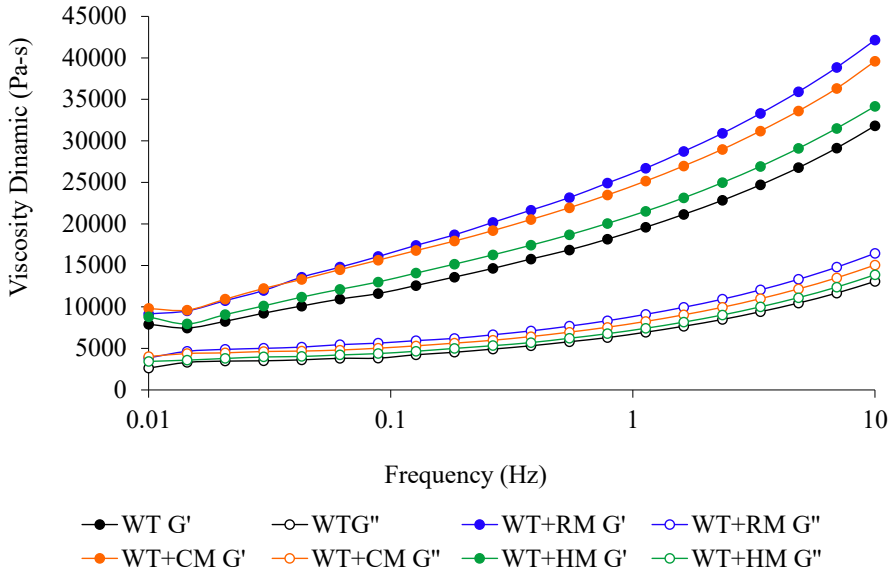


Figure 2.8. Frequency sweep of wheat dough, and wheat plus pea dough blends 15:85 (pea:wheat).

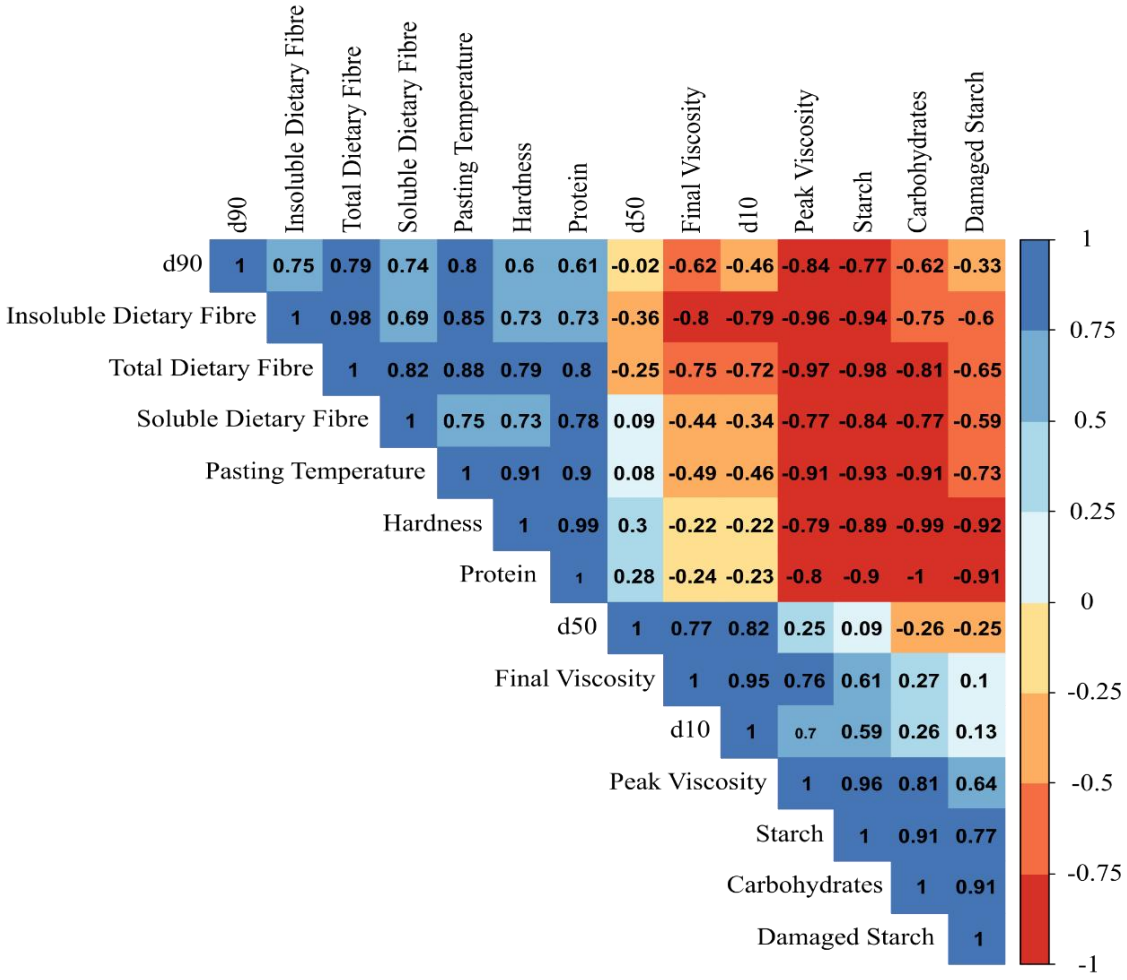
It was noticeable that pea flour incorporation led to a slight increase in storage and loss moduli. This observation may be related to the diverse botanical sources of starch and different protein and fibre composition of the pea source. Ziobro et al., (2016) and Jelena, (2020) conducted experiments to investigate the effects of legume proteins on dough rheology, and both found that the inclusion of pea protein led to modifications in the elastic and viscous components, increasing G' and G". Flour blends had higher fibre content than WT. The pea fibres produce strong bonds and act as a filler on the starch-protein matrix which can lead to higher G' and G" modules (Coţovanu & Mironeasa, 2022).

The frequency sweep of the doughs showed how the elastic modulus (G') was higher than the viscous modulus (G") through all the frequency ranges, and both moduli slightly increased with increasing frequency levels. This is the typical behaviour observed in dough rheological studies (Mohammed et al., 2012).

2.6.4 Pearson's correlation coefficient

Figures 2.9 A and 2.9 B show Pearson's correlation corrplots, depicting the inter-relationship among analyses. Pearson correlation analysis provided a range of significant correlation coefficients (r) for the relationship between flours properties, proximate composition and dough properties.

A)



B)

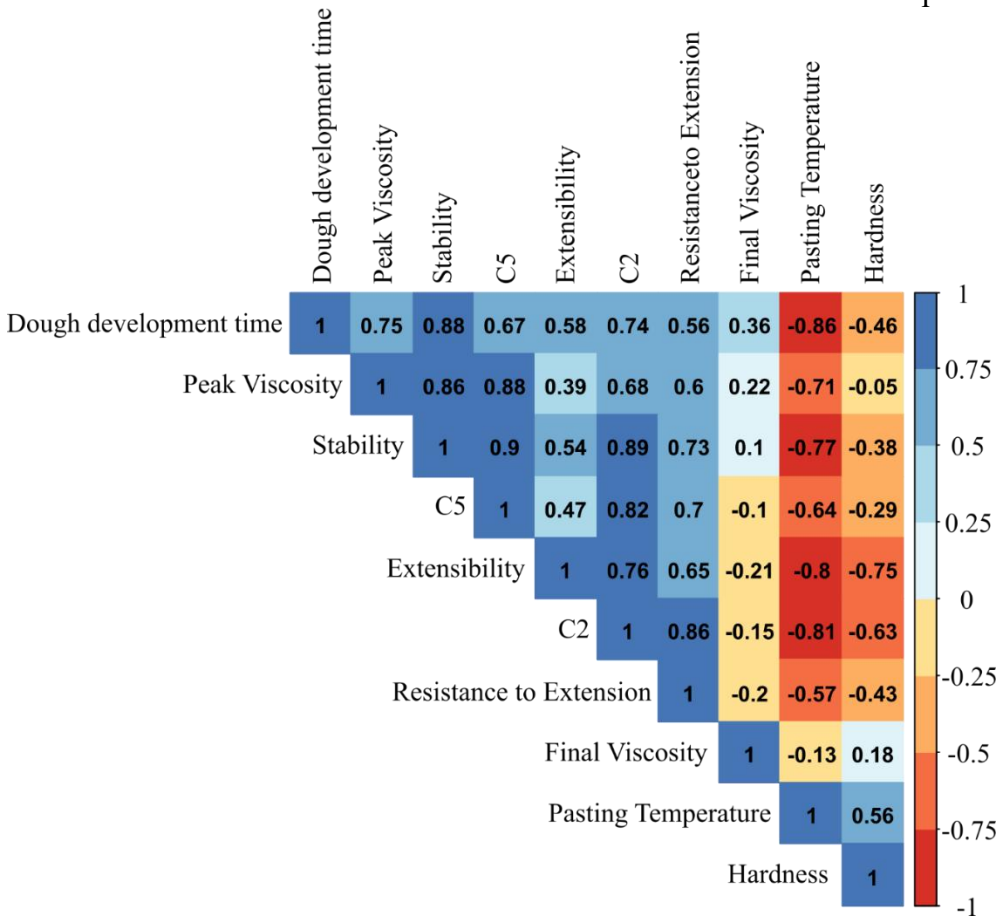


Figure 2.9. Corrplot based on Pearson's correlation coefficient depicting inter-relationship of analysis parameters for: A) Wheat flour and 100% pea flours (WT, RM, CM and HM); B) Wheat and pea flour blends 15:85 (pea:wheat) (WT, WT+RM, WT+CM and WT+HM). White to blue: positive correlation; white to red: negative correlation.

Some variables that show a strong correlation with final viscosity and peak viscosity are total dietary fibre (r= -0.75, -0.87) and particle size d90 (r= -0.62;-0.84), indicating that flours with lower total dietary fibre and lower particle size d90 served to increase both peak and final viscosities of the resultant RVA gels.

The pasting temperature of pea flour was found to be significantly higher ($p < 0.05$) compared to wheat flour. A strong positive correlation was found between this parameter and both protein (r= +0.9) and fibre content (r= +0.88). Higher protein and TDF contents were also positively correlated with higher gel hardness properties (r= +0.79, r= +0.99). The gel hardness of all pea flours was previously found to be higher ($p < 0.05$) compared to the wheat flour gel, thus supporting this correlation.

The Mixolab C2 parameter (protein weakening of the dough), was positively correlated with dough development time (DDT; r= +0.74), resistance to extension (r= +0.86) and extensibility (r= + 0.76). A higher protein weakening effect occurred when pea flour was added to the dough formulation, as a less strong protein network was created, subsequently affecting the development time of the dough. Moreover, the addition of pea flour was also shown to affect dough stretching properties such as resistance to extension and extensibility.

Dough stability, a Mixolab parameter, was strongly positively correlated with dough development time (r= +0.88) and C2 (protein weakening, r= +0.89). The stability of bread dough determines the mixing resistance of dough. The longer the stability, the stronger the flour (Interlang, 2007). Protein weakening and dough development time are dependent on the flour strength, which explains their correlation with the dough stability. Dough stability is a useful parameter when working with pea flour, as its incorporation results in dough weakening. Thus, the application of pea flour requires careful monitoring to not over-mix the dough and to obtain the best product quality and a standardised product.

2.7 Conclusion

The techniques employed to mill peas affected the flour composition, pasting properties and slight differences were observed in the rheological properties of the resulting doughs. Hammer-milled peas produced the highest yield of flour (93.9%). Vis-NIR Spectroscopy mirrored the proximate composition and PCA was able to discriminate among flour type and milling of pea flours (RM vs HM and CM). Near-Infrared spectroscopy demonstrated to be a valuable tool for flour characterization in this specific context, contributing to future practical applications. Larger starch and protein matrix aggregates were observed for cutting-milled flours, as evidenced by scanning electron microscopy. The roller mill produced flour with the highest pasting viscosity and gel texture hardness, due to the higher starch content and lower insoluble dietary fibre. No significant differences were observed between the wheat (WT),

WT+hammer milled flour and WT+cutting milled flour for starch gel hardness. The incorporation of 15% cutting-milled and hammer-milled flours may not have a significant influence the bread staling process. Hammer-milled pea flour is possibly the most suitable for bread applications as this flour had the highest yield, fibre and protein content. Further research is now being undertaken to study the influence of the pea milling process on bread-making performance, crumb texture and shelf-life.

3 Pea-wheat composite bread: Effects of milling methods and NIR spectral insights

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Pea-wheat composite bread: Effects of milling methods and NIR spectral insights

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ABSTRACT

Utilizing locally grown peas in bread-making offers environmental, nutritional, and economic advantages, yet pea milling remains underexplored compared to wheat. Optimizing pea flour processing is critical, as its integration with wheat can drastically alter baking performance. This study evaluated the impact of three milling techniques: roller mill (RM), hammer mill (HM) and cutting mill (CM). The pea flours were blended in a 15 %/85 % (pea/wheat) ratio and bread profiling was assessed. Additionally, flour and bread were analysed using NIR spectroscopy. Pea flour increased bread protein content. Considering the milling yield, bread characteristics and spectral analysis undertaken, this study suggests that the hammer mill is the most effective method for bread-making. NIR spectroscopy effectively distinguished formulations despite composition similarities enabling product standardization. Furthermore, NIR uniquely classified diverse breads by storage time, highlighting its potential for bread staling assessment.

1. Introduction

Pulses are the second most consumed food worldwide after cereals (Singh, 2017) and are valued for their high protein, fibre, and antioxidant content, as well as their low lipid levels (Kaiser, Barber, Manthey, & Hall III, 2019). Beyond nutritional benefits, pulses are affordable, adaptable to diverse soils, and contribute to environmental sustainability by improving soil health and reducing carbon footprints (Maphosa & Jideani, 2017; Uebersax et al., 2023).

Bread is a staple food worldwide, valued for its versatility, accessibility, and affordability, making it a suitable matrix for legume incorporation. Combining cereals and legumes in bread improves protein quality by providing a complete amino acid profile, while also offering environmental benefits (Kaiser et al., 2019). Nevertheless, the application of pulses in bakery systems remains under development, requires a comprehensive knowledge of the techno-functional properties of flour, dough and bread (Hall, 2021). Although the incorporation of higher levels of pulses is nutritionally more attractive, a low pea flour content has been previously used in bread formulations to avoid compromising the structure and acceptability of the final product (Hall, 2021).

Milling plays a crucial role in determining the properties of pea flour (Borsuk, Arntfield, Lukow, Swallow, & Malcolmson, 2012). Several studies have investigated how milling-related parameters, such as particle size, starch damage, and flour composition, affect pea flour functionality (Borsuk et al., 2012; Maskus, Bourre, Fraser, Sarkar, & Malcolmson, 2016; Zucco, Borsuk, & Arntfield, 2011). These factors, influence the dough matrix and the overall bread quality, thus, it is essential to identify the optimal pea milling process for optimal applications in bread.

Feed-mills such as roller mill, knife mill and hammer mill are

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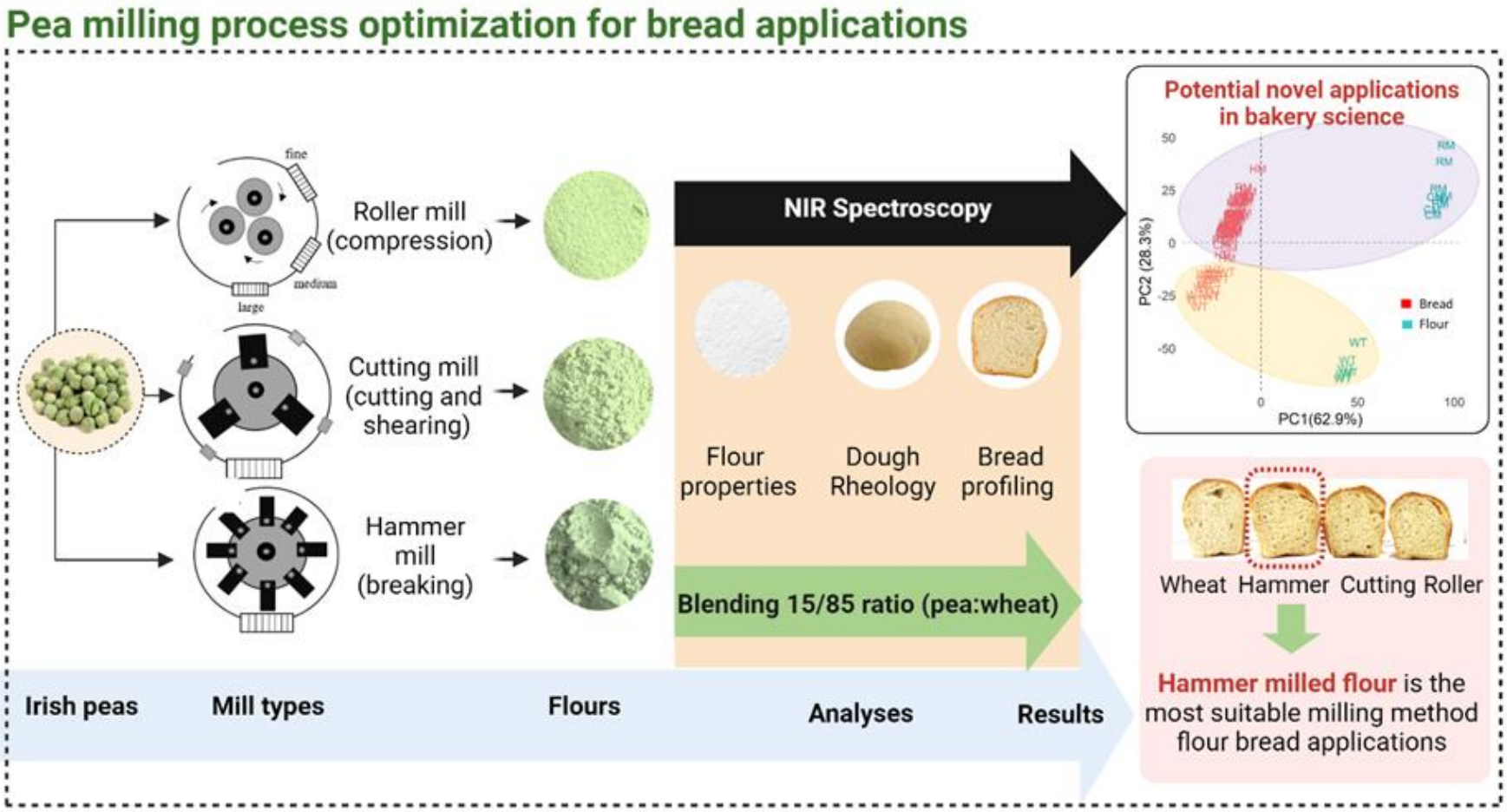
3.1 Graphical abstract



3.2 Abstract

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3.3 Introduction

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Milling plays a crucial role in determining the properties of pea flour (Borsuk et al., 2012). Several studies have investigated how milling-related parameters, such as particle size, starch damage, and flour composition, affect pea flour functionality (Borsuk et al., 2012; Maskus et al., 2016; Zucco et al., 2011). These factors, influence the dough matrix and the overall bread quality, thus, it is essential to identify the optimal pea milling process for optimal applications in bread.

Feed-mills such as roller mill, knife mill and hammer mill are commonly used for processing legumes. They are known for their automatic control and continuous feeder, which make them simple to operate and attractive to producers (Hoyle B, 1968). Roller mills are designed for wheat grains but can also be used for milling pulses. These mills comprise a

fractionation system producing large, medium and fine particle sizes and the principal particle size reduction mechanisms are compression and tearing (Arce & Kratky, 2022). The fractionation system of the roller mill can break and separate the outer coat of the grains from the internal part. The grains pass through a series of opposing cylinders and the flour is sieved between phases (Cappelli et al., 2020). The knife mills are characterised by a size reduction with static knives and rotating blades using a cutting and shearing mechanism. The leading pulse milling technique present nowadays is by hammer milling (Price et al., 2021). Hammer mills subject the grain to a dynamic effect of pressure and rotation towards a breaking plate, and hence breaking is the dominant reduction mechanism (Arce & Kratky, 2022).

Near-infrared (NIR) spectroscopy, when adopted in bakery, can enable a rapid assessment of product composition. Bread characteristics such as protein, wet gluten, moisture, and water absorption has traditionally been identified and predicted using this technique (Jirsa et al., 2007). Also, the chemical processes that occur during bread staling, such as starch retrogradation and water loss, can be followed with near-infrared spectroscopy (Ringsted et al., 2017). With regard to milling, NIR can capture both physical and chemical characteristics of flour, and when combined with particle-size data, can provide valuable insights for flour mill process control. Multi-way modelling approaches have shown that NIR spectra, retain significant information on particle-size properties, highlighting their potential for real-time milling quality assessment (Nielsen et al., 2001). In addition, NIR has been investigated for predicting wheat milling characteristics, showing significant correlations in cross-validation but limited precision in independent validation, with satisfactory accuracy achieved only for semolina extraction rate (Blazek et al., 2005). NIR spectroscopy is an area under development where the limits of its applications are yet to be fully understood. Further research will provide potential to expand its use in bakery science, particularly to improve accuracy by developing calibrations that incorporate a broader range of ingredients, formulations, and processing conditions, ultimately enhancing its versatility.

The primary aim of this study was to evaluate the performance of Irish-grown peas in bread production, utilizing pea flours produced by roller, knife, and hammer mills. Building upon previous research published by Maçãs et al. (2023) **(Chapter 2)**, on composition, pasting properties and dough rheology of flours from each of these milling processes and their blends, this study focused on bread baking analysis and spectral techniques of newly formulated bakery products. Additionally, it intended to determine the quality of bread produced with a relatively low quantity of pea flour (15%/85% pea/wheat), thus establishing baseline data for their future applications at higher percentages of addition. The resulting breads underwent a detailed characterization over 7 days. Wheat bread was used as a standard product, not to emulate its properties, but to deeply understand the techno-functional properties of blended flour (pea/wheat) for onward bread applications. Flour and bread classification, as well as evaluations of bread staling, were analysed using conventional methods and NIR spectroscopy.

3.4 Materials and methods

3.4.1 Raw materials and milling process

Strong wheat spring flour was purchased from Nelstrops Family Flour Millers, UK. Spring green peas, harvested in 2022, were supplied by Teagasc Oak Park, Ireland. The bakery ingredients, including salt, yeast, and unsalted butter, were purchased locally, while the improver (Voltex, Ireks, UK) was purchased from suppliers. Whole peas were milled as described by (Maçãs et al., 2023) **(Chapter 2)**. Three mills were used: roller (CD1, Chopin®, France), cutting (SM300, Retsch®, Germany) and hammer (Labmill 3100, Perten®, Sweden). The resulting flours were labelled as RM (roller mill), CM (cutting mill), and HM (hammer mill). Results for the milled flours and bread dough, including flour composition, particle size, pasting properties, and dough mixing properties, were reported by Maçãs et al. (2023) **(Chapter 2)**. This present paper focuses on bread production and analysis. A preliminary focus

of the study concentrates on bread properties at one day post baking, and the second focus of the study monitored the staling profile (days 1, 3 and 7 post baking) of the breads using instrumental and spectral tools. Each milled pea flour was blended with strong wheat flour to obtain a 15%:85% (pea:wheat) ratio. A strong wheat flour was used as a control sample. Each baking trial was conducted three times, and each analysis was carried out in triplicate.

3.4.2 Bread making

The breads were prepared using 100% wheat or blended flour (15%:85%), salt (2%), unsalted butter (1%), improver (1%), yeast (1.5%) and water (% based on absorption results from Mixolab). The bread-making procedure and equipment utilized mirror those outlined by Biduski et al. (2023).

Bread descriptors:

WT: Control bread with 100% wheat flour.
CM: Bread with a 15%:85% ratio of pea/wheat, using Irish peas milled with a cutting mill.
HM: Bread with a 15%:85% ratio of pea/wheat, using Irish peas milled with a hammer mill.
RM: Bread with a 15%:85% ratio of pea/wheat, using Irish peas milled with a roller mill.

An incorporation level of 15% was selected based on extensive preliminary trials, with the aim of illustrating the significant impact of milling techniques on bread quality, supporting quality assessment, and enabling discrimination through NIR analysis.

3.4.3 Proximate composition, phytic acid, moisture, water activity and amino acid analysis

The total proximate composition of the breads was assessed. Protein content was determined using LECO (FP-628, LECO Corp., USA) (Nx5.7), following the method 968.06, (AOAC, 2000b). ORACLE Rapid NMR Fat Analyser (CEM Corp., USA) (AOAC, 2005) was

utilized to calculate the fat content. The total dietary fibre (TDF), comprising insoluble dietary fibre (IDF) and soluble dietary fibre (SDF), was assessed utilizing the Ankom automated dietary fibre analyzer (Ankom, USA) through the enzymatic-gravimetric method, following protocol 991.43 (AOAC, 1994). The ash content was determined using a Gallenkamp Muffle Furnace, with pre-drying, conducted overnight at 550°C, following the AOAC method 08–01.01 (Gallenkamp, UK) (AOAC, 2000a). The total carbohydrate content (%) was calculated by subtracting the sum of fat, protein, ash, and moisture from 100%. The total starch content was determined by subtracting the total dietary fibre (TDF) from the total carbohydrate content (Charrondiere et al., 2004).

Phytic acid analysis was performed using a Phytic Acid Assay Kit (K-PHYT 11/15) (Megazyme International Ltd.) following the manufacturer's instructions.

Crumb moisture was obtained using a 2-stage method according to method 44-15.02 (AACCI, 2010c). Water activity was determined using an Aqua Lab Lite (Decagon Devices, WA, USA). Crumb of the two centre slices were cut (approx. 0.5 x 0.5 x 0.5 cm) and analysed in triplicate per bake. Moisture content was undertaken on days 1, 3 and 7.

The amino acid profiles of 100% wheat bread and hammer-milled composite bread (HM) were determined, initially by hydrolysis and neutralization of the samples according to a protocol adapted from (Healy et al., 2022). A portion of the treated bread sample (200 µL) was combined with an equal volume of an internal standard (50 µM of 6-aminocaproic acid) and passed through 0.22 µm regenerated cellulose membrane filters. Following this, the samples underwent derivatization with o-phthalaldehyde (for primary amino acids) and 9-fluorenylmethoxycarbonyl chloride (for secondary amino acids) before being injected into a UHPLC-FLD system (Thermo Ultimate 3000 RS, Thermo Scientific, USA) equipped with an Agilent AdvanceBio AAA column (100 mm × 3.0 mm ID × 2.7 µm particle size, Agilent Technologies, USA). Two mobile phases were used for the separation: mobile phase A (10 mM Na2HPO4 in 10 mM Na2B4O7 decahydrate, pH 8.2) and mobile phase B (mixtures

45:45:10, v:v:v of acetonitrile, methanol and water). The separation was carried out at a flow rate of 0.62 mL/min following a gradient program. Fluorescence detection was conducted at excitation and emission wavelengths of 340 nm and 450 nm, respectively. The results are reported as milligrams of amino acid per gram of fractionated sample.

3.4.4 Loaf dimensions, crumb and crust colour, crumb structure and crumb texture and confocal microscopy

Loaf specific volume and density were measured with the VolScan Profiler (VSP600C, Stable Micro System, UK) following the method 10-16.01(AACCI, 2010a).

Loaf crust and crumb colour were assessed using a colourimeter (CR-410, Konica Minolta, UK). The colour was recorded and expressed using L*(0: black, 100: white), a* (-green, +red), and b* (-negative, +yellow) colour scale.

Three loaves of each bread were sliced into 1 cm thick slices and the two central slices of each loaf were used for digital image analysis and texture profile analysis (TPA). The C-Cell Bread Imaging System (Calibre Instruments Ltd, UK) was used to study the internal structure of the bread. The texture of the crumb was profiled with a texture analyser (TA-XT2i, Stable Microsystems, UK) using a 20 mm cylindrical probe and a test speed of 1 mm/sec. The centre slices were compressed to 50% of their initial height. Analysis was undertaken on days 1, 3 and 7 post baking.

The internal crumb micro structure of each bread was assessed by confocal microscopy. The bread crumb was prepared with resin and dye according to Biduski et al. (2023). Fluorescein isothiocyanate, fluorescent brightener 28, and fast green FCF were used to dye protein (red), cellulosic material (blue) and starch (green). The resin sections, treated with a triple-staining technique, were captured using an Olympus FluoView FV3000 microscope equipped with a 60x PlanApo 1.4 NA oil immersion objective. Subsequently, the images were

combined using Adobe Photoshop v24.5 and colour-enhanced in a pseudo-colour scheme: cellulose material was represented in blue, starch in green, and protein content in red.

3.4.5 Bread profiling through NIR spectroscopy

Spectral data of the breads was collected with a Vis/NIR spectrometer (Hi-Res ASD Labspec 4, Malvern Panalytical, UK), which operates across a wavelength range of 350–2500 nm, capturing data at 1 nm intervals, with each sample measured in triplicate. Prior to analysis, the spectra were individually processed with Standard Normal Variate and Detrend (SNVD) to reduce the scattering effect and correct the baseline. Each data point was then normalized by subtracting the mean and dividing by the standard deviation. The spectral processing was carried out in R Studio (R Studio Team 2022) using the same method as employed by Maçãs et al. (2023) **(Chapter 2)**, who studied the flour used to produce the bread in this study. Principal components analysis (PCA) was employed for all the different studies. The PCA was conducted using the "mdatools" and "ggplot2" R packages, and the first 10 principal components were examined and selected for visualization based on their significance to the study.

- Spectral analysis of flour and bread: The flour dataset, 100% wheat (WT) and 100% pea flour (RM, CM, and HM) from Maçãs et al. (2023) **(Chapter2)** was combined with the bread dataset (WT, RM, CM, and HM samples for days 1, 3, and 7 post baking) to differentiate samples based on product type (bread and flour) and composition (wheat and pea) (**Figure 3.3 A and B**).
- Spectral analysis of bread formulations: The bread dataset (WT, RM, CM, and HM samples for days 1, 3, and 7 post baking) was used to distinguish samples by formulation (**Figure 3.4 A and B**). However, as it was not possible to differentiate all samples, the dataset was narrowed down to include only RM, CM, and HM samples on day 7 to reduce variability (**Figure 3.4 C and D**).

- Spectral analysis of bread storage: The bread dataset (WT, RM, CM, and HM samples) was employed to distinguish samples by storage time (days 1, 3, and 7 post baking) (**Figure 3.5 A and B**).

3.4.6 Statistical analysis

To assess composition and baking parameters:

Analysis of variance (ANOVA) was completed using Minitab software (version 17.0) to assess the results of proximate composition, water activity and phytic acid analyses. For colour, volume and C-Cell a general linear model was employed, with experiment repetition and bread sample as the considered factors; Tukey's test was applied to identify significant differences in averages ($p < 0.05$). Each bread sample underwent triplicate analyses.

A two-sample T-test was performed to assess amino acid content, with the significance level set at $p < 0.05$.

To assess shelf life, focusing on Texture Profile Analysis (TPA) and moisture, two comparison tests were conducted:

- Comparison between samples within the same day: This comparison was carried out using Tukey's Pairwise Comparisons.
- Variable study over time, independent of the sample: Comparison conducted using Tukey Pairwise Comparisons. The general linear model was performed considering milling and analysis day as factors.

3.5 Results and discussion

3.5.1 Bread properties

3.5.1.1 Proximate composition, phytic acid and water activity

The proximate composition of breads is presented in Table 3.1. All breads containing pea flour showed a significant increase in protein content ($p<0.05$) compared with the wheat control. The inclusion of 15% of pea flour did not significantly affect the soluble dietary fibre, fat and ash contents of breads. However, the presence of insoluble dietary fibre significantly increased in all pea-containing breads, with CM and HM showing the highest results. Roller milled flour was obtained through a fractionation process where a larger fraction (mainly containing insoluble fibre) is discarded, resulting in a lower content of insoluble fibre (Maçãs et al., 2023) **(Chapter 2)**.

Table 3.1. Proximate composition of wheat bread and 15%:85% (pea:wheat) composite breads.

Composition (g/100 g)*	WT	RM	CM	HM
Protein	14.12 ± 0.44^{b}	15.40 ± 0.26^{a}	15.24 ± 0.20^{a}	15.32 ± 0.13^{a}
Total dietary fibre	6.33 ± 1.15^{b}	7.70 ± 0.66^{ab}	9.00 ± 0.26^{a}	8.75 ± 0.49^{a}
Soluble DF	2.73 ± 1.22^{a}	2.43 ± 0.35^{a}	3.07 ± 0.59^{a}	2.90 ± 0.14^{a}
Insoluble DF	3.60 ± 0.30^{b}	5.33 ± 0.29^{a}	6.00 ± 0.36^{a}	5.85 ± 0.35^{a}
Starch	75.71 ± 1.83^{a}	71.85 ± 0.88^{b}	70.82 ± 0.15^{b}	71.13 ± 0.23^{b}
Fat	1.51 ± 0.58^{a}	1.90 ± 0.39^{a}	1.82 ± 0.32^{a}	1.74 ± 0.44^{a}
Ash	2.81 ± 0.22^{a}	3.14 ± 0.08^{a}	3.13 ± 0.04^{a}	3.07 ± 0.25^{a}
Phytic acid	0.009 ± 0.010^{c}	0.173 ± 0.043^{bc}	0.275 ± 0.108^{ab}	0.399 ± 0.049^{a}
Water activity	0.9474 ± 0.01^{b}	0.9520 ± 0.01^{ab}	0.9608 ± 0.01^{a}	0.9570 ± 0.01^{ab}

* Wheat bread (WT); Composite breads with 15% pea flour from the roller mill (RM), cutting mill (CM) and hammer mill (HM). Data presented as means ± standard deviation. Values with a different letter (a-c) are significantly different ($p < 0.05$) along the row.

Phytic acid, recognized as the primary antinutrient in legumes (Nartea et al., 2023), exhibited lower levels in wheat control bread compared to composite breads. Legume flours are known for their higher phytic acid content compared to white wheat flour. Wu et al. (2023) documented that the phytic acid content in peas, lentils, and chickpeas was 8.55–12.40 mg/g,

8.56–15.56 mg/g, and 11.33–14.00 mg/g respectively, while Febles et al. (2002) noted that wheat endosperm (refined wheat flour) contained considerably lower values, mostly ranging between 2–4 mg/g.

The milling technique significantly influenced phytic acid levels in the composite breads. The roller milling fractionation process breaks and separates the seed coat from the inner parts of the seed (Cappelli et al., 2020). Consequently, bread produced with roller milled pea flour (RM) displayed the lowest levels, due to the ability of the mill to remove the seed coat (outer part) of peas. Unlike cereals, where most phytic acid is concentrated in the germ and bran (and thus largely removed during the production of white wheat flour), peas store phytic acid more uniformly throughout the seed, so removing the seed coat reduces phytic acid but to a lesser extent than in wheat Sarkhel and Roy (2022). This explains the lower phytic acid levels in wheat, the intermediate levels in roller-milled pea bread, and the higher levels in cutting and hammer milled pea breads. The phytate content in pea-containing breads remains well below this threshold, however it is still important to mitigate any potential effects. Various processing techniques during bread-making, such as prolonged fermentation and grain soaking, can effectively reduce phytate levels (Gupta et al., 2015). These techniques are particularly beneficial when working with pulses and will be a key focus in the next phase of this research.

Even though phytic acid is often considered an antinutrient due to its potential to reduce mineral bioavailability, low levels of phytic acid may offer health benefits, including antioxidant, antidiabetic and antibacterial activities (Boukid et al., 2019). Several human trials have indicated that a daily phytate intake of up to 2 g does not negatively affect mineral balance. However, excessive intake, particularly amounts exceeding 2 g per day in mineral-deficient diets, may pose risks to nutrient absorption (Pujol et al., 2023).

The water activity of pea-containing bread is similar to that of the control wheat bread. Maintaining a water activity level similar to that of wheat bread is crucial for food stability, as it directly impacts microbial growth and shelf life (Jakubczyk et al., 2008).

3.5.2 Loaf dimensions, crumb and crust colour, crumb structure

Specific volume and appearance are important quality parameters for bread (Clark & Aramouni, 2018; Dong & Karboune, 2021). Colour and shape are the consumer's first judgment and influence overall product acceptability (Garber et al., 2003). Typical desirable attributes are soft elastic crumb with a crisp thin crust and high specific volume (Maçãs et al., 2024b).

The specific volume, presented in Table 3.2, is significantly different between WT and composite flours, most likely due to gluten dilution which reduces the ability for gas incorporation and retention. As expected, the density follows the opposite trend, showing that higher volume correlates with breads of a lower density. Although no significant differences were observed between the composite breads, a trend can be observed in Table 3.2. Breads produced with the roller milled pea flour (RM) showed a tendency to a reduced volume, increased density, and low cell number, which may suggest that with higher levels of RM flour incorporation, a significant difference would be very evident.

Table 3.2. Crust and crumb colour and crumb structure of wheat bread and 15%:85% (pea:wheat) composite breads.

Breads[1]	**WT**	**RM**	**CM**	**HM**
Loaf Dimensions				
Specific Volume (ml/g)	3.27±0.30^{a}	2.91±0.29^{b}	3.02±0.31^{b}	3.01±0.39^{b}
Density (kg/m^3)	308.40±27.23^{b}	347.31±37.68^{a}	334.61±33.64^{a}	338.03±45.95^{a}
Colour				
L*-crust	45.43±3.57^{a}	41.68±4.08^{b}	41.29±3.79^{b}	43.30±4.43^{ab}
L*-crumb	72.07±1.29^{a}	69.06±0.55^{b}	69.24±1.98^{b}	69.01±2.61^{b}
b*-crumb	14.09±0.42^{b}	16.35±0.62^{a}	16.32±0.66^{a}	16.36±0.94^{a}
Digital imaging (C-Cell)				
Cell Diameter (mm)	2.09±0.32^{a}	1.91±0.19^{b}	1.95±0.25^{ab}	1.85±0.16^{b}
Number of Cells	1793.94±200^{a}	1657.11±124^{b}	1689.28±145^{ab}	1743.89±117^{ab}

[1] Wheat bread control (WT); Composite breads with 15% pea flour from the cutting mill (CM), hammer mill (HM) and roller mill (RM). Data presented as means ± standard deviation. Values with a different letter (a-b) are significantly different ($p < 0.05$) along the row.

Loaf crust and crumb colour, structure parameters and texture properties are also presented in Table 3.2. The addition of pea flour significantly lowered ($p < 0.05$) crust and

crumb L* (luminosity) value, except for HM crumb ($p > 0.05$). The higher protein content of pea flour compared to wheat flour, can lead to a darkened crust and crumb bread appearance due to an increase in Maillard browning reaction (Skendi et al., 2021). Additionally, the decrease in crumb luminosity (L*) is related to the addition of pea flour which contains a darker colour when compared to wheat flour. Pea flour dilutes the white wheat starchy endosperm, thus reducing the crumb luminosity (L*) (Skendi et al., 2021).

The chromaticity parameter a* for crust and crumb do not show significant differences among samples, indicating the intensity of red and green undertone was not changed (data not shown). The b* parameter for bread crumb increased significantly between wheat and pea-containing bread loaves, which means that pea flour inclusion resulted in increased yellow tones of the bread. Although there is a significant difference in some of the bread colour parameters, such differences are imperceptible under direct visual observation (**Figure 3.1 A**). The type of pea milling did not affect significantly the bread colour.

Digital imaging analyses of bread assess crumb structure, complementing subjective methods, such as visual and sensory methods (Smith, 2022). The cell diameter of bread crumb indicates the size of carbon dioxide bubbles formed during proofing and the number of cells corresponds to the production and stabilization of the air bubbles. Both parameters are related to volume, and impact the bread final properties (Sandhu et al., 2011). **Table 3.2** shows that cell diameter among samples ranges between 2.09 and 1.85mm. Breads comprising pea flour from the hammer mill and roller mill (RM and HM) showed significantly small different cell diameters to WT bread, however, no differences ($p < 0.05$) were observed between the composite breads. **Figure 3.1** and **Table 3.2** show that the presence of pea flour can influence the crumb structure causing the decreased bread volume and consequently a denser crumb and lower cell diameter. Similar results were observed by Frohlich et al. (2019). Bread produced with RM flour was the only sample to show a decrease ($p < 0.05$) in the cell number compared to WT, which is correlated with its lower volume and denser structure (**Table 3.1**). Density,

volume, cell diameter and number of cells are bread parameters that are interconnected and can be influenced by gluten dilution. According to Maçãs et al. (2023) **(Chapter 2)**, the addition of pea flour to the wheat bread dough matrix reduces dough extensibility, most probably caused by the higher non-gluten protein and higher fibre content of the pea flour. The lower cell number of RM bread can be related to the high volume density % (particle size) due to the larger particle size of this flour. Bourré et al. (2019) documented that bread produced with coarser flours had a lower number of cells. This supports the observation that larger particle sizes negatively impact the bread structure by reducing the cell number and overall volume.

Figure 3.1 A illustrates the cell distribution, where blue colour intensity corresponds to cell size. Darker blue areas indicate smaller cells, while lighter blue areas represent larger cells. It is evident that the wheat bread control has more extensive regions of lighter blue, indicating a higher average cell diameter compared to the other samples. This was also demonstrated in **Table 3.2**, where the cell diameter of the control (WT) bread is significantly larger than the pea composite breads. Parameters such as wall thickness, cell area, cell elongation, number of holes, and area of holes did not show significant differences among all breads ($p < 0.05$) (data not shown). These cell parameters are nonetheless crucial for understanding bread quality, and bread mouthfeel is also influenced by these cell parameters. According to Wang et al. (2017), thin walls and uniform cells are desirable characteristics in bread, as they contribute to a better texture and eating experience. These desirable characteristics are present in the wheat control bread and are also observed in composite breads.

Figure 3.1 B presents the confocal microstructure images of the bread crumb. The starch, protein and cellulosic material are represented by green, red and blue stains, respectively. Micrographs show an uneven distribution of starch and protein, which is common in bread (Dürrenberger et al., 2001; Kim Adrienne Millar et al., 2019). An evident increase in cellulosic material and protein is observed for the composite breads when compared with the WT bread, which reflects the previously discussed proximate composition results **Table 3.1**. Dietary fibre

is consistently located adjacent to the protein network, highlighted with white arrows in **Figure 3.1 B**. This suggests that cellulosic material can interrupt the formation and stabilization of a gas network during dough proofing, as it can interfere with the gluten matrix, potentially reducing the viscoelastic properties of the dough and thereby affecting the quality properties of the baked bread. This builds on our previous study **(Chapter 2)** on the milling of pea flour and its effects on dough (Maçãs et al., 2023).

Bread produced with roller milled pea flour (RM) exhibits cellulose material and protein networks surrounding starch granules in a highly organized manner, possibly influenced by its larger particle size and lower levels of damaged starch shown by Maçãs et al. (2023) in **Chapter 2**. Santetti et al. (2021) reported that non-gluten ingredients with smaller particle sizes have a less disruptive effect on the gluten structure during dough development when compared with ingredients of larger particle sizes. This could be attributed to the increased surface area of the smaller particles, which likely aids in their integration into the dough.

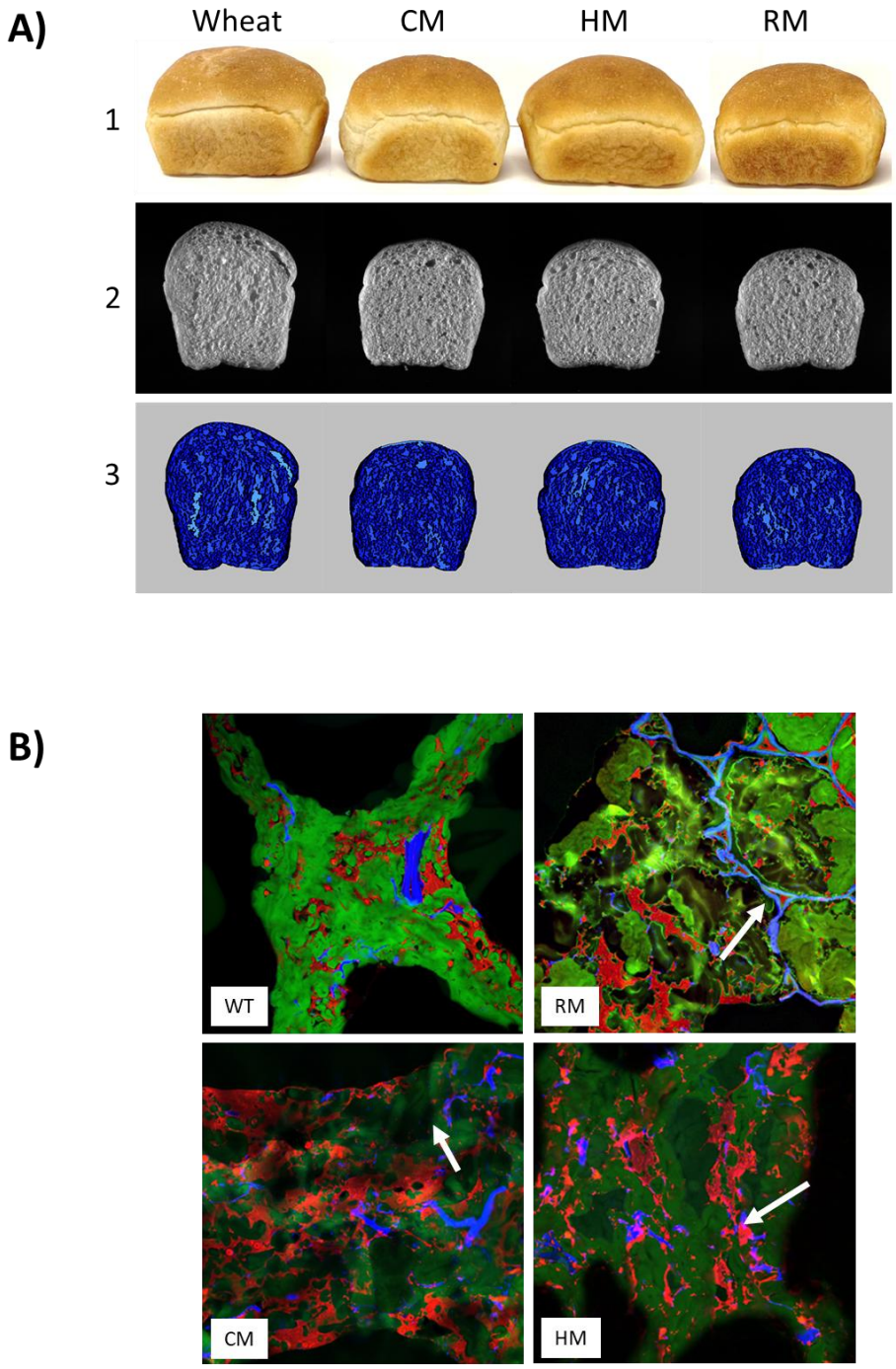

Figure 3.1. A) Images of bread from wheat flour and 15%:85% (pea:wheat) composite flour. 1. bread loaves; 2. centre slices (C-Cell); 3.cell structure (C-Cell). B) Confocal laser scanning micrographs of internal breads.

3.5.2.1 Bread profiling over a 7 day period: Texture profile analysis (TPA) and crumb moisture

Texture profiling of bread is crucial to evaluate product shelf life and textural changes over time. As expected, significant differences ($p < 0.05$) were observed in some TPA parameters between days 1, 3, and 7, as shown in **Figure 3.2**. No significant differences were found for crumb springiness between bread samples and analysis days (this data is not shown). Significant differences are indicated by the letters a-c (differences between bread samples for each day) and A-C (differences of mean of all the samples per day).

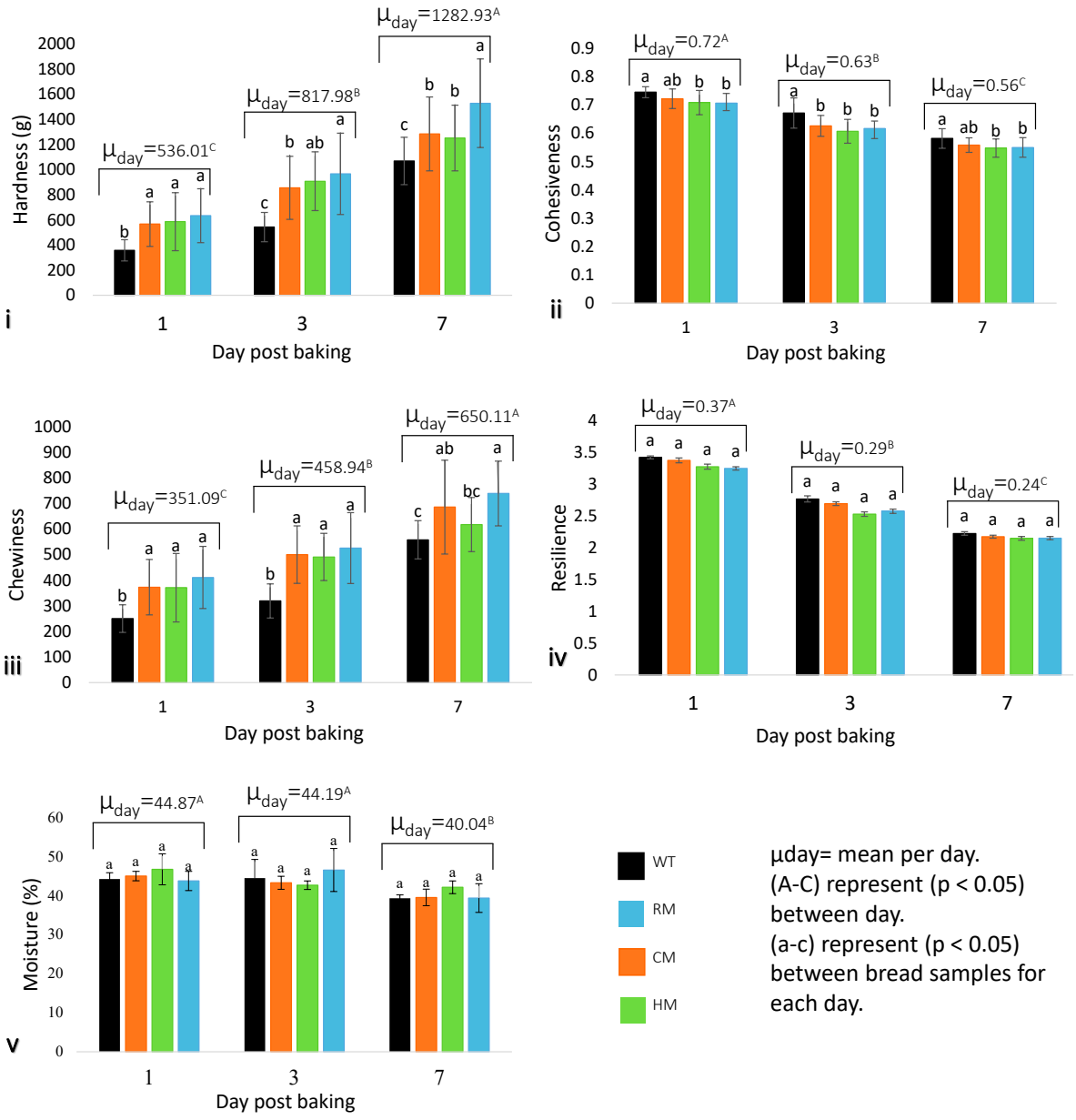

Figure 3.2. Bread TPA and moisture of wheat bread and 15%:85% (pea:wheat) composite breads, monitored seven days post baking. Each chart represents a different parameter; i) hardness; ii) cohesiveness; iii) chewiness, iv) resilience and v) moisture.

Texture profile analysis conducted 1 day post baking revealed that, while bread resilience was not significantly affected among samples, the parameters of hardness, cohesiveness and chewiness varied significantly ($p < 0.05$). Composite breads were firmer ($p < 0.05$) which is most likely related to starch retrogradation. Maçãs et al. (2023) evidenced that pea gels (starch gel produced via rapid visco analyser, 24 h after the standard test) were much harder ($p < 0.05$) than wheat gel. Pulses contain higher amylose content that retrograde at a faster rate when compared to amylopectin. In addition, the molecular structure of the starch increases the retrogradation process, when compared to cereal starches (Ahmed, 2021). Moreover, gluten dilution and the greater presence of dietary fibre cause network weakening and can reduce gas retention, producing a denser internal structure, and consequently increasing crumb hardness (Biduski et al., 2023). A decrease in cohesiveness ($p<0.05$) was observed for composite breads when compared to the wheat control, most probably due to gluten dilution and the addition of non-gluten proteins and dietary fibre. The loss of cohesiveness can increase crumbly texture, thus negatively impacting product acceptability (Rafał Ziobro et al., 2016). Composite bread loaves also showed an increased chewiness. Chewiness, defined as the combination of hardness, cohesiveness, and springiness, reflects the amount of energy needed to chew a solid food (Woolnough et al., 2010).

No significant differences were observed on day 1 among the three composite breads. However, over time, the rate of change in hardness differed, with the HM bread showing a slower increase in hardness (HM staling rate=18.91%/day) compared to the other composite breads (CM staling rate=21.10%/day and RM staling rate=23.52%/day). In addition, bread produced with the roller milled pea flour (RM) was significantly harder ($p < 0.05$) than other composited breads on day 7 post baking. The higher rate of staling observed in roller-mill pea bread (RM) over time is likely influenced by the milling technique, as it has been shown that

this process results in flour with a higher starch content, and lower IDF (Maçãs et al., 2023) and exhibits a greater rate of retrogradation compared to flours produced from hammer and cutting mills HM and CM. As a result, the RM breads, showed an increased hardness over time during storage. The lower fibre content of the roller-milled flour, compared to other pea flours, facilitates starch molecule re-association during cooling, resulting in a higher final bread hardness. Also, pea flour milled via the roller mill showed a higher pasting profile (RVA) and higher starch retrogradation (Maçãs et al., 2023) **(Chapter 2)**, contributing to an increase in the crumb hardness of this product. As consumers prefer a softer crumb texture, the hardness of the RM pea flour even at lower levels of addition will likely impact the final quality and consumer acceptance (Buehler, 2021). Crumb cohesiveness was shown to reduce ($p < 0.05$) over time. No significant differences were observed among composite breads. The decrease in cohesiveness can be attributed to bread staling. The loss of intramolecular attraction among the ingredients and consequently bread crumbly texture is commonly seen during ageing (Biduski et al., 2023). The chewiness of the bread samples increased over time, showing the same trend of hardness due to the correlation between both parameters. This increase in chewiness is likely attributed to changes in the crumb structure caused by starch retrogradation and moisture redistribution during storage, both of which are common in the bread ageing process. Crumb resilience reduced significantly ($p < 0.05$) from day 1 to day 7 in all breads, with no effects caused by the flour used.

The presence of moisture influences food stability, eating quality and physical properties. The incorporation of pea flour did not affect the crumb moisture within all bread samples. Having these parameters resembling wheat bread can be beneficial as it has the potential to provide a similar mouthfeel to wheat bread. As expected, there was a noticeable decrease in moisture content during storage. The staling process involves changes in bread properties, such as a reduction in moisture content due to water molecules distribution (Feizollahi et al., 2021). As the bread aged, the moisture within the crumb decreased, while the crust experienced an

increase in moisture, reflecting a movement of water from the crumb to the crust during the staling process (Ribotta & Le Bail, 2007).

3.5.2.2 Amino acid profile

Combining cereals with pulses or pseudocereals can create products with a balanced amino acid composition, resulting in a complete amino acid profile (Maçãs et al., 2024b). The amino acid profile of wheat bread and bread produced from hammer-milled flours were analysed and presented in Table 3.3. The HM bread was selected due to the quality attributes of the bread and the lowest staling rate. In addition, the hammer milled flour when considering flour yield, exhibited superior efficiency (93.89%), in contrast to the roller mill (55.64%) and the cutting mill (39.15%) (Maçãs et al., 2023) **(Chapter 2)**. This analysis did not aim to compare different milling methods, but rather to evaluate the improvements achieved by incorporating 15% pea flour into wheat bread.

Table 3.3. Amino acid profile of wheat bread and 15%:85% (pea:wheat) HM bread.

Breads[A]	**WT**	**HM**	**RDA per person[B]**
Essential amino acids	(mg/100g bread)		
Histidine	260±0	329±36	700
Threonine	399±4	485±33	1050
Valine	546±37	679±36	1820
Methionine	42±1	54±1	1050
Tryptophan	19±1	0±0	280
Phenylalanine	757±13	858±23	1750
Isoleucine	438±8	521±17	1400
Leucine	989±21	1154±20	2730
Lysine	314±3	519±4	2100
Nonessential amino acids	(mg/100g bread)		
Aspartate	725±47	1122±156	-
Glutamate	5516±158	5697±228	-
Serine	736±19	830±26	-
Glycine	536±0.00*	637±6*	-
Arginine	512±14	698±19	-
Alanine	447±8	543±14	-
Tyrosine	430±14	465±18	-
Total AA	12667±264	14591±626	-

[A] Wheat bread control (WT); Composite breads with 15% pea flour from the hammer mill (HM). [B] Recommended Daily Allowance (mg per 70 kg of body weight per day) (Joint et al., 2007). *Means values significant ($p > 0.05$) different.

Of the essential amino acids, lysine content exhibited a significant increase when comparing wheat bread with HM-produced bread. This amino acid can contribute to lower cholesterol levels, regulate the faecal excretion of steroids and remove bile acid (Kowalski et al., 2022). Common limiting amino acids of cereal are usually lysine, threonine, or leucine (Kowalski et al., 2022). When comparing the two breads, it can be seen that the inclusion of 15% pea flour significantly increases the amount of essential amino acids compared to wheat bread, making it easier to meet the Recommended Daily Allowance (RDA) for most essential amino acids. However, methionine and tryptophan remain notably low in pea bread.

To ensure a complete amino acid profile, it could be beneficial to incorporate tryptophan-rich foods and methionine sources. A practical approach to improving the nutritional quality of the bread would be to fortify it with nuts and seeds, enhancing its amino acid composition and making it a more complete protein source (Dodevska et al., 2022).

The non-essential amino acid glycine is significantly higher for pea-containing breads. This aids in the treatment of intestinal and gut diseases (Razak et al., 2017). Overall, the total amino acid content increased by approximately 15% (from WT=12667 mg/100g bread to HM=14591 mg/100g bread). The pattern observed indicates that the amino acid profile improves with the addition of pea flour, and a further impact would be achieved with higher quantities of pea incorporation. Additional research is now being undertaken to explore higher incorporations, using thermal and non-thermal technologies.

3.5.3 Near infrared spectroscopy (NIR) profiling

3.5.3.1 Spectral classification of ingredients

The spectra from 100% wheat flour (WT) and 100% pea flour from three different mills (CM, HM, and RM), from the study by Maçãs et al. (2023) **(Chapter 2)**, were combined with the spectra from each bread to evaluate whether the samples could be distinguished by product type (bread and flours) and composition (100% wheat vs containing pea flour). The spectra plot, along with PC1 (62.9% variability) and PC2 (28.3% variability), are presented in **Figure 3.3**, with the corresponding loadings shown in Appendix **Figure 3.A1**. As expected, the spectra of flour and bread are distinctly different, making it quite simple to separate them by product type. This separation is also confirmed by the Principal Component analysis (PCA), more specifically PC1. The loadings from PC1 (see Appendix **Figure 3.A1**) indicate that the observed differences are likely related to factors such as colour, moisture content, and the structure of starch and protein.

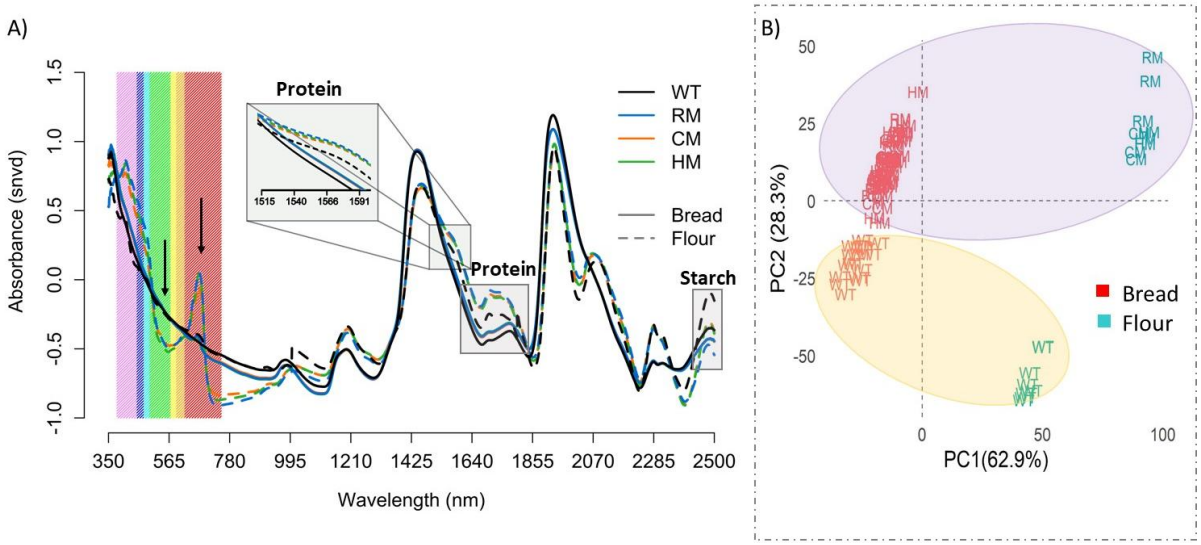

Figure 3.3. SNVD Vis-NIR spectra A) and Score plots B). Manley (2014) identified that the moisture content is related with the absorbance in the spectral ranges between 1440-1470 nm and 2148-2200 nm. In this study, these ranges on the spectra correspond to higher absorption levels for bread. This observation is also supported by the loadings in Appendix **Figure 3.A1**.

PC2 effectively separated WT bread and flour from samples containing pea flour, even though this distinction was not obvious in the spectra. The loadings from PC2 (Appendix Figure 3.A1) indicate that the separation of WT samples is mainly due to colour differences, with high peaks for pea flour in the red and green visible spectrum. Flour and bread containing pea flour reflected more light in the red wavelengths of the visible range and absorbed more in the green wavelengths when compared to wheat. This is likely due to the greenish undertone of the pea flour as a consequence of the presence of chlorophyll. Additionally, pea flour samples show higher absorption in the 1500-1600 nm and 1600-1850 nm spectral ranges related to protein bonds, aligning with their higher protein content (Table 3.1). The 2400-2500 nm range, associated with starch absorption, shows higher values for wheat, consistent with its higher starch content. These findings align with the protein and starch areas identified by Maçãs et al. (2023) in **Chapter 2** and suggest that specific traits in the flour could predict its impact on bread quality.

3.5.3.2 Spectral classification of bread formulations

Figure 3.4 displays the SNVD Vis-NIR spectra, along with the score plot and loadings (PC1 and PC6), used to differentiate bread based on formulation. The primary spectral ranges that stand out include 600–700 nm, 1650–1850 nm, 1900–2000 nm, and 2400–2500 nm. PC1 which represents 37.9% of variability, separates the two distinct groups: WT bread and composite breads. The loading of PC1 (Appendix Figure 3.A2) is quite broad and encompasses all of the mentioned spectral ranges. These ranges are associated with protein, starch and fat, as previously identified by Maçãs et al. (2023), and are clearly illustrated in Figure 3.4 A.

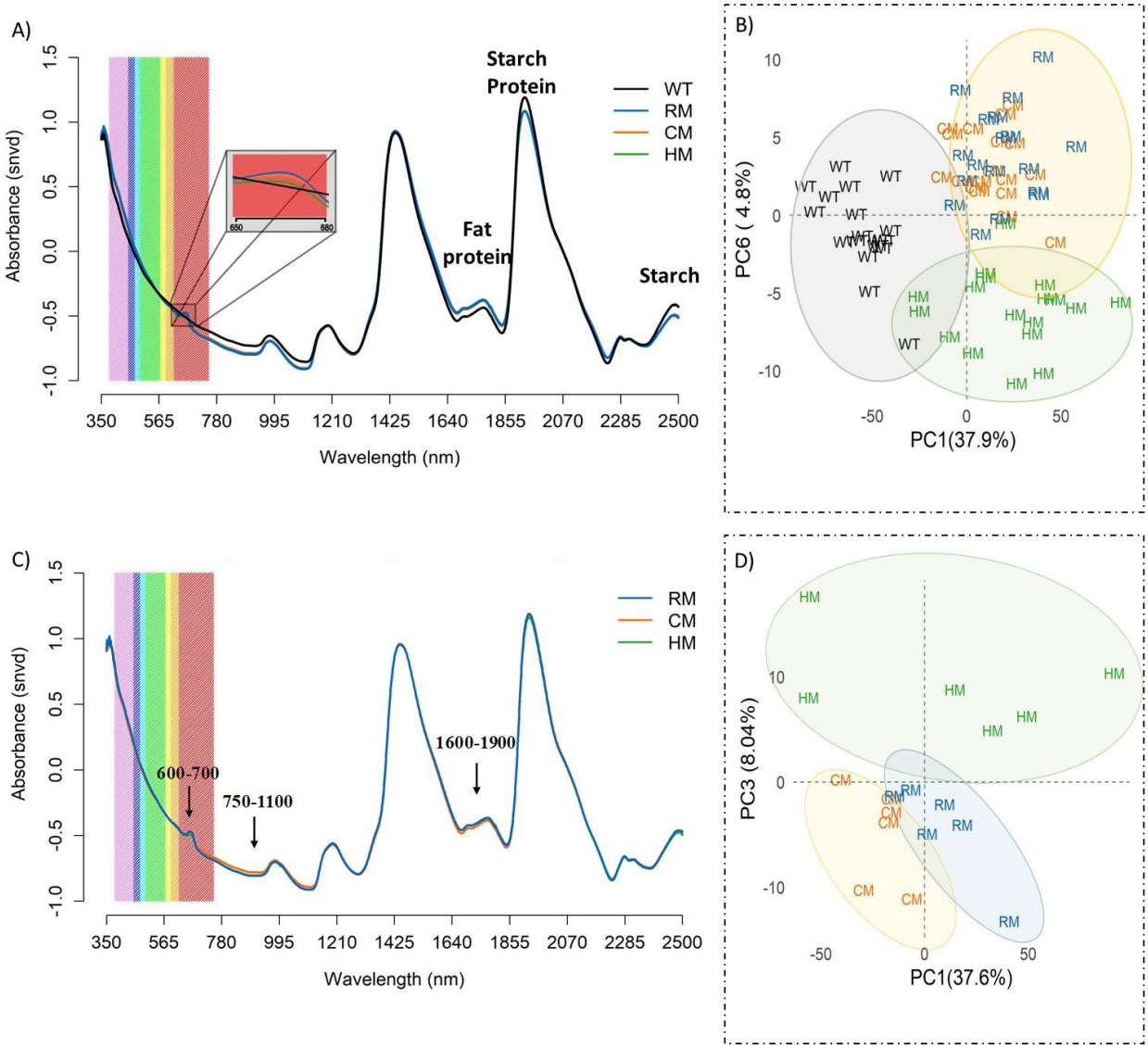

Figure 3.4. SNVD Vis-NIR spectra A) and Score plot B) of PC1 and PC6 of bread (15%:85% pea:wheat) on day 1, day 3 and day 7 post baking. SNVD Vis-NIR spectra C) and Score plot D) of PC1 and PC3 of all composite breads (15%:85% pea:wheat) on day 7 post baking. In the spectra, breads produced using the hammer mill (HM) showed lower absorption in the 600-700 nm range (red visible region) compared to other samples. This variability occurred due to colour changes, indicating that the flour milling technique can influence the colour of the bread. This pattern is shown by PC6, which groups HM on the negative side relative to composite breads and is confirmed by the loadings (Appendix Figure 3.A2). This was not apparent in the colour analyses or visually detectable, underscoring the capability and precision of NIR spectroscopy in distinguishing or categorizing products, even when the colour differences are not statistically significant.

As CM and RM could not be distinguished through PCA, likely due to variability introduced by storage time (spectra collected on days 1, 3, and 7 post-baking) and the high similarities between the composite breads, an alternative approach was adopted. The dataset was reduced to include only bread from day 7, where RM displayed the most distinct behaviour in texture results, a key parameter that reflects physical and chemical changes in the bread. **Figure 3.4** displays the spectra for bread (C) along with the respective PC1 and PC3 scores (D). The loadings for these components are shown in Appendix **Figure 3.A2**.

PC1 can separately group CM and RM breads. The loadings for PC1 reveal multiple absorbing areas, making it challenging to pinpoint a single cause. The proximate composition of the composite breads is similar. Therefore, another factor contributing to their separation could be the milling process, which affects particle size and damaged starch content (Maçãs et al., 2023), potentially altering the starch structure and gluten matrix post baking.

PC3 distinguishes HM from the other composite breads. By examining the loadings in Appendix **Table 3.A2**, it is clear that the cause of this separation aligns with the data presented in **Figure 3.4 A**. This separation is due to the colour differences between HM and the other composite breads, as previously highlighted in **Figure 3.4 B** (PC6).

The variations between these samples are mainly due to differences in the milling of the pea flour, despite each bread containing pea flour at lower levels (15%). This demonstrates that NIR spectroscopy can be effectively used for quality monitoring, as it can detect even subtle physical and chemical differences. NIR spectroscopy is already employed in the industry to detect adulterations, such as identifying margarine in butter or sunflower oil in olive oil. NIR shows further potential not only for detecting adulterations but also for verifying product standardization rapidly and non-destructively. It can assist in identifying outliers in online food products, ensuring companies consistently meet consumer expectations and maintain product quality.

3.5.3.3 Spectral classification by storage time

The SNVD Vis-NIR spectra, along with the score plot for bread discrimination based on each storage day, are presented in **Figure 3.5 A and B** and respective PC2 (20.1%) and PC3 (11.6%) loadings on Appendix **Figure 3.A3**. Differences in bread staling within the NIR region were primarily observed in the following spectral ranges: 1160–1240 nm, 1400–1500 nm, 1650–1750 nm, 1910–2000 nm, 2200-2350 and 2450–2500 nm. These spectral regions are associated with typical starch and water bond vibrations. Peiris et al. (2021) identified absorption peaks at 1160 and 1240 nm as being linked to starch groups involved in the second overtone of C–H bonds. The peaks at 1210 nm, 1480 nm, and 1955 nm are associated with C–H stretching vibrations in carbohydrates (Beć et al., 2020). According to the findings by De Géa Neves et al. (2023) 1709 nm, 1938 nm and 2300 nm are related to absorption bands that represent starch chemical functionalities that increase during aging due to crystallization. Furthermore, Maçãs et al. (2023) in **Chapter 2** confirmed the association of these absorption ranges with starch molecules by analysing wheat starch, as a standard to identify the primary absorption zones for starch. The loadings from PC2 and PC3, presented in Appendix **Figure 3.A3**, highlight the relevance of the mentioned wavelengths, showing trends that align with those observed in the referenced studies.

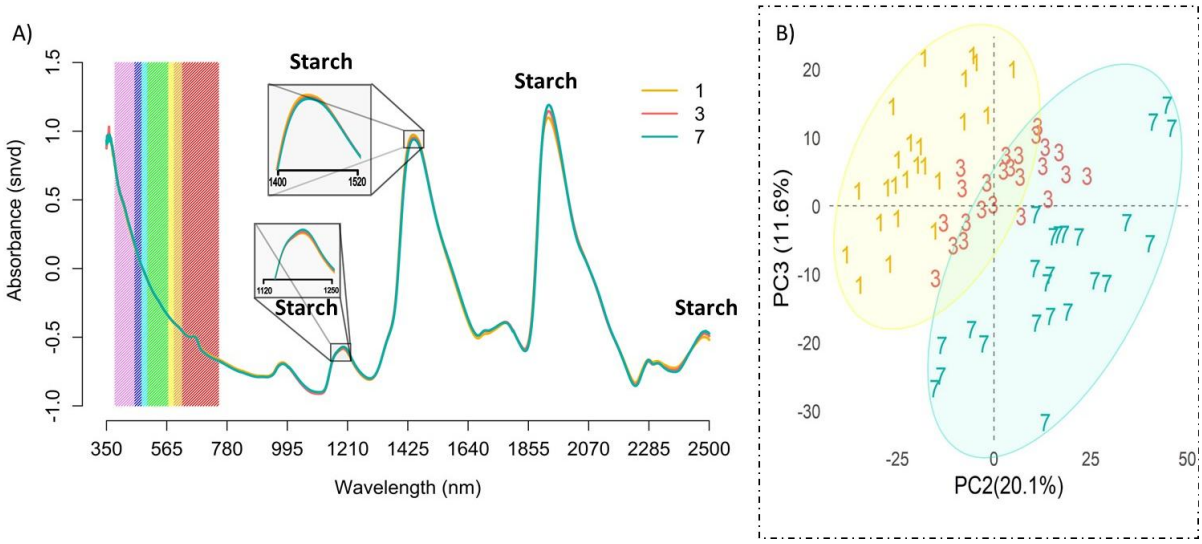

Figure 3.5. SNVD Vis-NIR spectra A) and Score plot B) of PC2 and PC3 of all breads (15%:85% pea:wheat) on day 1, day 3 and day 7 post baking.

De Géa Neves et al. (2023) also noted that the peak at 1883 nm corresponds to the OH combination bands of weakly hydrogen-bonded water. This peak decreases over time as a result of evaporation and diffusion from the centre of the crumb to the crust. This peak is not visible in the spectra but can be identified in the PC2 and PC3 loadings at 1880 nm, highlighted with a blue arrow.

From PC2 and PC3 scores plot **Figure 3.4 B**, three main clusters can be clearly identified, corresponding to days 1, 3, and 7 post baking. The **Figure 3.4 B** shows an elliptical shape, suggesting that PC2 and PC3 share substantial variance. This is supported by the loadings (Appendix **Figure 3.A3**), shown by the blue arrows, where many variables contribute similarly to both components but with opposite signs. This indicates that PC2 and PC3 capture the same underlying patterns in the data, but in inverse directions, resulting in the observed clustering. PC2 and PC3 capture the variability associated with starch structures and water, likely reflecting changes in the starch-gluten matrix over time, which directly influences bread staling. This variability can be attributed to starch retrogradation and crystallinity, as during storage, amylopectin reverts to double helical structures and reorganizes within crystalline regions (Zucco et al., 2011).

Researchers including Ghoush et al. (2007), Zucco et al. (2011) and Amigo et al. (2021) have highlighted the effectiveness of Near-Infrared (NIR) spectroscopy in analysing the staling profile of baked products. These studies consistently demonstrate that NIR spectroscopy is a valuable tool for determining changes during storage. This present study is unique as it has shown that bread storage changes are largely independent of composition. PCA showed that, despite differences in composition and physical properties such as hardness, bread samples could still be grouped by storage time. This suggests the potential for a calibration model

capable of rapidly assessing the shelf life of various bread types. However, further research is needed to validate these initial findings.

3.6 Conclusion

This study highlights the significant impact of milling technologies on the performance of Irish-grown pea flour in bread-making.

Inclusion of pea flour was shown to increase the protein and insoluble dietary fibre content of bread, with breads produced from the cutting mill (CM) and hammer mill (HM) yielding the highest fibre levels. Among the composite breads, only bread made with roller milled pea flour (RM) showed a significant reduction in cell number compared to wheat bread. Overall, roller-milled pea flour appeared the least suitable for bread production, as the resulting bread exhibited reduced quality in key markers. HM bread showed the slowest increase in hardness and crumb staling compared to the other composite breads. Overall, the addition of pea flour increased the total amino acid content of bread by approximately 15%.

NIR spectroscopy was shown to be an effective tool for distinguishing samples containing wheat versus non-wheat flour, whether in bread or flour form, underscoring its predictive potential for flour quality in bread applications.

NIR spectroscopy provided valuable insights by detecting on bread milling-induced differences. Also, while NIR is conventionally used to detect fraud, such as identifying margarine substitution for butter in cakes due to their distinct compositional differences, this research advances the field by showcasing the capability of NIR to identify subtle outliers in products with identical compositions.

This study demonstrated that, despite significant differences between the control wheat and pea-containing breads, NIR was nevertheless able to classify all samples by storage time (days 1, 3, and 7 after baking). This highlights the potential of NIR spectroscopy as a non-destructive tool for rapid staling assessment across bread types.

4 Physical modifications of Irish peas through advanced processing technologies

Physical modifications of Irish-grown peas through advanced processing technologies

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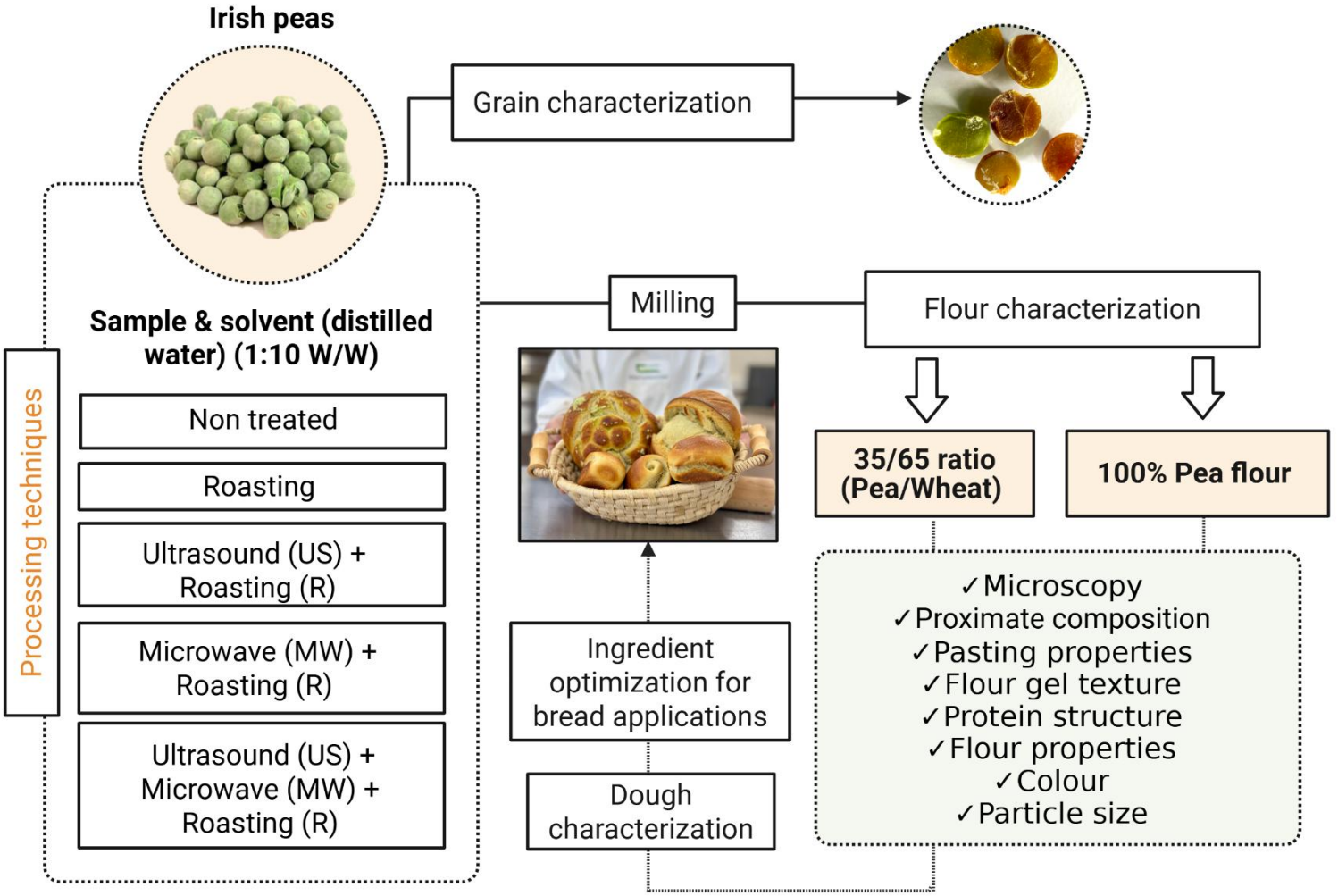
4.1 Graphical abstract



4.2 Abstract

With growing interest in plant-based ingredients, Irish-grown peas offer a promising opportunity to enhance the nutritional quality of cereal-based foods. However, their wider use is limited by functional limitations, including low solubility and off-flavours. This study evaluated the effects of processing techniques, roasting (R), ultrasound (US) and microwave (MW), applied individually and in combination, on the compositional, functional, and structural properties of pea flour and a pea:wheat blend (35:65) for bread applications. Commercial pea (CP) flour showed higher water solubility, pasting viscosity, and gel hardness than Irish pea (IP). Processing modified flour properties, with roasting most strongly affecting starch gelatinisation, protein denaturation, and water absorption. FTIR showed reduced starch crystallinity and a protein transition from α-helices to random coils, especially after roasting. SEM revealed that combined US+MW (IP+US+MW+R) produced the highest microstructural breakdown. Future work will investigate incorporating these treated flours into bread-making to evaluate their impact on product quality.

4.3 Introduction

The global food landscape continues to undergo a remarkable transformation, fueled by a growing interest in incorporating more plant-based foods into human diets. This shift is underscored by an increase in consumer preference towards diets rich in plant-derived sources, driven by health, and sustainability concerns (Fuentes & Fuentes, 2022). Pulses, including peas, beans, lentils, and lupins, are dietary staples known for their nutritious composition, rich in protein and fibre. Also, they are high in lysine, an essential amino acid lacking in cereals (Hall, 2021a). The nutritional profile of pulses, combined with their ability to the enhancement of soil organic matter, leads to improved crop yields and potentially greater profitability, positioning pulses as a key player in the ongoing dietary shift towards plant-based foods (Cusworth et al., 2021). Peas, well-suited to the Irish climate, are rich in essential vitamins, minerals, protein and dietary fibre (Binti Mohd Zaini et al., 2021). The inclusion of Irish-grown peas in food products such as baked goods has the potential to not only enhance their nutritional value but also holds promise for boosting the local economy and promoting sustainability.

Although pulses offer notable nutritional and health advantages, their use in food formulations can be limited by certain flavour and functional challenges. Incorporating pea flour into products may introduce off-flavours, which can affect consumer acceptance (Young et al., 2019).

Several processing techniques are being explored to improve flavour and modify flour functionality to maximise the use of plant-based protein sources in baked products (Bourré et al., 2019; Guldiken et al., 2022; K. A. Millar et al., 2019; Nilufer-Erdil et al., 2012). Conventional methods such as roasting have long been used to improve the sensory experience, the protein and starch digestibility and to decrease antinutritional compounds (Young et al., 2019). Furthermore, previous research demonstrated that using roasted pulse flours in bread formulations can improve quality. Kim Adrienne Millar et al. (2019) observed higher loaf

volume and softer crumb texture in bread made with toasted pea flour compared to non-toasted pea flour.

Alongside traditional approaches, emerging technologies such as ultrasound (US) and microwave (MW) treatments are gaining attention for their ability to enhance both the physicochemical properties (e.g., molecular weight, particle size and zeta potential) and functional properties (e.g., pasting behaviour, water solubility, foaming and emulsifying capacity) of pea flour and other pulse-derived ingredients (Maçãs et al., 2024b).

Ultrasound operates by generating cavitation, the formation and collapse of microscopic bubbles, using high-intensity, low-frequency sound waves (A. G. A. Sá et al., 2022). Microwave processing utilises non-ionising electromagnetic radiation. This occurs through interactions with the food's chemical constituents via ionic conduction and dipolar rotation, where penetration depth and the material's dielectric properties are key factors influencing the heating efficiency (A. G. A. Sá et al., 2022). Wanyu Qin et al. (2022) reported that treating semi-dry milled rice flour with microwave or ultrasound prior to its use in bread formulations enhanced the specific volume of the bread. This effect was linked to changes in the flour structure and particle size. M. Zhang et al. (2022) showed that ultrasound-assisted dough fermentation decreased free sulfhydryl (SH) groups and increased wet gluten content, which enhanced dough extensibility and produced bread with greater specific volume and reduced crumb hardness. Garske et al. (2023) found that microwave treatment of cassava starch and chickpea flour improved crumb hardness in gluten-free bread.

Roasting, microwave, and ultrasound can improve the functional and nutritional properties of pea flour, but they are usually applied separately. Therefore, this study aimed to investigate, for the first time, the combined effects of these treatments on Irish-grown pea flour and its blends with wheat flour to determine whether their integration would yield further beneficial effects on flour properties and, consequently, on bread quality.

4.4 Materials and methods

4.4.1 Raw material and grain processing

Grains of the Irish-grown peas (Carrington) were provided by Wynnes Ltd (Kildare, Ireland). The cleaned grains were processed using ultrasound, microwave, and a combination of both techniques using ultrasound-microwave assisted extractor model E200 developed by Idco SAS (Marseille, France), equipped with a cooling system to ensure sample temperatures remained below 30°C. Given the limited existing literature on the subject, several preliminary studies (data not included) were conducted to determine the most appropriate parameters. For each treatment, 1kg of Irish-grown pea grains were submersed with 2L of tap water and placed into the E200, working at 1000 W for the microwave and/or 100% amplitude level (200W) with a frequency of 25 kHz for ultrasound, with continuous agitation. These parameters were selected based on preliminary optimization trials. The pea grains were exposed to ultrasound (US), microwave (MW) and combined ultrasound and microwave (IP+US+MW+R) for a total of 12 minutes, divided into four cycles of 3 minutes each, with a 5-minute cooling interval between cycles to prevent overheating. The grains were then dried with roasting (R) at 170 °C for 16 min in a deck oven (MacPan, Thienne, Italy). As roasting is an additional processing step, its individual impact on the peas was also assessed using roasted Irish pea grains (IP+R). Before roasting, the pea grains were submerged in water for 30 minutes. The flour was milled with hammer mill as reported by Maçãs et al. (2023). Non-treated Irish pea grains (IP) and a commercially available pea flour (CP), of Hungarian origin (supplied by Mills & Folks, UK), were utilized for comparative purposes. Strong wheat flour (Shakleton's Milling, Ireland) was used in the composite blends to assess dough rheological properties. The abbreviations for grains, flours, and flour composites used in this study are specified in the Appendix (**Table 4.A1**).

4.4.2 Flour proximate composition

Proximate composition of the flours was analysed. Total nitrogen content was measured via the combustion method based on the Dumas principle with LECO equipment (FP-628, LECO Corp., USA) (protein was determined as Nx5.7), following method 968.06 (AOAC, 2000b). Fat content was analysed using the ORACLE Rapid NMR Fat Analyser (CEM Corp., USA) (AOAC, 2005). Total dietary fibre (TDF), including insoluble (IDF) and soluble dietary fibre (SDF) fractions, was assessed through an enzymatic-gravimetric method with an Ankom automated dietary fibre analyser (Ankom, USA) following AOAC (1994) method 991.43. Ash content was quantified using a Gallenkamp Muffle Furnace, pre-dried overnight at 550°C, following method 08-01.01 (Gallenkamp, UK) (AACC, 2010a). Moisture content was assessed as per method 44-15.02 (AACC, 2010b). The percentage of total carbohydrates was calculated by subtracting the combined values of protein, fat, ash, (dry basis) from 100%. Total starch content was then determined by subtracting the TDF value from the total carbohydrate content, as described by Charrondiere et al. (2004).

4.4.3 Colour

The colour of each flour sample was evaluated using a colourimeter (CR-410, Konica Minolta, UK). Measurements were recorded and expressed using L* (0 = black, 100 = white), a* (- = green, + = red), and b* (- = negative, + = yellow) colour scale.

4.4.4 Particle size

Flour particle size was determined using a laser scattering technique with a Mastersizer 3000 (Malvern Instruments Ltd, UK). The Hydro EV system was employed for wet dispersion, utilising a refractive index of 1.53 and an absorption index of 0.01. Samples were diluted in reverse osmosis water until they achieved the acceptable obscuration range of 10–20%. The

particle size of each sample was then recorded at the 10th (d10), 50th (d50), and 90th (d90) percentiles of the volume distribution.

4.4.5 Scanning electron microscopy (SEM) of grains and flours

To analyse the internal structure of the pea grains, they were subjected to cryo-sectioning by immersing them in liquid nitrogen to enhance brittleness and subsequently fragmented into smaller pieces. Grains and flours were fixed onto sample holders using double-sided carbon tape and sputter-coated with gold at 80 mA for 1 min using a Emitech K575X sputter-coater (Quorum Technologies, UK). Imaging was performed using a Gemini field emission SEM (Supra 40VP, ZEISS, Germany) operating at an accelerating voltage of 2 kV and a working distance of 4-8 mm. The images reported were acquired using the secondary electron detector of the instrument.

4.4.6 Characterization of protein and starch structures

Fourier Transform Infrared Spectroscopy (FTIR) was used to characterise the protein and starch structures of the flour samples. Measurements and data processing were carried out following the approach outlined by Sengar et al. (2025). Spectral analysis was conducted using a Spectrum Two™ FTIR Spectrometer (PerkinElmer, UK) fitted with an attenuated total reflection (ATR) unit. Spectra were collected in the 4000–400 cm^{-1} range, using 30 scans at a resolution of 4 cm^{-1}, with air as the background reference. For protein structure, the data underwent baseline correction and smoothing via the Savitzky-Golay method. Peak identification in the amide I region (1600–1700 cm^{-1}) was enhanced using Fourier self-deconvolution and second derivative analysis. Individual Gaussian peaks were assigned to secondary structural components based on their position, following the classification of Sadat and Joye (2020): 1610–1627 cm^{-1} for intermolecular β-sheets, 1628–1642 cm^{-1} for β-sheets, 1643–1650 cm^{-1} for random coils, 1650–1659 cm^{-1} for α-helices, and 1660–1699 cm^{-1} for β-

turns. Spectral data were analysed using OriginPro 2021 (OriginLab, USA). Baseline correction was applied to the FTIR spectra prior to starch structural evaluation. The ratio between the absorbance at 1047 cm^{-1} (crystalline region) and 1022 cm^{-1} (amorphous region) was used to evaluate the degree of molecular order in the carbohydrate matrix.

4.4.7 Water absorption index (WAI) and water solubility index (WSI)

The water absorption index (WHI) and water solubility index (WSI) were determined using a 1:4 (g/g) flour-to-water ratio to ensure proper hydration. The mixture was then placed in an orbital shaker set at 150 rpm and left to mix for 24 hours at room temperature. After this period, the hydrated mixture was transferred into 2 ml centrifuge tubes and centrifuged at 4000 rpm for 15 minutes to separate the components. This process resulted in two distinct phases: the supernatant and the sediment, which were separated and dried at 40°C until fully dehydrated. The water absorption index (WAI) and water solubility index (WSI) were calculated using the following formulas:

$$(1) \quad WAI(g/g) = \frac{Weight\ of\ wet\ sediment(g)}{Dry\ weight\ of\ flour\ (g)}$$

$$(2) \quad WSI(\%) = \frac{Weight\ of\ dried\ supernatant\ (g)}{Dry\ weight\ of\ flour\ (g)} \times 100$$

4.4.8 Flour pasting properties

Pasting properties of the flours were determined using Standard Profile 1 on the Rapid Visco Analyser (RVA-4, Newport Scientific Pty. Ltd., Australia). To 25 g distilled water, 4g of flour with moisture adjusted to 14% according to method 76–21.02 (AACCI, 2010b). Peak viscosity, breakdown, setback viscosity, final viscosity and pasting temperature were quantified. Starch pasting properties were assessed for both pure and composite flours. The composite flours were specifically studied to evaluate their starch behaviour, to determine their suitability for future bread baking trials.

4.4.9 Texture profile analysis (TPA) of the RVA gel

The gels from RVA were sealed with film to prevent weight loss and stored at room temperature for 24 hours. The gel texture was evaluated using a texture analyser (TA-XT2i, Stable Microsystems, UK). Texture profile analysis (TPA) was conducted with a 20 mm cylindrical probe, applying a 40% deformation and a test speed of 1 mm/sec. The properties of both pure and composite flours were assessed. Composite flours were evaluated to determine their suitability for future bread baking trials, as gel texture after 24 hours is likely correlated with staling behaviour (Biduski et al., 2024).

4.4.10 Dough mixing properties

Flour blends with a 35:65 ratio (pea:wheat) were examined using Mixolab (Mixolab-2, Chopin, France) according to the standard Chopin + Protocol as a preliminary study to determine their suitability for future bread baking trials. Flour moisture content was measured beforehand (AACC, 2001) to determine the appropriate flour weight. The mixing curve obtained from this analysis provided data on optimal dough water absorption (necessary to achieve a consistency of 1.1 ± 0.05 Nm); dough stability (torque duration maintained at 1.1 Nm); dough development time, C2 (protein quality, Nm), C3 (starch gelatinization), C4 (starch gel stability, Nm) and C5 (starch retrogradation, Nm) as per method 54-60.01 (AACC, 2010c).

4.5 Results and discussion

4.5.1 Flour proximate composition and colour

Table 4.1 presents the proximate composition and colour of the flours. Significant differences were found in all the composition parameters. As expected, wheat flour had the lowest protein and fibre content. Irish pea (IP) flour contained significantly ($p < 0.05$) more protein and fibre than commercial pea (CP) flour, thus highlighting the potential of Irish soil

for pea production. When roasting was applied, the protein content decreased ($p < 0.05$). This reduction may be due to denaturation and protein loss, likely caused by Maillard reactions. During heating, the reaction between the aldehyde groups of reducing sugars and free amino groups can lead to the formation of insoluble brown polymers known as melanoidins (Oboh et al., 2010). An increase in protein content was observed when processing the roasted pea flour with ultrasound (US), microwave (MW), or a combination of both. This could be due to structural alterations, the disruption of protein-starch and protein-fibre complexes, making the protein more available (Mahalaxmi et al., 2022; Xu et al., 2025).

Wheat flour (W) had the lowest fat and ash contents ($p < 0.05$), while commercial pea (CP) flour had the highest. All Irish pea flour samples fall in the middle, showing similar values, meaning that the roasting, ultrasound and microwave did not significantly affect the fat and ash content.

Moisture content was highest in wheat flour ($p < 0.05$), followed by raw pea flours, and then the samples where roasted treatment was applied (IP+R, UPR+US, IP+MW+R and UPR+US+MW). As expected, the roasting process decreased their water content.

Table 4.1. Proximate composition and colour of wheat and pea flours.

Flour*	W	CP	IP	IP+R	IP+RT+US	IP+RT+MW	IP+RT3+US+MW
Proximate composition							
Ash	0.67±0.13^{c}	2.93±0.12^{a}	2.72±0.04^{ab}	2.75±0.06^{ab}	2.73±0.08^{ab}	2.7±0.05^{ab}	2.61±0.04^{b}
Fat	0.32±0.06^{b}	0.9±0.07^{a}	0.72±0.03^{a}	0.86±0.10^{a}	0.76±0.10^{a}	0.83±0.12^{a}	0.74±0.18^{a}
Protein	16.11±0.10^{e}	22.79±0.03^{c}	23.86±0.14^{a}	21.82±0.05^{d}	23.81±0.07^{a}	23.48±0.02^{b}	23.47±0.04^{b}
IDF	1.25	7.85	14.85	14.8	14.8	15.4	14.3
SDF	4.25	5.6	5.65	6.4	6.6	7.6	7.4
TDF	5.5	13.45	20.5	21.2	21.4	23	21.7
Starch	65.96	49.58	43.18	44.10	43.27	42.40	42.95
Moisture	12.11±0.01^{a}	11.96±0.00^{b}	11.35±0.04^{d}	11.77±0.01^{c}	10.22±0.01^{f}	9.86±0.01^{g}	10.89±0.02^{e}
Colour							
L*	91.15±0.3^{a}	84.54±0.42^{b}	83.81±2.42^{b}	80.68±2.7^{b}	80.37±1.7^{b}	79.79±1.09^{b}	79.4±3.27^{b}
a*	0.03±0.04^{a}	-9.74±0.49^{e}	-9.43±0.41^{e}	-1.99±0.29^{b}	-3.18±0.18^{c}	-1.9±0.16^{b}	-4.52±0.25^{d}
b*	12.017±0.39^{d}	28.44±1.48^{ab}	24.57±1.18^{c}	28.32±0.75^{ab}	26.28±0.41^{bc}	30.23±0.60^{a}	28.45±0.52^{ab}

*W: Wheat commercial flour; CP: Commercial pea flour; IP: Irish pea flour non-treated; IP+R: Irish pea flour + roasting; IP+US+R: Irish pea flour + ultrasound + roasting; IP+MW+R: Irish pea flour + microwave + roasting; IP+US+MW+R: Irish pea flour + ultrasound + microwave + roasting. Data presented as means ± standard deviation. Values with a different letter (a-g) are significantly different ($p < 0.05$) along the row.

Product colour is a crucial parameter, playing a significant role in consumer purchasing decisions, as it is often associated with quality, reliability, and overall appeal (Singh, 2024). As these flours are being optimised for use as ingredients in value-added products, their colour is a crucial factor to consider. The measured colour values of the flours, along with their corresponding images, are presented in **Table 4.1**.

As expected, the L* value, representing lightness, indicated that wheat (W) was the lightest among all the flours ($p < 0.05$). The greenish hue of pea flours is reflected in the a* parameter (where negative values indicate green undertones and positive values indicate red). All pea flours exhibited negative a* values; however, the roasted pea flours showed values closer to zero, influenced by chlorophyll degradation and Maillard reactions during roasting, indicating a reduction in greenness compared to the raw samples.

This shift toward a less green tone may improve the visual appeal of the flour, making it more acceptable to consumers, especially in applications where colour uniformity and a neutral appearance are preferred. The b* parameter (blue) and (yellow) was significantly higher for pea flours, indicating their yellow hue.

4.5.2 Particle size

The particle size of flour and its distribution can influence its functional properties, including water absorption capacity and staling behaviour. In addition, it affects the texture, appearance, and overall sensory attributes of the final product (Zeng & Chen, 2018). **Figure 4.1** provides a visualisation of the particle size volume density (%), and **Table 4.A2** (Appendix) reflects the distribution of particle sizes.

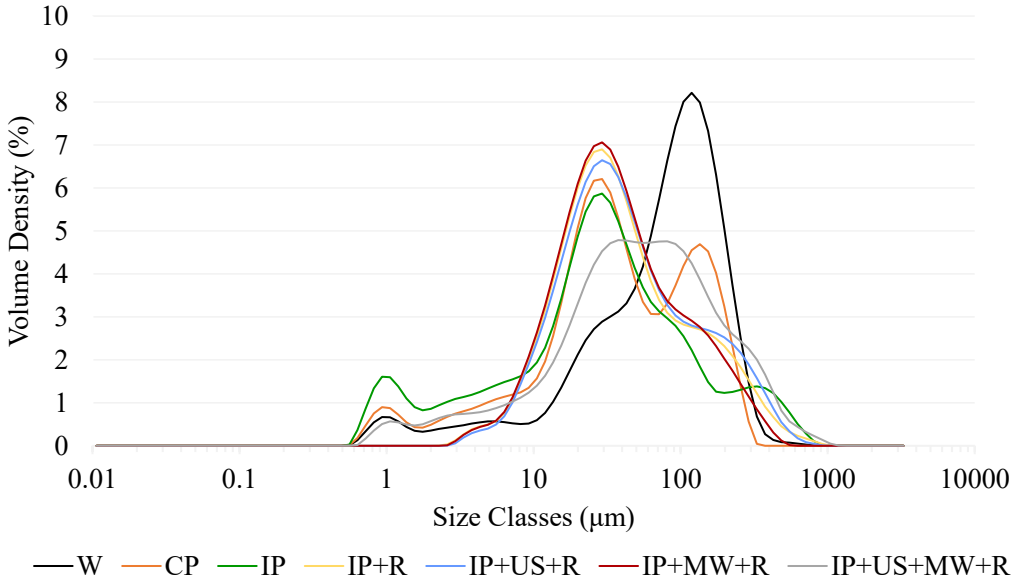


Figure 4.1. Particle size distribution curves of flours. Wheat flour (W) showed the highest peak at a higher size class (≈70-300 µm) compared to all other samples, indicating that it contains coarser particles. This is likely due to the standard milling process used for wheat, in which the endosperm is separated from the bran and germ, unlike the non-standard methods typically applied to pea milling (Dziki et al., 2024). Additionally, the distinct grain morphology between wheat and peas also contributes to these differences. All other samples exhibited higher peaks (volume density percentage) at smaller size classes (~10-100 µm).

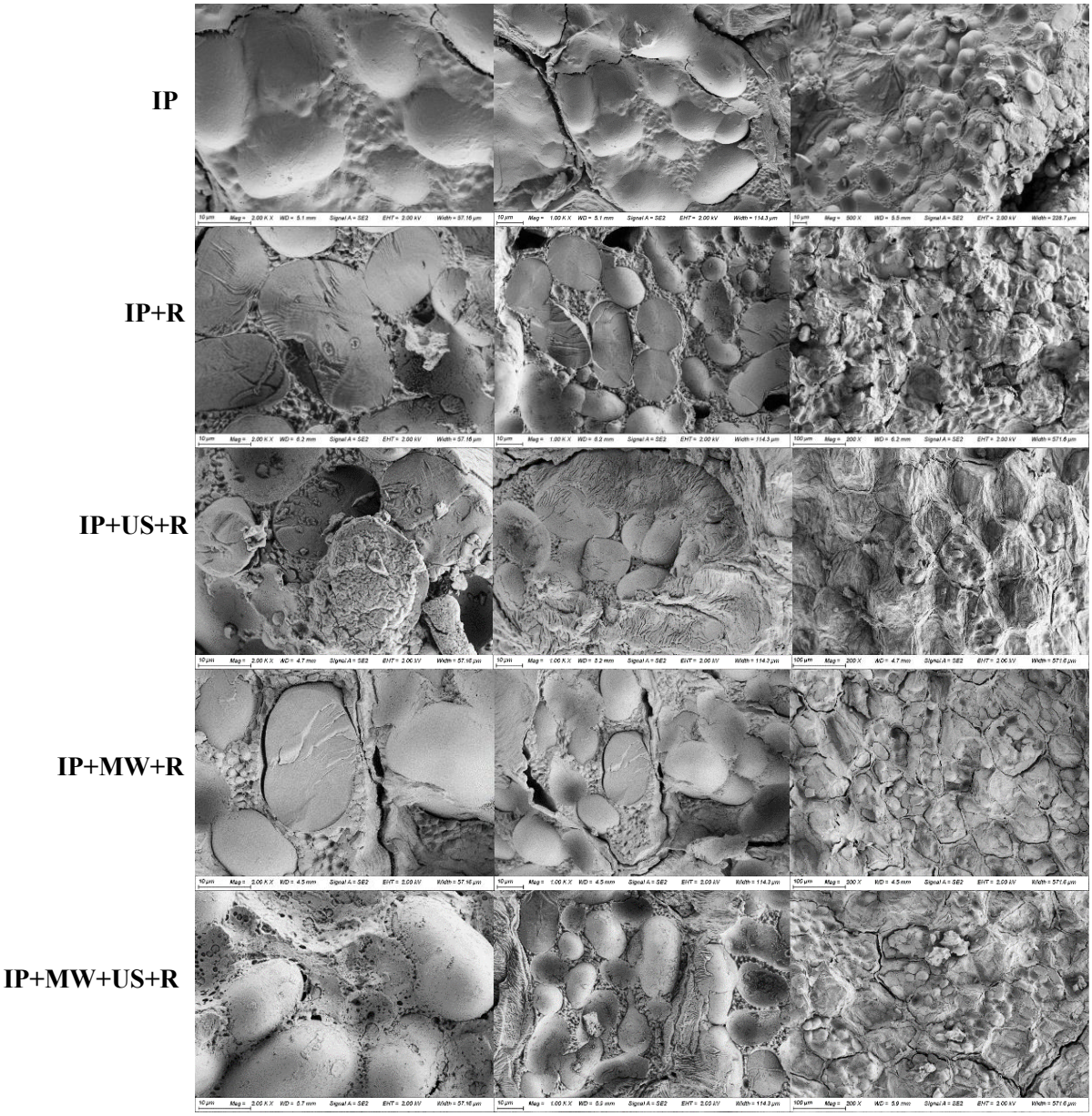
Commercial pea (CP) also showed another relevant peak (≈70-300 µm), similar to wheat. This suggests that CP flour contains both finer and coarser particles which may be attributed to its specific milling technique or pea variety. Additionally, CP exhibited the smallest particle size distribution in Dx90 and Dx99 **Table 4.A2** (Appendix), and is visible in **Figure 4.2 B**, suggesting a particle size distribution in a smaller range. Wheat (W) and commercial pea (CP) contained a higher proportion of larger particle sizes compared to the other samples. However, all other samples, despite having a lower volume density percentage, contained particles up to ≈800 µm, with W and CP having a range of only up to ≈300, showing a broader particle size range.

IP, IP+R, IP+US+R, and IP+MW+R exhibited a similar shape throughout their graphs (Figure 4.1). IP+US+MW+R shows a broader peak but with smaller and more uniform particles due to the synergistic effects of ultrasound and microwave processing, which enhance fragmentation and promote homogeneity.

4.5.3 Microstructural analysis of grains and flour

Figure 4.2 A and B present the SEM of grains and flours, respectively, providing valuable insights into their structural characteristics and properties. The SEM analysis reveals clear differences in the protein matrix organization, starch granule morphology and cell structure across the samples: wheat flour (W), commercial pea flour (CP), Irish pea grain/flour (IP), and Irish pea roasted grain/flour samples.

A) Grains



B) Flours

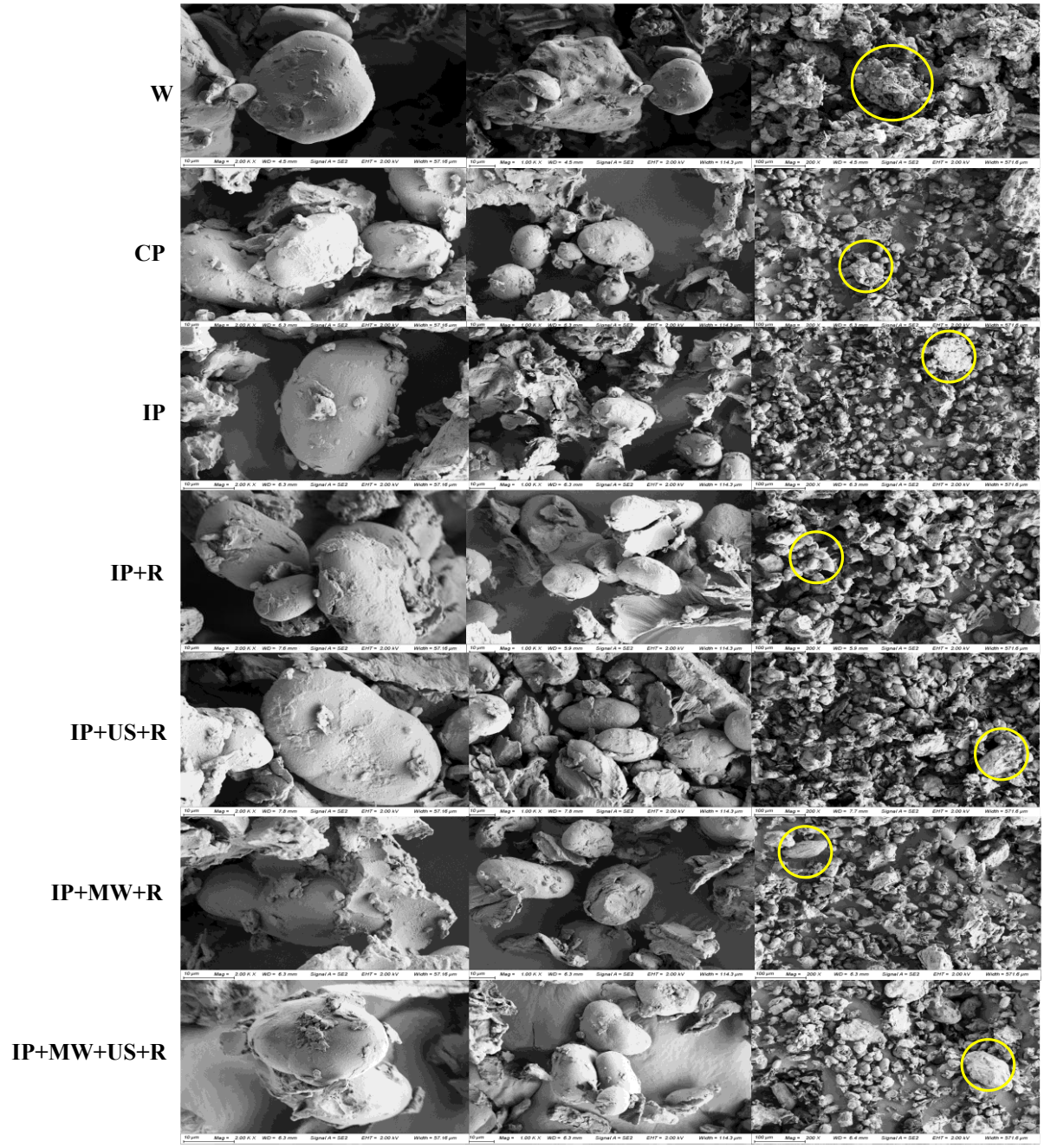


Figure 4.2. Scanning electron micrographs of A) cryo-fractured grains and B) flours.

The non-treated Irish pea grains (IP) (Figure 4.2 A), displayed a denser, more compact matrix with intact, smooth, and round starch granules.

The roasting of Irish pea grains (IP+R) led to a loosening of the cell walls and matrix, resulting in more porous starch surfaces and protein layers becoming visible, likely due to protein denaturation and partial starch pre-gelatinisation. A similar profile was observed for

peas both before heating at 20°C and after heat treatment at 90°C (Pelgrom et al., 2013). The more fractured starch surfaces observed in the roasted samples were due to the cryo-fracturing process used during sample preparation. The roasted starch granules were more fragile and thus more prone to breakage upon cryo-fracturing, while the unroasted ones remained more intact under the same conditions.

Ultrasound roasted grains (IP+US+R) exhibited increased structural loosening compared to IP+R. The cell walls are more pronounced in a hexagonal shape (visible in image (A) IP+US+R 3), and the starch granules appear rougher.

The IP+MW+R sample grains, which combined roasting and microwave treatment, also exhibited significant microstructural alterations compared to the IP+R and IP+US+R. The cell structure appeared strongly fractured and heterogeneous upon cryo-fracturing, unlike the non-treated IP, indicating their increased fragility, with evident starch granules but smoother than IP+US+R. This indicates that microwave treatment promoted the disruption of the pea matrix, resulting in the loosening of starch granules, a structural change that could be advantageous for improving compound extractability.

IP+US+MW+R grains showed the highest degree of microstructural breakdown, characterised by irregular, fragmented/damaged starch granules and a highly porous matrix of components, resulting from cumulative processing effects. The combination of roasting, ultrasound, and microwave treatments likely caused extensive protein denaturation and mechanical disruption, leading to a porous and heterogeneous protein and starch matrix and cracked cell wall. These microstructural changes could influence functional properties, such as enhanced water absorption (as shown in **Table 4.A4**), and improved interaction with other food components. Nutritional aspects may also be affected, with increased digestibility and a potentially altered glycemic response due to greater surface exposure; tests on these effects will be conducted in the next study. Additionally, these structural modifications offer processing

benefits, including the potential for developing tailored textures and improved processability in various food applications (Cheng et al., 2024).

The SEM of the flours are represented in **Figure 4.2 B**, wheat flour displayed small, spherical and oval starch granules embedded within a dense protein matrix, as expected for this type of flour (Ahmed, 2021). All pea flours exhibited smaller starch-protein aggregates compared to wheat flour. Among them, commercial pea (CP) flour and Irish pea flour (IP) showed smoother starch granules than the other processed flours (IP+R, IP+US+R, IP+MW+R, and IP+US+MW+R), though they were still rougher than those observed in the non-treated Irish pea (IP) grains. The increased surface roughness and disruption of the protein matrix in the flours are likely the result of the milling process.

Processed flours displayed more damaged starch granules and greater exposure of the other components (protein regions and fibrous structures) also became apparent in the interstitial spaces. However, these microstructural changes were much more evident in the grain samples than in the flours. This is likely because milling caused a separation of the protein matrix from the starch granules, making it more difficult to observe the individual effects of each processing treatment in the flour samples.

4.5.4 Characterization of protein and starch structures -FTIR

The FTIR spectra in the amide I region (1600–1700 cm^{-1}), associated to stretching vibrations of the C=O bond of the amide, is widely used to assess the secondary structure of proteins (Animesh Singh et al., 2025). Deconvolution of the amide I band (1600–1700 cm^{-1}) enabled identification of specific secondary structures, with absorption peaks assigned as follows: 1610–1627 cm^{-1} for intermolecular β-sheets, 1628–1642 cm^{-1} for β-sheets, 1643–1650 cm^{-1} for random coils, 1650–1659 cm^{-1} for α-helices, and 1660–1699 cm^{-1} for β-turns (Sadat & Joye, 2020). The deconvoluted spectra corresponding to all treatments are shown in Appendix **Figure 4.A1**.

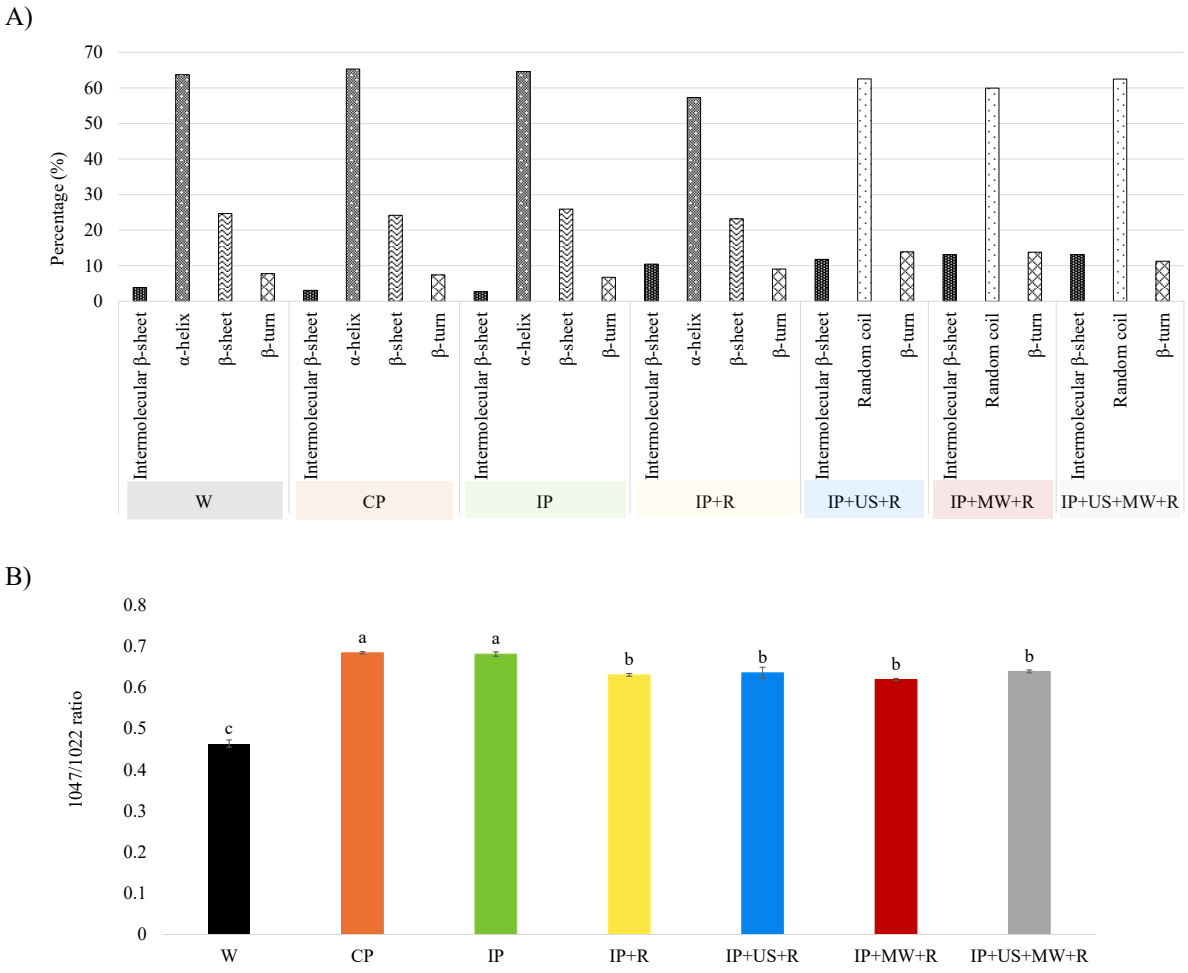


Figure 4.3. FTIR A) protein secondary structure of flours B) absorbance ratio at 1045/1022 cm^{-1} to estimate the relative proportion of crystalline vs. amorphous regions.

The secondary structure of proteins in the pea and wheat flour samples was evaluated through deconvolution of the amide I and is represented on **Figure 4.3 A**. The non-treated samples, commercial wheat (CP) and Irish pea flour (IP) and wheat flour (W) exhibited dominant α-helix structure. This results are consistent with FTIR literature, where native wheat gluten typically exhibits the highest α-helix content (Kłosok et al., 2021) and legumes such as fava bean flours display α-helix dominance (Sengar et al., 2025).

Following roasting treatment on pea flour (IP+R), a reduction in α-helix and β-sheet content was observed, accompanied by an increase in intermolecular β-sheet structures which is associated with protein aggregation, likely due to high-temperature stress (Lao et al., 2023).

More prominent structural alterations were noted in samples treated with ultrasound (IP+US+R), microwave (IP+MW+R), or their combination (IP+US+MW+R). The α-helix and β-sheet structure is not detectable, and an increase in random coil structures and intermolecular β-sheet was observed. This suggests significant disruption of the native protein conformation, likely caused by cavitation (in ultrasound) and thermal effects (in microwave treatment).

Ultrasound processing is a commonly used, environmentally friendly technique for protein modification. The collapse of cavitation bubbles generated by ultrasound creates intense shear forces and vibrations within a localized zone, which can readily disrupt protein structures and induce conformational changes (Qiu et al., 2025). Tian et al. (2020) showed that an increased ultrasound exposure of soy protein isolate hydrolysates significantly reduced α-helix content, indicating notable alterations in protein secondary structure.

Microwave energy excites polar groups within protein molecules, leading to the formation of free radicals and the disruption of stabilizing interactions such as hydrogen bonds and van der Waals forces. This process reduces structural order and compromises protein stability. Moreover, the alternating electric field may interfere with the dipole moment of peptide bonds, resulting in significant degradation of the α-helix structure (Zhang et al., 2023). Zhang et al. (2023) demonstrated that in Spirulina protein, microwave treatment transformed the α-helix content into β-turn and random coil conformations. Sun et al. (2020) decrease in the ordered structures, α-helix and β-sheet, and increase in the random coil for the microwave-treated pigeon pea. This molecular rearrangement is likely affecting water absorption and solubility index functionality, protein stability and interaction with other biopolymers, such as starch and fibre.

Starch structure was assessed through the 1100–900 cm^{-1} region of the FTIR spectrum, which reflects its degree of molecular order particularly in terms of short-range molecular order. The peak around 1045 cm^{-1} is typically linked to the organized, crystalline regions (mainly amylopectin), while the band near 1022 cm^{-1} is related to less ordered, amorphous

areas often associated with amylose (Tiozon Jr et al., 2022). The absorbance ratio at 1045/1022 cm^{-1} was used to estimate the relative proportion of crystalline vs. amorphous regions and is represented in **Figure 4.3 B**.

Wheat flour (W) showed the lowest 1045/1022 ratio ($p < 0.05$), indicating a lower degree of short-range molecular order (lower crystallinity) compared to the pea-based samples. Similar findings were reported by López-Barón et al. (2018), who observed higher absorbance at 1047 cm^{-1} in extruded products containing both pea and wheat, compared to a control sample made solely from wheat.

The non-treated samples, commercial pea flour (CP) and Irish pea flour (IP), exhibited the highest 1045/1022 ratios, indicating a greater degree of molecular order in their starch granules. This is expected, as native starch typically maintains intact granule structures with a higher proportion of crystalline amylopectin chains arranged in double helices.

All samples subjected to roasting (IP+R) and further ultrasound (IP+US+R), microwave (IP+MW+R), or combined treatments (IP+US+MW+R) showed a reduction in the 1045/1022 cm^{-1} ratio, indicating a loss of short-range starch crystallinity. This decrease is likely due to partial gelatinization from roasting (Peng et al., 2025), mechanical shear (ultrasound) (Tiozon Jr et al., 2022) and dielectric heating (microwave) (Lee et al., 2007), which disrupt hydrogen bonds and double-helix structures in amylopectin and may affect starch functionality and digestibility.

4.5.5 Water absorption index (WAI) and water solubility index (WSI)

The water absorption index is the ability of the flours to associate with water molecules, reflecting its capacity to absorb and retain water. The water solubility index measures the quantity of soluble components released from the flour when mixed with water. These are descriptive characteristics used for the processing of legumes and cereals (Schneeweiß, 2018) nd are presented in **Figure 4.4**.

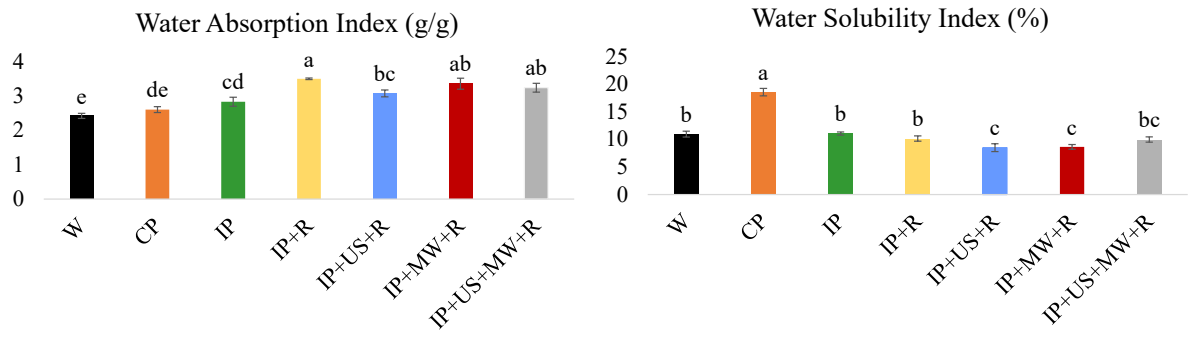


Figure 4.4. Water absorption and water solubility of flours.

Wheat (W) flour has the lowest water absorption index (WAI) ($p < 0.05$), followed by raw pea flours, with roasted pea flours exhibiting the highest WAI. Pea flour has a higher protein and fibre content than wheat flour, which competes with starch for water, contributing to its greater water absorption capacity (B. Dang et al., 2022). In addition, legume proteins tend to exhibit stronger hydrophilic properties, allowing them to bind more effectively with water (K. Kotsiou et al., 2021; Oscar Moreno-Araiza et al., 2023).

Thermal treatments may have led to the formation of pre-gelatinized starch, denaturation of the protein fraction, and/or modifications in the fibre structure, facilitating their interaction with water (Oscar Moreno-Araiza et al., 2023). Additionally, structural changes induced by roasting may have increased porosity and surface area, thus further enhancing water absorption capacity (B. Dang et al., 2022). No significant differences ($p > 0.05$) were observed in the water absorption index (WAI) between roasted flours processed with ultrasound, microwave, or their combination. The higher WAI of the roasted flours can be advantageous when the goal is to enhance the softness, smoothness, and viscosity of food products. (Lapčíková et al., 2021).

Commercial Pea (CP) had the highest WSI and was significantly ($p < 0.05$) different from all other flours. The higher solubility of the CP flour can be attributed to the pea variety, as no treatment was applied and, based on particle size, it does not have finer particles than other flours. This suggests higher solubility of proteins and starches, making it more suitable for applications where solubility is beneficial (e.g., soups, beverages).

Wheat (W), Irish Pea (IP), Irish Pea Roasted (IP+R), and IP+US+MW+R all have similar water solubility index (WSI) values, which are higher than those of IP+US+R and IP+MW+R. The individual application of ultrasound or microwave (IP+US+R, IP+MW+R) may have induced molecular rearrangements, leading to reduced solubility. However, combining ultrasound and microwave (IP+US+MW+R) appears to have disrupted molecular aggregates, partially restoring solubility to levels similar to IP+R.

4.5.6 Flour pasting properties

The flour pasting properties can be affected by various factors, including the flour processing methods, the type and variety of grains used, growing conditions, flour particle size, the extent of starch damage, and the composition of proteins, fibres, and lipids (Nahar et al., 2024; Zhang et al., 2020). **Figure 4.5** and **Table 4.A3** (Appendix) present the pasting curve for each flour. Composite flours (35:65 pea:wheat) were also assessed to better understand starch behaviour when combining both flours, and to explore the potential of these flours for future bakery product applications at this level of incorporation.

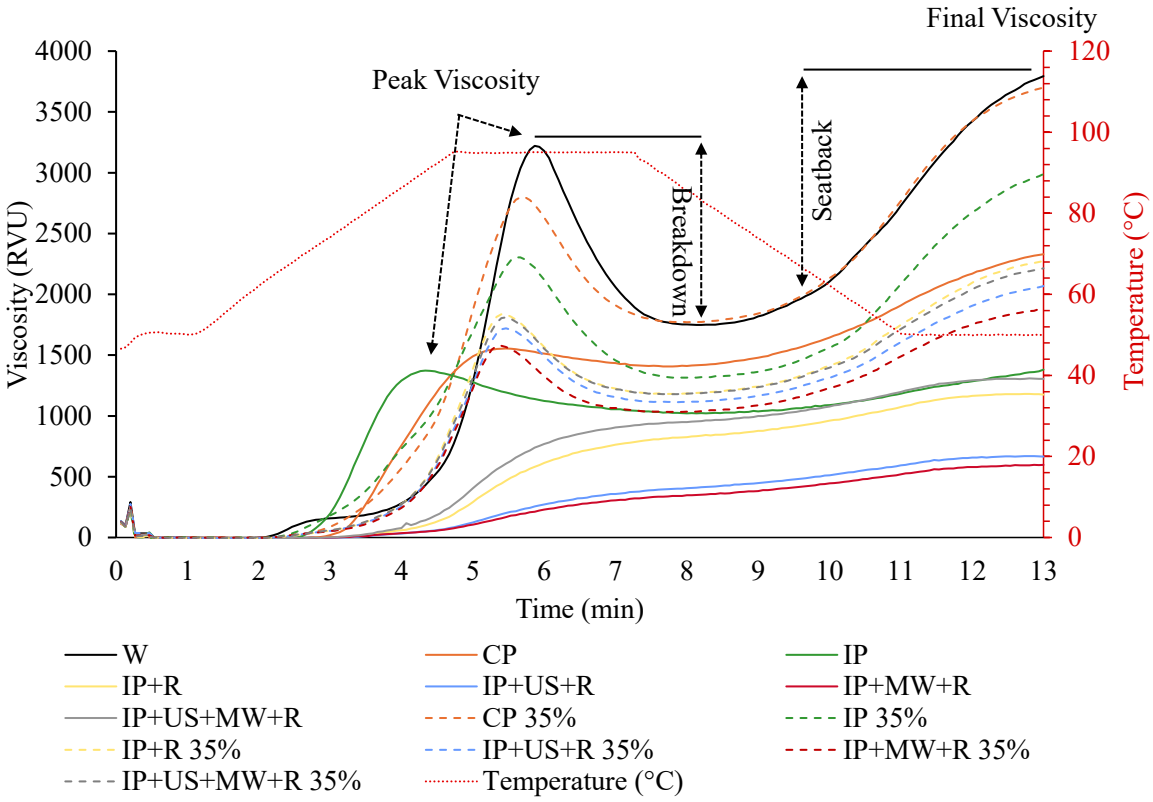


Figure 4.5. RVA starch pasting curves of wheat flour, pea flours and flour blends 35:65 (pea:wheat).

Wheat flour, followed by the pea:wheat flour blends, exhibited the highest pasting profile, showing the curves with the highest viscosities. Next were the non-roasted pea flours, while the roasted pea flours showed the lowest pasting profile. The higher pasting curves observed in wheat flour compared to pea flour can be attributed to its higher starch content and increased gelatinisation ability. In general, pea flour tends to show reduced swelling, likely due to its higher amylose content (24–65%) compared to wheat (18–35%), as well as its higher fibre and lipid content, which can interfere with starch gelatinisation. (Gosa et al., 2023; Maçãs et al., 2023; O. Moreno-Araiza et al., 2023). The higher pasting profile observed in non-roasted pea flours compared to the roasted flours can be attributed to the structural and compositional changes induced by roasting. Heating often induces partial starch gelatinisation/damage and protein denaturation, reducing the availability of intact starch granules for further swelling during pasting, resulting in lower viscosity profiles (Gu et al., 2022). Similar findings were

reported by Oscar Moreno-Araiza et al. (2023) when comparing wheat flour to raw, under-pressure-steamed, and pre-cooked pea flour. The pasting curves followed the same trend, with lower values observed for the under-pressure-steamed and pre-cooked pea flours.

The commercial pea flour (CP) had a higher pasting profile when compared to Irish pea flour (IP), which is likely due to the variety of grains used, growing conditions and milling technique.

The effects of the processing treatments are evident from **Figure 4.5**. The IP+US+R and IP+MW+R, showed lower pasting curves compared to IP+R. However, when both processing techniques are combined (IP+US+MW+R), the pasting curve increases to a level similar to IP+R (**Figure 4.5**). The lower pasting curves observed in IP+US+R and IP+MW+R suggest that ultrasound and microwave treatments when individually applied, can disrupt the starch structure and increase amylose content, leading to a reduction in viscosity (Wang et al., 2020; Zhu, 2015a). However, when combined (IP+US+MW+R), these treatments may promote structural reorganization or enhance starch-protein interactions, restoring pasting properties to levels similar to IP+R by improving gelatinization, a trend also observed by Wang et al. (2020). The pasting curves that include processed flours presented a distinct shape, with lower peaks and flatter curves, indicating less swelling and gelatinisation capacity and more stable viscosity profiles.

Peak viscosity was significantly higher ($p < 0.05$) in wheat-containing flours, followed by non-roasted pea flours, with roasted pea flours exhibiting the lowest values. The higher peak viscosity in wheat and non-roasted pea flours may be attributed to the greater availability of intact starch granules capable of swelling during pasting.

All pea flours displayed reduced to no breakdown ($p < 0.05$) compared to wheat flour. According to Maçãs et al. (2023), the reduced disintegration of swollen starch granules in pea flours, attributed to their higher amylose content compared to wheat flours, suggests improved thermostability under shear stress, leading to minimal breakdown viscosity.

Final viscosity was significantly ($p < 0.05$) higher in wheat-containing flours compared to pea flours, showing the higher ability of wheat starch to reassociate and form a harder gel upon cooling. The lower final viscosity observed in pea flours can be attributed to their higher amylose content (24–65%), which reduces starch retrogradation and limits gel formation. Final viscosity can be an indicator of bread hardness, as a lower final viscosity is often associated with a softer crumb, an essential quality in bread (Buehler, 2021). Therefore, the lower final viscosity observed in roasted flour could potentially result in softer breads compared to those made with non-roasted pea flour. However, final viscosity is just one-factor influencing bread texture. Wheat, even though it has a higher final viscosity, remains the most suitable flour for soft bread due to its gluten network, allowing for a light and airy crumb.

4.5.7 Texture profile analysis (TPA)

The texture of the RVA gel provides valuable information relating to the retrogradation of starch paste 24 hours after the RVA pasting tests are completed. As retrogradation is a key factor in bread staling, minimising its rate is beneficial for bread production (Denardin & Silva, 2009) Gel textural parameters for each sample are shown in **Table 4.2**. Composite flours (35:65 pea:wheat) were also evaluated to better understand retrogradation behaviour, as this parameter is correlated with bread staling, and to explore the potential of the combination of these flours for future bakery applications at this level of incorporation. The firmest gels were formed with the non-treated commercial pea (CP) and Irish pea (IP) ($p < 0.05$), followed by wheat flour ($p < 0.05$), with roasted pea flour producing the softest gels. The same trend was observed when 35% pea flour was incorporated into the wheat flour, whereby raw pea flours (CP and IP) formed the hardest gels, and roasted pea treated the softest. The hardness parameter reflects the peak force that occurs during the first compression of the TPA test.

Focusing specifically on the samples treated with microwave, ultrasound, and their combination, it is evident that after 24 hours of gel formation, these flours (IP+R, IP+US+R,

IP+MW+R, and IP+US+MW+R) follow the same trend as the final viscosity on the RVA chart. IP+R and IP+US+MW+R exhibit higher hardness and final viscosity compared to IP+US+R and IP+MW+R. This is likely due to better starch-protein interactions and enhanced water retention facilitated by the combined effects of ultrasound and microwave.

Adhesiveness, which measures the force required to detach the material from a surface, was highest in wheat, indicating strong stickiness most likely due to its gluten content. Roasting, ultrasound and microwave treated pea samples (IP+US+R, IP+MW+R, and IP+US+MW+R) exhibited significantly lower adhesiveness properties ($p < 0.05$) compared to raw pea samples (IP and CP), most likely due to starch pre-gelatinization during roasting, cavitation and electromagnetic radiation that induced protein denaturation and starch modification.

Wheat, CP and CP 35% showed significantly higher ($p < 0.05$) cohesiveness (the ability of the gel to adhere to itself under compression), indicating stronger internal bonding.

Table 4.2. Texture profile analyses of wheat flour, pea flours and flour blends 35:65 (pea:wheat).

Flours	Hardness (g)	Adhesiveness (g sec)	Springiness	Cohesiveness	Gumminess	Resilience
W	230.57±4.76^{c}	-323.1±73.77^{de}	0.87±0.057^{a}	0.56±0.045^{ab}	128.68±10.5^{b}	0.12±0.006^{ab}
CP	425.9±99.17^{a}	-157.2±89.92^{abc}	0.98±0.01^{a}	0.53±0.031^{abcd}	227.4±48.67^{a}	0.18±0.034^{a}
IP	303.65±40.73^{b}	-131.7±136.3^{abc}	0.95±0.037^{a}	0.43±0.06^{g}	131.14±5.5^{b}	0.12±0.012^{ab}
IP+R	67.85±39.45^{ef}	-112.93±63.4^{abc}	1.29±0.399^{a}	0.46±0.032^{efg}	31.26±22.06^{d}	0.13±0.079^{ab}
IP+US+R	62.3±37.07^{ef}	-81.13±75.4^{a}	0.95±0.046^{a}	0.47±0.016^{defg}	29.37±19.35^{d}	0.05±0.02^{b}
IP+MW+R	60.49±37.64^{f}	-76.02±76.2^{a}	0.93±0.015^{a}	0.49±0.016^{cdefg}	29.75±19.01^{d}	0.06±0.015^{b}
IP+US+MW+R	86.63±28.8^{e}	-101.4±70.2^{ab}	0.93±0.036^{a}	0.43±0.055^{fg}	37.65±19.69^{d}	0.07±0.011^{b}
CP 35%	247.22±99.17^{c}	-306.4±89.92^{de}	0.98±0.01^{a}	0.57±0.031^{a}	141.91±48.67^{b}	0.12±0.034^{ab}
IP 35%	229.7±40.73^{c}	-354.5±136.27^{e}	0.92±0.037^{a}	0.54±0.06^{abc}	123.65±5.5^{b}	0.11±0.012^{ab}
IP+R 35%	139.18±39.45^{d}	-228.15±63.4^{cde}	0.91±0.399^{a}	0.51±0.032^{abcde}	71.45±22.06^{c}	0.09±0.079^{ab}
IP+US+R 35%	129.88±37.07^{d}	-216.2±75.4^{bcd}	0.88±0.046^{a}	0.5±0.016^{bcdefg}	64.64±19.35^{c}	0.09±0.02^{ab}
IP+MW+R 35%	128.2±37.64^{d}	-214.32±76.2^{bcd}	0.91±0.015^{a}	0.5±0.016^{bcdef}	64.03±19.01^{c}	0.09±0.015^{ab}
IP+US+MW+R 35%	138.98±28.8^{d}	-208.8±70.16^{bcd}	0.92±0.036^{a}	0.53±0.055^{abcde}	73.49±19.69^{c}	0.09±0.011^{ab}

*W: Wheat commercial flour; CP: Commercial pea flour; IP: Irish pea flour non-treated; IP+R: Irish pea flour + roasting; IP+US+R: Irish pea flour + ultrasound + roasting; IP+MW+R: Irish pea flour + microwave + roasting; IP+US+MW+R: Irish pea flour + ultrasound + microwave + roasting; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated: wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65). Data presented as means ± standard deviation. Values with a different letter (a-j) are significantly different ($p < 0.05$) along the column.

As expected, gumminess (hardness*cohesiveness) shows a similar trend to hardness, due to the high relative weight of hardness when calculating the gumminess parameter, and measuring the energy required to disintegrate a sample. Commercial pea (CP) exhibited the highest gumminess ($p < 0.05$), consequently the blend containing commercial pea (CP 35%) followed the same trend, indicating more resistant gels. Wheat and Irish pea (IP) showed similar gumminess values. The lowest gumminess values for the IP+R, IP+US+R, and IP+MW+R flours suggest that roasting weakens the gel structure. However, the IP+US+MW+R treated flour showed a slight recovery in gumminess values, reinforcing the idea that using ultrasound and microwave together has a distinct effect on the product compared to applying them separately as separate processes.

4.5.8 Mixolab

Figure 4.6 and Table 4.A4 shows the dough mixing behaviour, including dough hydration, which is essential for the formation and development of the gluten network in the flours, as measured by the Mixolab. Water absorption in the wheat flour (W) sample was the highest (62%) among the non-treated flours, compared to 60.5% for Irish pea flour (IP) and 56.9% for commercial pea flour (CP). Notably, all samples subjected to roasting showed a substantial increase in water absorption, reaching approximately 69%, indicating that thermal treatment significantly enhances the hydration capacity of flour. The thermal degradation of native structures during roasting transforms pea flour into a more open and reactive matrix, capable of binding significantly more water. This effect is also evident in the SEM images (Figure 4.2 A), where the processed grains exhibit more porous structures compared to the non-treated samples, as well as in the WAI results.

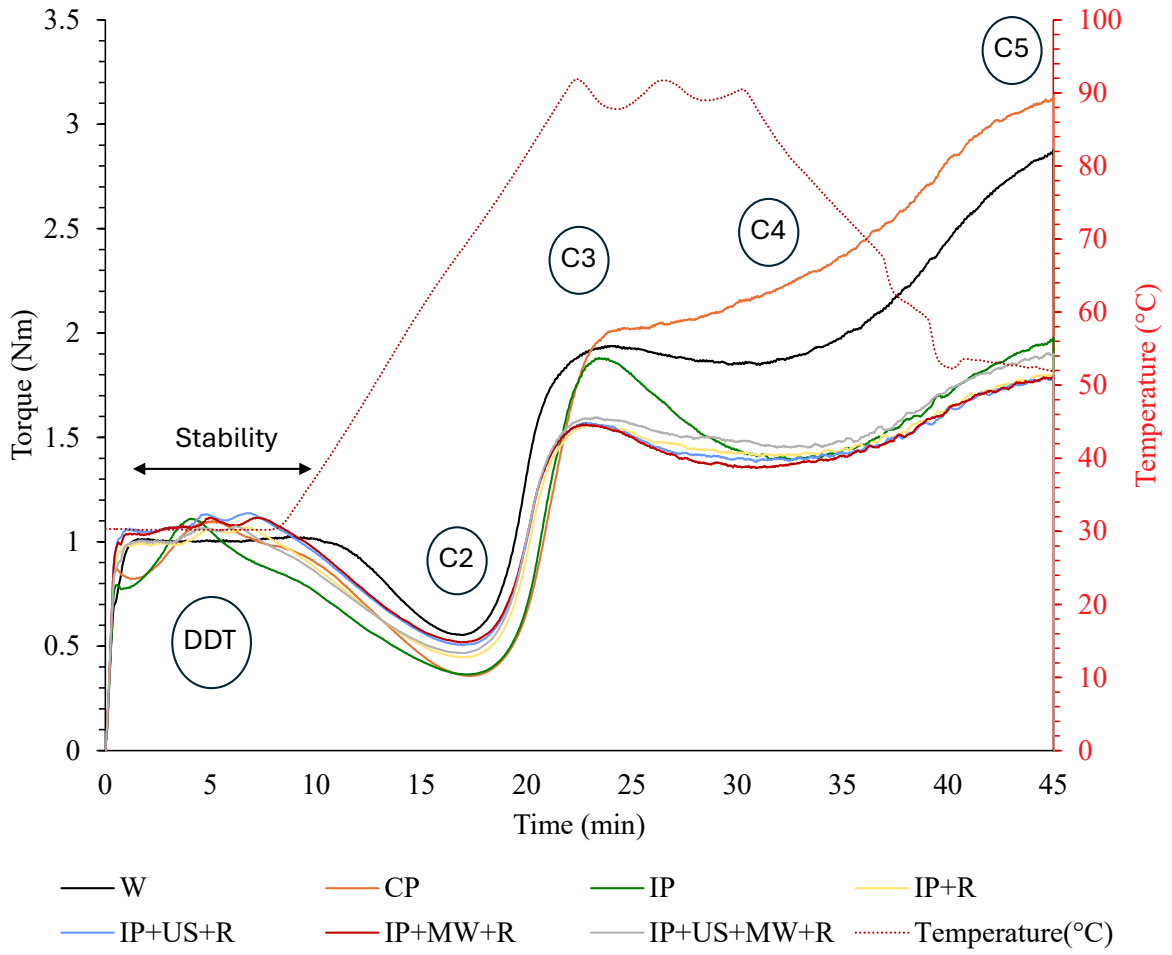


Figure 4.6. Mixolab dough curves of wheat flour and pea flour blends 35:65 (pea:wheat), using the Chopin + test.

The resistance to mechanical mixing of the bread dough can be effectively evaluated through key parameters such as dough development time (DDT), dough stability, and C2, which reflects protein weakening. The flour blends exhibited significantly lower values ($p < 0.05$) for dough stability, DDT, and C2 compared to wheat flour (W). This reduction is expected, as gluten is primarily responsible for dough strength and elasticity, and its functionality is diluted upon the incorporation of pea flour. Additionally, the higher fibre content in pea flours can disrupt the structure of the starch-gluten matrix (Sadowska et al., 2003). However, a significant increase in dough stability ($p < 0.05$) was observed in all samples subjected to roasting, with the effect becoming more pronounced in the IP+MW+R sample, where roasting was combined with microwave treatment. Stability is a critical parameter, as it indicates the resistance of the dough to overmixing and protein weakening. Higher stability

implies a dough that is easier to handle and more tolerant to mechanical mixing. Regarding dough development time (DDT), all the flours that underwent roasting showed values more similar to wheat flour, suggesting that roasting helps align the dough development behaviour of pea-based flours with that of traditional wheat. Protein weakening (C2) values were reassembling to those of wheat flour across all roasted samples, with IP+US+R and IP+MW+R+US showing the closest resemblance, indicating improved thermal and shear stability.

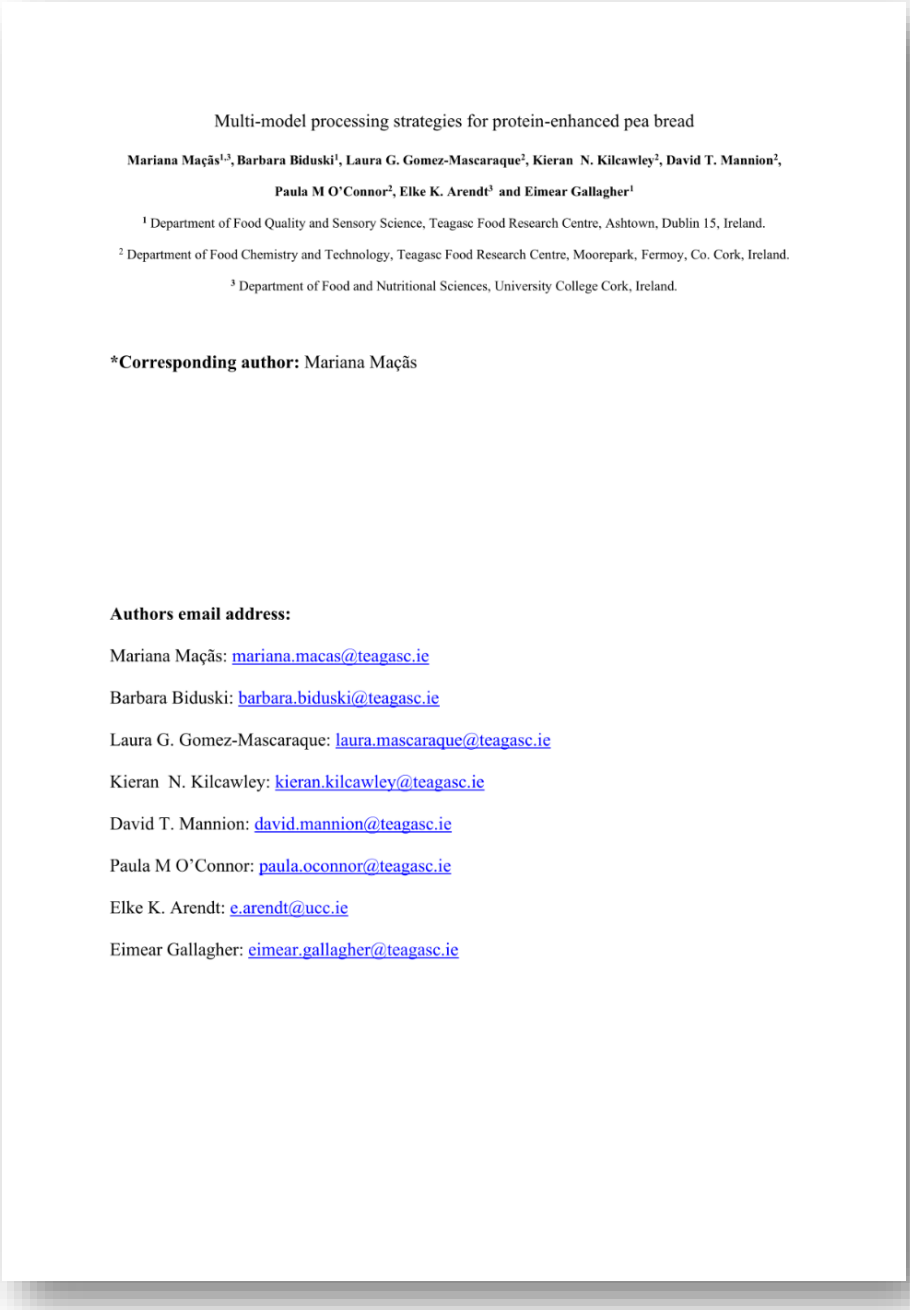
C3 values indicate the extent of starch gelatinisation, the C4 parameter relates to the stability of the starch gel under heat, and C5 values correspond to the behaviour of the starch during the cooling phase. The trends observed in C3, C4, and C5 values are consistent with those obtained from the RVA profiles. Wheat flour (W) and blends containing non-treated pea flours (IP and CP) exhibited higher ($p < 0.05$) gelatinization levels (C3). This trend is consistent with the observations shown in **Figure 4.5** and can be explained by partial starch gelatinization and protein denaturation, which reduce the number of intact starch granules available for further swelling during the gelatinization process. The high C5 value ($p < 0.05$) observed for the commercial pea (CP) may indicate a greater tendency for starch retrogradation, which is often associated with firmer or harder bread. This suggests that CP flour may not be ideal for baking applications where a softer texture is desired.

4.6 Conclusion

This study confirmed that roasting, ultrasound, and microwave treatments, applied individually and in combination, affected the structural, functional of Irish-grown pea flour. Distinct functional and structural properties exist between wheat and pea flours. Additionally, the growing conditions of pea had a significant effect ($p < 0.05$) on flour functionality. Commercial pea (CP) flour showed higher water solubility, pasting viscosity, and gel hardness than Irish-grown pea (IP), highlighting the influence of cultivar and origin on flour

performance. Roasting was found to have the greatest impact on protein denaturation, starch crystallinity and gelatinisation properties, and water absorption capacity. FTIR analysis, on roasted samples confirmed a decrease in starch crystallinity, alongside a transition in protein secondary structure from α-helices and β-sheets to more disordered random coil conformations. FTIR did not reveal significant differences between the samples treated with ultrasound and microwave previous to roasting, probably likely the structural changes were minor and not detectable by this technique. In terms of functionality, IP+US+R and IP+MW+R had lower pasting profiles than roasted flour alone, but the combination (IP+US+MW+R) restored peak viscosity to levels similar to IP+R, likely due to enhanced starch-protein interactions. Gel texture results confirmed this trend, with higher gel hardness and final viscosity in IP+R and IP+US+MW+R, suggesting improved water retention and structure formation. The effects of ultrasound and microwave treatments were most evident when examining the SEM images of the grains, where IP+US+MW+R grains showed the highest degree of microstructural breakdown. The effects on pasting and dough behaviour when studying the flour blends for future bread applications were complex and treatment-dependent, suggesting that the choice of processing method should be tailored to the specific food application. Overall, the integration of these clean-label technologies shows promising potential for optimising the use of pea flour in bread formulations.

5 Multi-model processing strategies for protein-enhanced pea bread



Multi-model processing strategies for protein-enhanced pea bread

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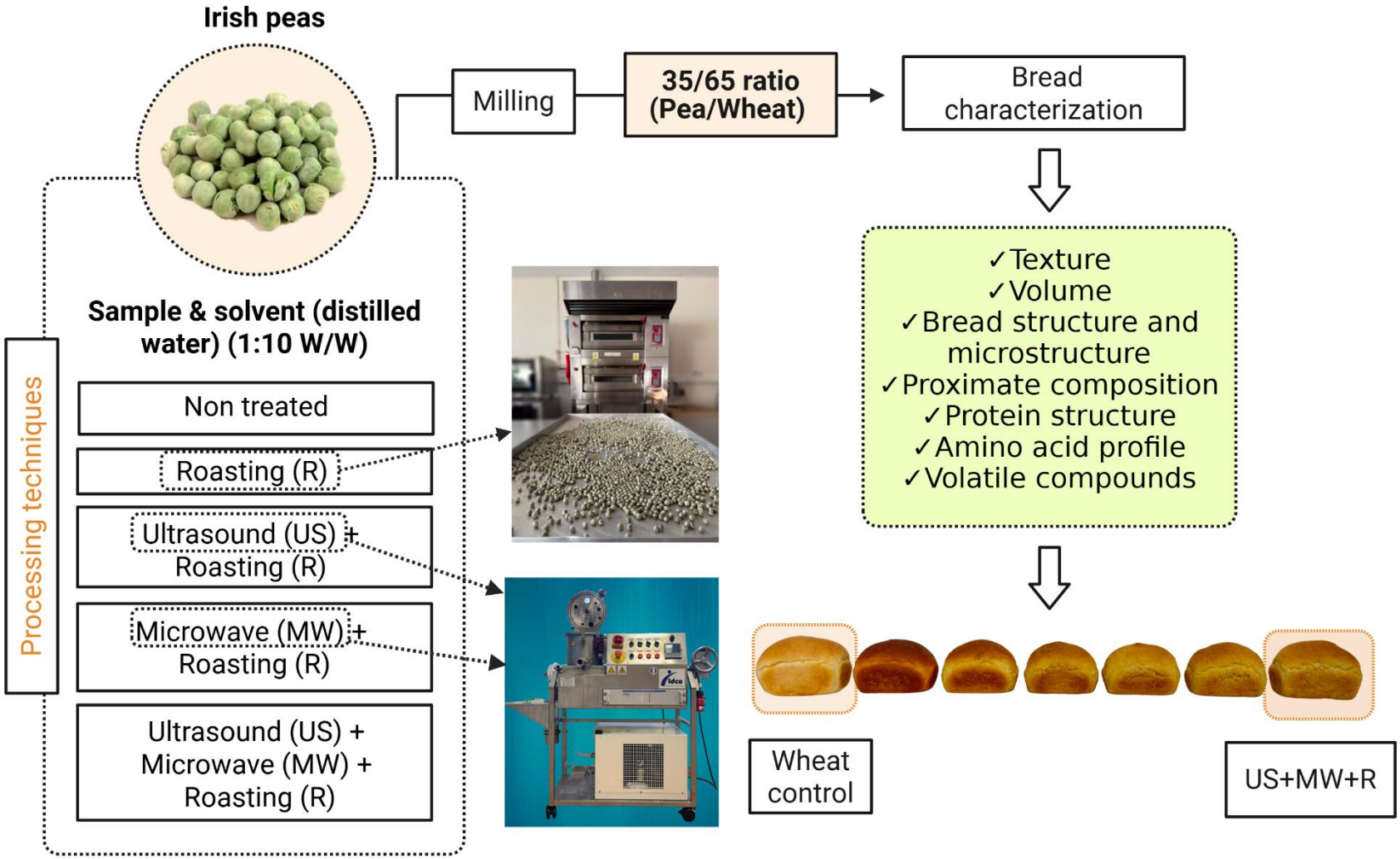
5.1 Graphical abstract



5.2 Abstract

Incorporating peas into bread formulations presents environmental, nutritional, and economic benefits. Optimizing the processing and functional properties of pea flour is crucial for consumer acceptability, yet it remains relatively underexplored. This study investigated multi-model processing strategies, roasting (R), ultrasound (US), and microwave (MW), to increase the incorporation of 35% pea flour, (Irish pea (IP) and commercially available pea (CP)) in wheat bread. The addition of pea flour significantly improved the nutritional profile, particularly protein and fibre content. The roasting process on Irish pea bread (IP+R) increased water absorption and helped mitigate off flavours. Among all treatments, the combination of roasting, US and MW (IP+US+MW+R) resulted in a bread with higher specific volume, softer crumb texture, and a more balanced, complex flavour profile. Non-treated Irish pea bread (IP) had better nutritive perspectives but lower loaf volume compared to commercial pea bread (CP). Pea growing location and milling strongly affected bread quality. These findings highlight the potential of combining processing technologies to improve the structural and aroma properties of legumes-enriched breads.

5.3 Introduction

The continued emphasis on health and environmental sustainability is reshaping global food preferences, with a clear shift toward more plant-based diets (Fuentes & Fuentes, 2022; Venter de Villiers et al., 2024). This trend is driving innovation in the development of nutrient-enriched products, particularly baked goods fortified with plant-derived proteins and fibres (Biduski et al., 2024; Singh et al., 2025). Pulses such as peas, beans, chickpeas and lupin are among the most extensively studied ingredients for their nutritional potential and functional benefits in reformulated foods (Asif et al., 2013; Maçãs et al., 2024b; Sozer et al., 2017).

Peas, are a valuable source of essential vitamins, minerals, protein and dietary fibre (Binti Mohd Zaini et al., 2021). Their ability to improve soil fertility and fix atmospheric nitrogen makes them a sustainable crop choice, helping to boost yields and farm profitability (Cusworth et al., 2021; Uebersax et al., 2023). Peas are a cost-effective ingredient that can improve the nutritional value of bread and support sustainable agriculture. As bread is a widely consumed staple, there are ongoing opportunities for legume inclusion, creating high-protein products with a balanced amino acid profile (Kaiser et al., 2019).

Despite their benefits, when used in bakery applications such as bread, peas interfere with the gluten network and dough matrix, resulting in functional limitations (e.g. harder texture, less aerated volume) (Young et al., 2019). Their presence may also cause off flavours. Therefore, while promising, their use in bakery formulations still requires improvements for optimized product functionality and consumer acceptance (Hall, 2021a). To address this, various processing methods have been explored to enhance techno-functional properties of pulse-enriched breads (Bourré et al., 2019; Guldiken et al., 2022; K. A. Millar et al., 2019; Nilufer-Erdil et al., 2012). However, this area still remains under research, especially in understanding how different processing strategies, alone or in combination, influence the structural, functional, sensory and nutritional properties of pulse-enriched breads.

Traditional methods such as roasting, fermentation and germination of grains have been effective in improving their taste, reducing antinutrients and enhancing their nutritional properties (Kahraman et al., 2022; Setia et al., 2020a; Villacrés et al., 2020; Xing et al., 2021). More recently, emerging technologies such as ultrasound, microwave and pulsed electric field technology have shown promise in improving solubility, emulsification, and pasting behaviour (Maçãs et al., 2024b).

The roasting process applies dry heat, which induces Maillard reactions, protein denaturation and moisture reduction in food (Young et al., 2020). Roasting increases the variety of aromatic compounds and can result in an appealing flavour for peas. The process can alter protein structure, enhance starch digestibility and reduce antinutritional factors (van Rooyen et al., 2022; Young et al., 2019). Previous studies have shown that incorporating roasted pulse flours can improve bread quality. Kim Adrienne Millar et al. (2019) reported increased loaf volume and reduced crumb hardness when comparing bread containing toasted pea flour to that made with non-toasted pea flour. Similarly, Kahraman et al. (2022) observed that roasted chickpea flour led to better volume and texture in gluten-free bread. These improvements are likely due to protein denaturation and partial starch gelatinization during roasting, which enhance gas retention and crumb structure.

Ultrasound produces cavitation (microscopic bubbles that rapidly form and collapse) through the application of low-frequency, high-intensity sound waves (Amanda Gomes Almeida Sá et al., 2022; Singh et al., 2025). Ultrasound technology significantly affects both the physicochemical (e.g., molecular mass, particle size, zeta potential) and functional properties (e.g., solubility, foaming, emulsifying) of food. It's application in foods has also been shown to influence protein structure, amino acid composition, digestibility, and reduce anti-nutritional factors (Rivera et al., 2025; Amanda Gomes Almeida Sá et al., 2022).

Microwave treatment relies on non-ionizing electromagnetic waves that generate energy through mechanisms such as ionic conduction and dipolar rotation. The effectiveness of this

heating regime depends on factors such as the dielectric properties of the material and the depth of wave penetration (Rivera et al., 2025; Amanda Gomes Almeida Sá et al., 2022). Microwave processing can improve the nutritional value of food by changing starch structure and digestibility, as well as influencing its pasting behaviour (Deng et al., 2022a). According to Wanyu Qin et al. (2022), applying microwave or ultrasound treatments to semi-dry milled rice flour before incorporating it into bread formulations led to higher specific volume than using untreated flour. This improvement was attributed to modifications in the structure and particle size of the flour.

This study explores how the application of roasting, ultrasound and microwave technologies, applied individually and in combination to Irish pea grains, affect the techno-functional and nutritional properties of the resulting bread, an approach that remains largely unexplored in current literature.

5.4 Materials and methods

5.4.1 Raw material, grain processing and bread-making

Raw materials

Irish-grown pea grains (variety Carrington) were provided by Wynnes Ltd (Kildare, Ireland) and a commercially available pea flour, of Hungarian origin (supplied by Mills & Folks, UK), were utilized for comparative purposes. Strong wheat flour (Shakleton's Milling, Ireland) was used in the wheat bread control and blends to assess bread properties.

Pea treatments:

Irish-grown pea grains were treated through ultrasound, microwave and a combined ultrasound-microwave method, utilising an E200 extractor (Idco SAS, Marseille, France), which includes a cooling mechanism to keep the sample temperature under 30°C. Due to the

scarcity of available studies on the topic, multiple preliminary trials (data not shown) were carried out to establish suitable parameters for the final experimental design.

Each treatment involved processing 1 kg of Irish-grown pea grains combined with 2 L of tap water using the E200 system. The microwave was operated at 1000 W. The ultrasound was applied at a 100% amplitude level (200 W) and 25 kHz frequency. The ultrasound treatment lasted for 12 minutes. The microwave and the combined ultrasound-microwave treatment were conducted over a total of 12 minutes, divided into four 3-minute cycles, with 5-minute cooling breaks between each cycle to avoid overheating. Following these treatments, each sample was then roasted at 170 °C for 16 minutes using a deck oven (MacPan, Thienne, Italy). As roasting represents an additional processing step, a separate roasting study was also carried out to evaluate its individual impact. Irish pea grains subjected only to roasting were soaked in water for 30 minutes prior to roasting, to ensure consistency with the other treatments (microwave and ultrasound), which were carried out in water.

Pea milling and flour blend preparation:

After treatments, the whole Irish peas were ground using a hammer mill (Labmill 3100, Perten®, Sweden), following the optimized procedure described by Maçãs et al. (2023) **(Chapter 2)**. The flours were blended in a 35:65 ratio of pea to wheat flour for bread-making. Control samples included commercial wheat flour, commercial pea flour and untreated Irish pea flour.

Bread formulations:

Details of the bread formulations, including abbreviations used in this study, are presented in **Table 5.1**. Breads were prepared using either wheat flour (as control) or pea:wheat blended flour, along with salt (2%), unsalted butter (1%) (both purchased locally), improver (1%), yeast (1.5%) (McDougall's, UK), and water, with water addition adjusted according to absorption

values determined by the Mixolab analysis. The bread-making process and equipment followed the methodology described by (Maçãs et al., 2025).

Table 5.1 Abbreviations of bread formulations used in this study.

Abbreviation	Bread Formulations
W	Strong wheat flour
CP	Commercial pea flour: wheat flour (35:65)
IP	Irish pea flour: wheat flour (35:65)
IP+R	Irish pea flour + roasting: wheat flour (35:65)
IP+US+R	Irish pea flour + ultrasound + roasting: wheat flour (35:65)
IP+MW+R	Irish pea flour + microwave + roasting: wheat flour (35:65)
IP+US+MW+R	Irish pea flour +ultrasound + microwave + roasting: wheat flour (35:65)

5.4.2 Crumb confocal microscopy

Cubes (approximately 2 mm in dimension) were excised from the bread samples using a blade and fixed in a solution of 2.5% (w/w) glutaraldehyde in 80% aqueous ethanol. The samples were then dehydrated and embedded in activated LR White Acrylic Resin, following the procedure outlined by Fitzgerald et al. (2014). Thin sections, 5 μm thick, were obtained from the resin blocks using a Leica EM UC7 ultramicrotome (Leica Microsystems, Mannheim, Germany), mounted onto microscope slides, and triple-stained with 0.1% (w/w) fluorescein isothiocyanate (FITC) in ethanol, Calcofluor White (1:20 aqueous), and 0.1% (w/w) Fast Green FCF in water. The staining solutions were applied in sequence by placing a drop of each dye onto the sections, allowing them to react for 10 seconds, and then gently removing the excess with tissue paper. The stained sections were mounted with 0.13 mm glass coverslips and visualised using a Leica TCS SP5 confocal laser scanning microscope (Leica Microsystems CMS GmbH, Wetzlar, Germany) fitted with a 63×/1.4 oil immersion objective. Calcofluor White was excited using a 405 nm diode laser, FITC with a 488 nm Argon laser, and Fast Green FCF with a 633 nm Helium-Neon (He/Ne) laser. The corresponding emission filters were set to 415–470 nm, 525–593 nm, and 650–705 nm, respectively. Digital images with a resolution of 1024 × 1024 pixels were captured using Leica LAS AF software (version 2.7.3.9723).

5.4.3 Bread proximate composition

The proximate composition of the bread samples was evaluated. Protein content was measured using LECO equipment (FP-628, LECO Corp., USA) applying a nitrogen-to-protein conversion factor of 5.7, by AOAC method 968.06 (AOAC, 2000b). Fat content was determined using the ORACLE Rapid NMR Fat Analyser (CEM Corp., USA) following the AOAC (2005) guidelines. Total dietary fibre (TDF), including both insoluble (IDF) and soluble dietary fibre (SDF), was measured using the Ankom automated dietary fibre analyser (Ankom, USA) and the enzymatic-gravimetric method, according to the AOAC protocol 991.43 (AOAC, 1994). Ash content was measured using a Gallenkamp Muffle Furnace (Gallenkamp, UK), following pre-drying overnight at 550 °C, in accordance with AOAC method 08–01.01 (AOAC, 2000a). Total carbohydrate content (%) was calculated by difference, subtracting the combined percentages of fat, protein, ash (dry basis) from 100%. Total starch content was estimated by subtracting the total dietary fibre (TDF) from the total carbohydrate content (Charrondiere et al., 2004). Crumb moisture was determined using a two-stage procedure in accordance with AOAC method 44-15.02 (AACCI, 2010c). Water activity (Aw) was measured using an Aqua Lab Lite device (Decagon Devices, WA, USA). Moisture content and water activity were undertaken on days 1, 3 and 7 post baking.

5.4.4 Amino acid profile

Amino acid analysis was performed on all bread samples, initially using microwave-assisted acid hydrolysis, based on the method described by Moore and Stein (1963). Following this, the amino acid profile was quantified through ion exchange chromatography with post-column derivatisation using ninhydrin. Before hydrolysis, cysteine and methionine were oxidized into cysteic acid and methionine sulfone, respectively, by treating the samples with performic acid and phenol (9:1 formic acid: 30% hydrogen peroxide containing phenol). The

samples were then hydrolysed in 6 M HCl at 160°C for 15 minutes using a Discover 2.0 microwave synthesizer (CEM, North Carolina, USA). Post-hydrolysis, the pH of the samples was adjusted to 2.2 on ice using 7.5 N NaOH, and the volume was adjusted to 20 ml with 0.2 M sodium citrate buffer (pH 2.2). Samples were then diluted with the internal standard, norleucine, to achieve a final concentration of 125 nm/ml. Amino acids were quantified using an amino acid analyser (Jeol JLC-500/V, UK) with a Jeol Na+ high-performance cation exchange column.

5.4.5 Bread properties

The specific volume and density of the loaves were determined using the VolScan Profiler (VSP600C, Stable Micro System, UK) following AACCI method 10-16.01 (AACCI, 2010a).

Crust and crumb colour were measured with a colourimeter (CR-410, Konica Minolta, UK), with colour values recorded and expressed using the L*(0: black, 100: white), a* (-green, +red), and b* (-negative, +yellow) scales.

Three loaves were sliced into 1 cm thick pieces, with the two central slices of each loaf selected for digital image analysis and texture profile analysis (TPA). The internal structure was assessed using the C-Cell Bread Imaging System (Calibre Instruments Ltd, UK). Crumb texture profile was evaluated with a texture analyser (TA-XT2i, Stable Microsystems, UK) using a 20 mm cylindrical probe at a test speed of 1 mm/sec. The centre slices were compressed to 50% of their original height. Texture analysis was conducted on days 1, 3, and 7 post-baking. To compare treatments with different initial hardness values, the staling rate was expressed as the relative percentage change in hardness per day, calculated as:

$$(3) \quad R(\%) = \frac{H2-H1}{H1} \times \frac{100}{t2-t1}$$

- $H1$ = hardness at the initial time (e.g., day 1)

- *H2* = hardness at the later time (e.g., day 3 or 7),
- *t2−t1* = number of days between measurements

5.4.6 Volatile compounds

Volatile analysis was performed using solid phase micro-extraction (SPME) and was automated using a Gerstel MPS Autosampler (Gerstel AG, Switzerland) equipped with a heated agitator and SPME bakeout station. Bread crumb was prepared by adding an internal standard (100 µL) to 4 g of material (5 ppm) in a 20 mL amber headspace vial and capped using a magnetic screw cap with blue silicone/white PTFE. The vial was equilibrated at 40 °C for 10 minutes with 5-second agitation pulses at 500 rpm. A 50/30 µm DVB/CAR/PDMS (Carboxen/ divinylbenzene/ polydimethylsiloxane) SPME fibre (Agilent Technologies Ltd, Cork, Ireland) was used to extract volatiles by exposing it to the sample headspace (1 cm depth) at 40 °C for 20 minutes. After extraction, the fibre was inserted into the GC inlet and desorbed at 250 °C for 2 minutes. The SPME fibre was conditioned in between runs for 3 min at 270 °C to eliminate carry-over between sample injections. Volatile compounds were analysed using a Shimadzu 2010 Plus GC (Mason Technology Ltd, Dublin, Ireland) equipped with a DB5-MS column (60 m × 0.25 mm × 0.25 µm) (Agilent Technologies Ltd, Cork, Ireland) and a split/splitless injector operated in splitless mode, with a Merlin microseal septum. The oven temperature started at 35 °C, ramped at 6.5 °C/min to 230 °C (held for 5 min), then at 15 °C/min to 260 °C, held for 15 min, resulting in a 52-minute run. Helium was used as the carrier gas at a constant flow rate of 1.2 mL/min. Detection was performed with a Shimadzu TQ8030 mass spectrometer (Mason Technology Ltd, Dublin, Ireland) operating in single quadrupole mode. The ion source and interface temperatures were set at 220 °C and 260 °C, respectively. Electron ionisation at 70 eV was applied, with mass scanning from 35 to 250 amu. Compound identification was based on mass spectral matching with the NIST 2014 library (Mason Technology Ltd, Dublin, Ireland), the Shimadzu FFNSC 2 flavour and fragrance database

(Mason Technology Ltd, Dublin, Ireland), and an in-house library of authentic standards, using target/qualifier ions and linear retention indices calculated via the Kovats index as per van Den Dool and Dec. Kratz (1963). AMDIS (Automated Mass Spectral Deconvolution and Identification System; www.amdis.net) was used for spectral deconvolution, and when reference linear retention indices or standards were unavailable, identification relied on spectral matches from the NIST library and was considered tentative.

5.5 Results and discussion

5.5.1 Bread proximate composition and amino acid profile

The proximate composition, presented in **Table 5.2**, shows that the inclusion of pea flour led to significant differences ($p < 0.05$) compared to the wheat control, with all enriched formulations displaying higher protein, ash, fat, and fibre contents.

Table 5.2. Proximate composition and moisture of bread samples.

Bread	W	CP	IP	IP+R	IP+US+R	IP+MW+R	IP+US+MW+R
Ash	2.79±0.19^{b}	3.51±0.15^{a}	3.49±0.13^{a}	3.71±0.10^{a}	3.61±0.10^{a}	3.63±0.10^{a}	3.6±0.11^{a}
Fat	1.49±0.23^{b}	1.97±0.31^{ab}	1.76±0.39^{ab}	2.16±0.10^{a}	1.89±0.36^{ab}	2.02±0.24^{a}	1.82±0.31^{ab}
Protein	15.14±0.18^{c}	17.38±0.06^{b}	17.5±0.10^{ab}	17.66±0.06^{a}	17.64±0.10^{a}	17.62±0.07^{a}	17.61±0.21^{a}
IDF	1.55	4.4	8.7	7.8	7.3	7.8	7.3
SDF	4.75	4.55	6	5.35	5	4.4	4.5
TDF	6.3	8.95	14.7	13.15	12.3	12.2	11.8
Starch	74.28	68.19	62.55	63.32	64.56	64.53	65.17
Moisture	43.49±0.55^{b}	42.07±1.06^{b}	43.79±1.0^{2b}	49.81±1.26^{a}	49.63±2.16^{a}	49.95±0.78^{a}	49.05±1.79^{a}

*W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65). Data presented as means ± standard deviation. Values with a different letter (a-c) are significantly different ($p < 0.05$) along the row.

Bread made with untreated Irish pea flour (IP) showed higher protein (but non-significant) content compared to that formulated with commercial pea flour (CP). This difference became significant ($p<0.05$) following processing of the Irish pea flour. This may be attributed to structural changes that disrupt protein-starch and protein-fibre interactions, thereby increasing the availability of proteins in the final product. (Mahalaxmi et al., 2022; Xu et al., 2025)

The ash content shows that the substitution of wheat flour with pea flour significantly ($p<0.05$) enhanced the mineral profile of the breads, in line with previous studies that highlight legumes as mineral-rich ingredients (e.g., calcium, iron, potassium) (Olanipekun et al., 2018; Roy et al., 2010). The treatments did not significantly change the ash content of the pea breads. Bread containing treated pea flours (roasting, US and MW) showed significantly higher moisture levels (≈49–50%) compared to that of the wheat control and other untreated formulations (≈ 42–43%). This is a consequence of the processing methods applied to the Irish pea flour, particularly roasting, which can increase its water absorption capacity. This effect is likely due to protein denaturation and partial starch gelatinisation during roasting, which may increase porosity and surface area, thereby enhancing the water absorption capacity of the flours (Bin Dang et al., 2022; Oscar Moreno-Araiza et al., 2023).

The combination of cereals with pulses enhances the overall protein quality by compensating for each other's amino acid deficiencies, resulting in a nutritionally complete amino acid profile (Maçãs et al., 2025). The amino acid profile analysis (**Table 5.3**) reveals the differences that were found between wheat-based bread and bread containing pea flour. The incorporation of the legume flours notably enhanced the presence of essential amino acid (EAAs) contents, particularly in leucine, histidine, lysine and arginine. Cereals commonly lack sufficient levels of certain essential amino acids, with lysine, threonine, and leucine being the most frequently limiting (Kowalski et al., 2022). The incorporation of pea flour improves the levels of all three of these amino acids, thereby enhancing the breads overall protein quality.

Table 5.3. Total amino acid profile of bread samples.

Breads	W	CP	IP	IP+R	IP+US +R	IP+MW +R	IP+US +MW+R
Essential Amino Acids (g kg^{-1} of sample)							
Threonine	3.516	4.633	4.666	4.670	4.800	4.729	4.520
Valine	6.197	7.155	7.253	7.225	7.388	7.212	6.994
Isoleucine	4.086	5.506	5.473	5.522	5.576	5.511	5.283
Leucine	8.488	10.854	10.578	10.667	10.785	10.656	10.293
Phenylalanine	6.794	7.549	7.264	7.334	6.644	7.282	6.106
Histidine	4.766	6.138	6.444	6.379	6.102	6.540	7.334
Lysine	6.661	8.561	8.854	9.137	9.169	9.251	9.178
Arginine	5.266	8.027	8.203	8.447	8.485	8.292	7.945
Total EAA	45.774	58.423	58.735	59.381	58.949	59.473	57.653
Non-Essential Amino Acids (g kg^{-1} of sample)							
Cysteic Acid	5.186	5.077	4.971	4.973	5.015	4.763	4.750
Taurine	0.942	0.650	0.715	0.770	0.793	0.834	0.829
Meth Sulfone	3.029	2.494	2.520	2.543	2.542	2.505	2.518
Aspartic Acid	6.175	11.009	11.069	11.183	11.423	11.396	10.858
Serine	6.313	7.034	6.936	7.125	7.102	7.129	6.749
Glutamic Acid	45.73	40.27	38.831	39.235	39.68	39.35	38.27
Glycine	5.427	6.383	6.494	6.592	6.605	6.560	6.369
Alanine	4.385	5.677	5.747	5.886	5.982	5.899	5.694
Tyrosine	2.941	2.818	2.343	2.482	2.093	2.167	1.639
Ammonia	1.851	1.521	1.521	1.637	1.535	1.637	1.678
Total non-EAA	83.902	84.738	82.938	84.223	84.709	84.073	81.152

*W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65) along the row.

The primary variations in the amino acid profile depend on the incorporation of pea flour, with processing treatments having minimal or negligible influence. In terms of non-essential amino acids (non-EAAs), the total content remained relatively consistent across all samples.

5.5.2 Physical and structural properties of bread loaves

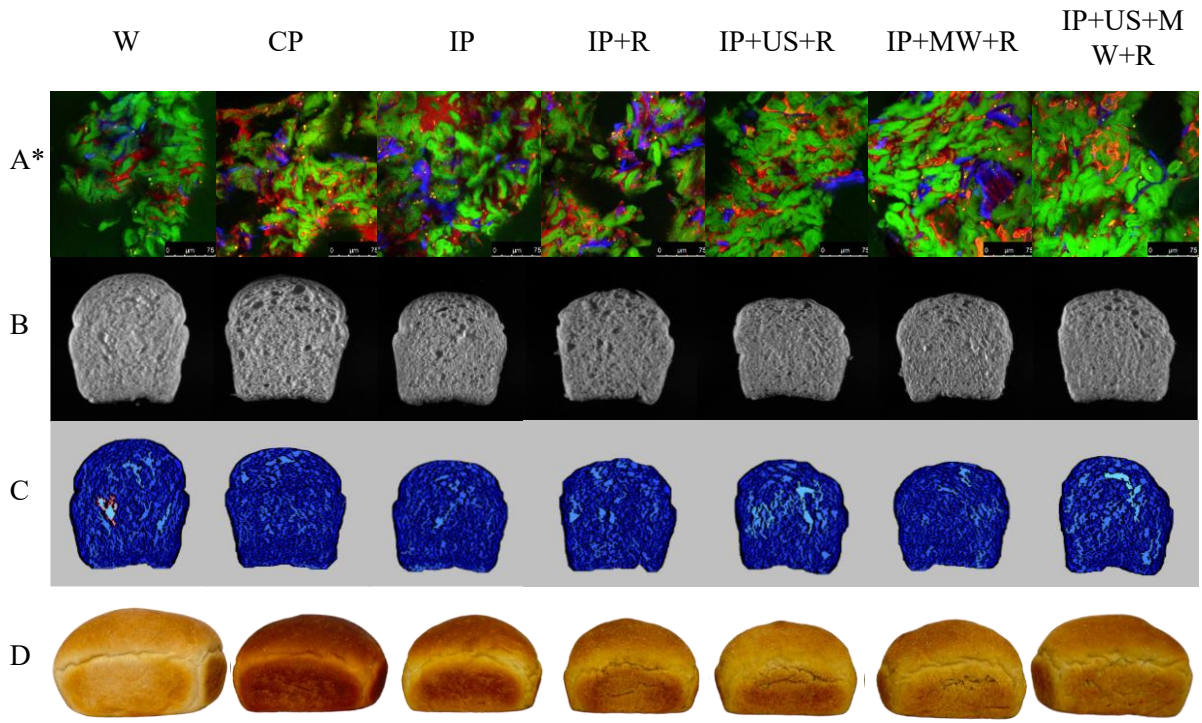


Figure 5.1. Images of bread made with 100% wheat flour and 35%:65% (pea:wheat) flour. A*) Confocal laser scanning micrographs of internal bread crumb, labelled as starch (green), protein (red) and cellulosic material (blue); B) centre slices (C-cell); C) cell structure (C-cell); D) bread loaf images. W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65).

Figure 5.1 A illustrates the confocal laser scanning microscopy (CLSM) images of the bread crumb for all formulations. In the wheat control bread (W), starch (green) appears relatively well-distributed, with a more open structure and less visible protein (red) and fibre (blue), consistent with a typical gluten network that supports good gas retention and bread expansion.

The pea containing breads show visibly higher concentrations of red and blue regions, reflecting an increased presence of protein and cellulosic material, respectively. This matches the compositional data (Table 5.2), where higher protein and fibre contents were recorded in

pea flour breads. In particular, CP and IP samples show dense, uneven clusters of protein and fibre, suggesting a less homogeneous crumb matrix.

The processed pea flour breads (IP+R, IP+US+R, IP+MW+R, IP+US+MW+R) display some improvement in structural organization. Although protein and fibre are still prominent, the fibre (blue) appears slightly more fragmented and with improved dispersion in these samples. This could be the result of processing methods, which may break down cell wall structures and reduce fibre agglomeration. This may help improve the distribution and interaction of these components, potentially enhancing the functional properties of the final product.

Table 5.4 presents the dimensions, colour, crumb structure, and texture of the breads. Specific volume and density (Table 5.4) are critical indicators of bread quality, as they reflect the ability of the dough to retain gas during fermentation and baking, directly influencing texture and consumer appeal. All formulations containing pea flour showed significantly lower specific volumes and greater loaf density ($p<0.05$) compared to the wheat control bread. This reduction is commonly observed in legume-fortified breads and is mainly attributed to the dilution of gluten, the primary structure-forming protein in wheat dough, as well as potential interference in gas retention caused by the higher fibre and protein content of legumes.

Table 5.4. Dimensions, colour, C-cell and texture of bread samples.

Bread	W	CP	IP	IP+R	IP+US+R	IP+MW+R	IP+US+MW+R
Dimensions							
Specific Volume (ml/g)	3.21±0.12^{a}	2.59±0.19^{b}	2.39±0.10^{c}	2.37±0.06^{c}	2.27±0.08^{cd}	2.2±0.17^{d}	2.59±0.06^{b}
Density (kg/m^{3})	311.96±12.15^{d}	386.71±28.13^{c}	418.28±15.62^{b}	422.19±10.33^{b}	440.50±16.34^{ab}	457.82±33.73^{a}	385.9±8.84^{c}
Colour							
L* crust	56.80±1.82^{a}	36.77±2.09^{c}	38.95±2.74^{c}	51.73±1.88^{b}	51.92±2.44^{b}	53.96±2.93^{b}	49.76±3.25^{b}
a* crust	15.70±0.57^{c}	18.27±0.81^{a}	17.76±1.13^{ab}	16.11±0.67^{bc}	15.71±0.73^{c}	14.85±1.54^{c}	16.37±0.74^{bc}
b* crust	35.15±0.10^{a}	23.28±2.15b	25.66±3.85^{b}	36.10±1.089a	35.51±2.57^{a}	35.78±1.72^{a}	34.39±1.84^{a}
L* crumb	71.41±0.84^{a}	68.11±1.12^{b}	67.17±1.68^{b}	64.90±0.74^{c}	63.98±0.71^{cd}	62.74±0.66^{d}	64.09±1.21^{cd}
a* crumb	0.18±0.31^{c}	-1.37±0.10^{d}	-1.30±0.25^{d}	0.97±0.19^{b}	1.93±0.10^{a}	2.30±0.37^{a}	0.24±0.21^{c}
b* crumb	15.56±0.73^{d}	24.43±0.59^{c}	23.86±0.58^{c}	26.03±0.94^{abc}	27.07±1.03^{ab}	27.30±0.75^{a}	24.92±1.07^{bc}
C-cell structure							
Number of Cells	1722.9±174^{a}	1621.1±137^{b}	1590.6±150^{b}	1579.5±124^{b}	1479.9±130^{c}	1491.9±120^{c}	1485.0±132^{c}
Area of Cells	52.69±1.03^{a}	51.93±0.95^{b}	51.49±1.16^{b}	50.67±0.88^{c}	50.88±1.25^{c}	50.58±1.09^{c}	51.71±0.89^{b}
Cell Diameter (mm)	2.16±0.25^{a}	1.90±0.16^{b}	1.87±0.23^{bc}	1.81±0.19^{bc}	1.85±0.22^{bc}	1.78±0.21^{c}	2.08±0.20^{a}
Non-Uniformity	4.19±5.87^{a}	1.06±0.83^{b}	0.92±0.93^{b}	0.84±0.71^{b}	0.98±1.29^{b}	0.96±1.41^{b}	0.93±0.53^{b}
Texture							
Hardness (g)	386.6±47.97^{d}	1328.9±107.35^{a}	1342.4±90.78^{a}	909.3±114.19^{b}	975.5±98.86^{b}	974.5±116.89^{b}	700.3±59.94^{c}
Adhesiveness (g.sec)	0.73±0.64^{b}	0.37±0.27^{ab}	0.42±0.25^{ab}	0.27±0.22^{a}	0.36±0.31^{ab}	0.43±0.33^{ab}	0.30±0.23^{a}
Springiness	0.94±0.01^{a}	0.90±0.02^{b}	0.90±0.01^{b}	0.94±0.01^{a}	0.93±0.01^{a}	0.94±0.01^{a}	0.94±0.01^{a}
Cohesiveness	0.75±0.02^{a}	0.58±0.03^{d}	0.61±0.04^{d}	0.68±0.03^{c}	0.70±0.02^{bc}	0.71±0.02^{bc}	0.71±0.02^{b}
Chewiness	271.5±26^{f}	693.4±44^{ab}	727.0±67^{a}	580.1±57^{d}	635.7±59^{c}	642.3±73^{bc}	467.3±34^{e}
Resilience	0.38±0.02^{ab}	0.27±0.02^{c}	0.28±0.02^{c}	0.36±0.02^{b}	0.37±0.024^{ab}	0.38±0.01^{a}	0.38±0.01^{a}

*W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65). Data presented as means ± standard deviation. Values with a different letter (a-f) are significantly different ($p < 0.05$) along the row.

Among the Irish pea-enriched formulations, the IP+US+MW+R bread showed the highest ($p<0.05$) specific volume, suggesting that the combined ultrasound and microwave treatment had a positive effect on the pea-wheat matrix by improving its viscoelasticity, thereby allowing better loaf expansion. Additionally, differences between the commercial pea (CP) and Irish pea (IP) breads (both non-processed) indicate that likely the pea variety, growing conditions and milling process (Maçãs et al., 2023) can influence the flour functional properties and consequently affect fermentation and dough network, which is reflected in bread volume and structure.

Colour is a critical sensory attribute that strongly influences consumer perception and acceptance (Singh, 2024). Crust and crumb L*, a* and b* parameters are presented in **Table 5.4**. The addition of pea flour significantly reduced ($p < 0.05$) the crust L* values (lightness), particularly in bread containing raw pea flour (IP and CP). The roasting process crust luminosity values are closer to those of the wheat control bread. The same trend was observed by Kali Kotsiou et al. (2021) in their study involving roasting of split peas. All treated flours (roasting, ultrasound and microwave) showed lower a* crust values, suggesting that treatments reduced the effects of Maillard browning reactions and caramelization.

Crumb lightness (L*) was highest ($p<0.05$) in the wheat bread, followed by the breads made with IP and CP, while the lowest lightness values were observed in breads containing processed pea flour. The wheat bread shows a* crumb value close to zero. Raw pea flours tend to give the crumb a greenish hue, while processed flours, influenced by chlorophyll degradation during roasting, have a more reddish tone.

The overall differences in crust and crumb colours are likely associated with the higher protein content of pea flour compared to wheat flour, which enhances Maillard browning reactions during baking, resulting in a darker appearance (Skendi et al., 2021). Additionally, the darker colour of pea flour dilutes the bright white wheat flour and reduces overall luminosity (Skendi et al., 2021).

Digital imaging of bread provides an objective evaluation of crumb structure, serving as a valuable complement to traditional visual assessment methods (Smith, 2022). The C-cell imaging results are presented in **Table 5.4** and **Figure 5.1.B** and **5.1.C**. The wheat bread showed significantly higher values ($p < 0.05$) for cell number, area, and diameter, indicating a more open and aerated crumb compared to all pea flour bread formulations. This is likely due to the dilution of gluten and the interference of legume proteins and fibre in the dough matrix, which can reduce gas retention during fermentation and baking.

In the case of IP+US+MW+R, the combination of treatments may have improved dough interactions, as evidenced by a larger cell area and a cell diameter equal to that of the wheat control. The improved crumb structure is likely linked to the softer texture observed in this bread (**Figure 5.1**). The improved crumb structure in CP and IP+US+MW+R aligns with their higher specific volume and lower density. Breads containing processed pea flours showed significant ($p < 0.05$) lower number of crumb cells.

5.5.3 Storage profiling - texture, moisture and water activity

Texture profiling (TPA) of bread plays a crucial role in assessing the textural changes resulting from different treatments coupled with the natural staling process (**Figure 5.2** and **Table 5.4**). Texture profile analysis (**Table 5.4**) conducted 1 day post-baking revealed that samples show significant differences ($p < 0.05$) for all texture parameters. Composite breads exhibited higher hardness values ($p < 0.05$), which can be attributed to the higher amylose content in pulses, known to retrograde faster than amylopectin. Furthermore, the structural characteristics of pulse starch promote a quicker retrogradation process compared to that of cereal starches (Ahmed, 2021). In addition, the dilution of gluten and the increased presence of dietary fibre contribute to a weakened network structure, reducing gas retention and resulting in a denser crumb and higher hardness (Biduski et al., 2023). The breads made with raw pea flour (IP, CP) exhibited the highest hardness values ($p < 0.05$), likely due to the factors

mentioned above. The applied processing methods significantly reduced crumb hardness ($p < 0.05$), with the most effective reduction observed when combining roasting, ultrasound, and microwave treatments (IP+US+MW+R). These treatments likely modified starch and protein structures, improving dough gas retention and network integrity, reducing retrogradation and resulting in a softer crumb (W. Qin et al., 2022). Breads with processed pea flour required more water during dough preparation, which contributed to their higher moisture content (**Table 5.1** and **Figure 5.2**). The increased moisture content may also have contributed to the increased softness of the bread (Meriem Ouazib et al., 2016).

The raw pea flours (CP and IP) negatively affected the springiness, cohesiveness and resilience of the breads, compared to the wheat control ($p < 0.05$). Springiness refers to the ability of the bread crumb to recover its original shape after being compressed; lower springiness indicates a less elastic and more compact crumb structure. The loss of cohesiveness can increase crumbly texture, thus negatively impacting product acceptability (Rafał Ziobro et al., 2016). However, the application of the roasting, ultrasound and microwave improved springiness, cohesiveness and resilience parameters. These treatments have previously been shown to yield higher viscoelastic behaviour of dough, which can positively influence the springiness, cohesiveness and resilience of the final bread (W. Qin et al., 2022).

The incorporation of pea flours, particularly CP and IP, increased the chewiness of the breads compared to the wheat control ($p < 0.05$). Chewiness describes the extent to which a sample transitions from being chewable to becoming ready for swallowing (Y. Zhang et al., 2022); thus, higher chewiness values indicate a firmer and denser crumb, as observed in the hardness and number of cells results.

Evaluating the texture profile of bread is essential for assessing its shelf life and how its textural properties evolve over time. **Figure 5.2** shows the texture, moisture, and water activity of the samples across days 1, 3, and 7.

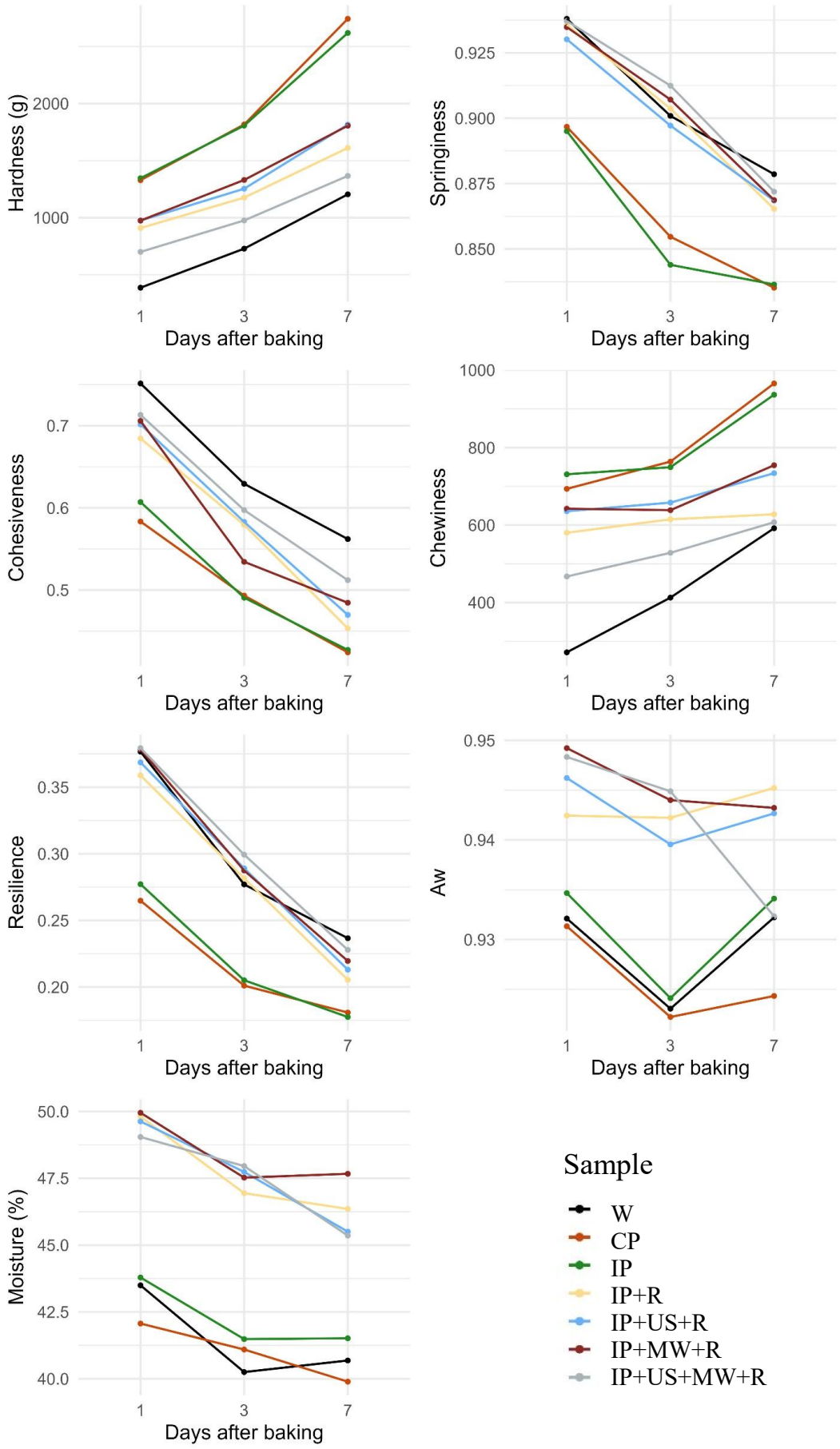

Hardness (g)
2000
1000
1
3
7
Days after baking
Springiness
0.925
0.900
0.875
0.850
Cohesiveness
0.7
0.6
0.5
Chewiness
1000
800
600
400
Resilience
0.35
0.30
0.25
0.20
Aw
0.95
0.94
0.93
Moisture (%)
50.0
47.5
45.0
42.5
40.0
Sample
W
CP
IP
IP+R
IP+US+R
IP+MW+R
IP+US+MW+R

Figure 5.2. Texture, moisture, and water activity of the samples across days 1, 3, and 7 after baking. W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65).

As expected, hardness and chewiness increased over time, indicating that the bread became firmer and more difficult to chew across all samples. The trend observed for these parameters on day 1 was consistently maintained through days 3 and 7. Wheat bread remained the softest sample overall, followed by the IP+US+MW+R formulation, the other processed flours, and finally the raw pea flour, which exhibited the highest values for both parameters. Although wheat initially presented lower hardness and chewiness, it experienced a higher staling rate over time (35.3%) compared to IP+US+MW+R (15.8%), presented in **Table 5.A1**. As both parameters are closely associated with bread staling, the proximity of their values by day 7 suggests that the textural differences between these two samples diminish during storage, potentially resulting in similar mouthfeel characteristics on day 7 after baking.

Over the 7-day storage period, all bread samples exhibited a decrease in moisture content, particularly between days 1 and 3. This trend aligns with the expected water migration and evaporation that occur post-baking and during storage. Notably, formulations containing processed pea flour (IP+R, IP+US+R, IP+MW+R, and IP+US+MW+R) retained the highest moisture levels by day 7, remaining above 45%. In contrast, breads made with raw pea flour (CP and IP) and the wheat control (W) showed lower moisture values, falling below 42.5%.

At day 1 after baking, breads produced with commercial pea flour (CP), wheat bread (W) and the sample with untreated Irish pea flour (IP) revealed the lowest Aw values. In contrast, the breads containing processed pea flours exhibited higher water activity values. Higher water activity generally reflects greater moisture availability, usually contributing to a softer crumb and improved sensory characteristics in fresh bread, which are generally desirable from a

consumer perspective. However, above a certain threshold a higher aw may also influence microbial stability and shelf-life (Bhise & Kaur, 2014).

The Aw values followed a V-shaped trend over the 7 days testing period, with a noticeable decrease on day 3, followed by a slight increase on day 7 for most of the samples. The relationship between moisture and Aw in this context indicates that while moisture content declines steadily, the availability of water (Aw) remains stable. This stability is important for maintaining softness and delaying staling (Puhr & D'appolonia, 1992).

5.5.4 Volatile compound analyses

Flavour is a key factor influencing consumer acceptance and the recognition of different bread products. The major chemical groups contributing to bread aroma include aldehydes, alcohols, ketones, esters, and acids, along with notable contributions from pyrazines, pyrrolines, hydrocarbons, furans, and lactones (Cayot, 2007). The overall aroma profile of bread is influenced by the ingredients used and the baking process. Additionally, baking-related processes such as thermal lipid oxidation reactions, especially Maillard reactions and caramelisation, play a crucial role in shaping bread aroma (Galoburda et al., 2020). The relative abundance of individual volatiles can provide some insight into its potential to influence sensory perception. The volatile profiles of the bread crumb samples were best expressed in the heatmap (**Figure 5.3**). The sensory descriptors are available in **Table 5A2** in the Appendix.

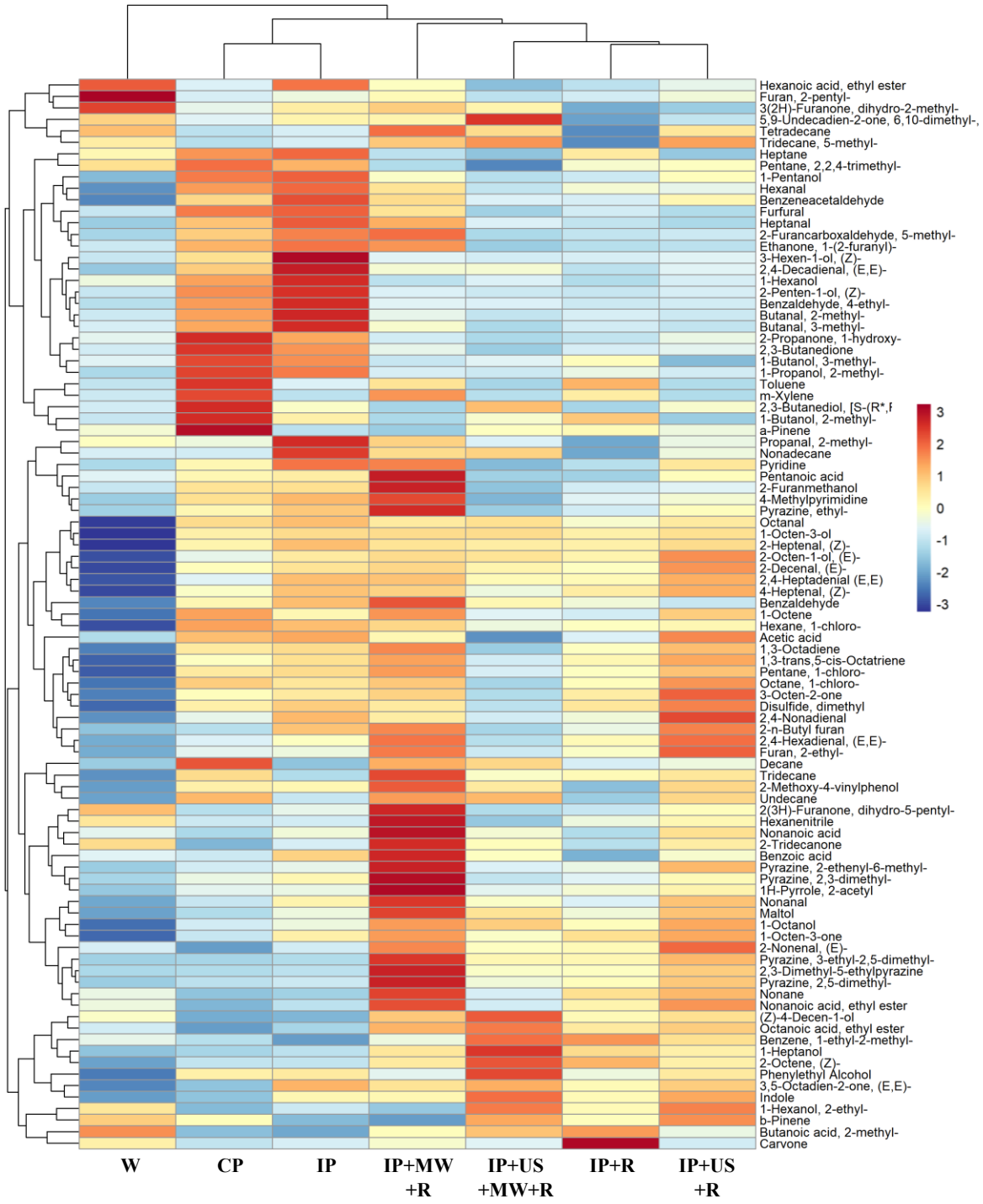


Figure 5.3. Heatmap of volatile compound profiles in bread samples. W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated : wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65).

Ninety three volatile compounds were identified in these samples consisting of 15 alcohols, 15 aldehydes, 9 alkanes, 5 ketones, 8 furans, 7 benzenes, 6 pyrazines, 4 acids, 4 alkenes, 4 hetrocyclic compounds, 3 esters, 3 terpenes, 3 chlorinated compounds, 1 each of the

following; hydroxy ketone, sulfur, nitrile, pyranone, phenol, lactone and a terpenoid ketone. Each volatile compound was identified in every sample, but the abundances varied considerably depending upon the bread sample type as evident in the heatmap (**Figure 5.3**).

The wheat control bread (W) was the most distinct sample, characterised by a lower diversity and abundance of most volatile compounds compared to the other bread samples. Therefore, the inclusion of the pea flour enhanced the abundance of most volatiles, except for ethyl hexanoate (apple peel, fruity), 2-pentylfuran (green bean, buttery), and dihydro-2-methyl-3(2H)-furanone (sweet, solvent-like, bready, buttery, nutty). Ethyl hexanoate is likely produced by yeast metabolism, 2-pentylfuran results from thermal lipid oxidation, and the furanone is formed via the Maillard reaction (Birch et al., 2014).

The samples most similar to W were the control commercial pea (CP) and Irish pea (IP) bread samples, although volatile differences within these samples were also evident. However, this is not surprising as previously mentioned that different pea varieties, growing conditions and milling process will differ (Maçãs et al., 2023). The CP sample was characterised by a high abundance of a wide range of volatiles. Some of the most prominent were the alcohols 3-methyl-1-butanol (whiskey, malt, burnt), 2-methyl-1-propanol (wine, solvent, bitter), and 2-methyl-1-butanol (wine, onion) which are all derived from Strecker degradation and impact the Maillard reaction. Other volatiles such as 2,3-butanediol [S-(R*,R)]* (fruity, creamy, buttery), 1-hydroxy-2-propanone (pungent, sweet, caramel-like, ethereal), and 2,3-butanedione (butter) were also more abundant and result from yeast metabolism. The terpene α-pinene (pine, turpentine) was also more abundant and is directly related to the pea component, whereas others such as toluene (paint), m-xylene (plastic), and decane (alkane) although more abundant are unlikely to influence sensory perception due to their high odour thresholds. The IP bread sample had a volatile profile most similar to CP but was distinguished by a greater abundance of 1-hexanol (resin, floral, green), 3-hexen-ol-Z (grass), and pentan-1-ol-Z (green, plastic, rubber), 2,4-decadienal-E,E (fried, waxy, fatty), 2-methyl butanal (cocoa, almond), 3-methyl

butanal (malt), and 2-methyl propanal (pungent, malt, green) all of which are products of thermal lipid oxidation. The branched-chain aldehydes (2-methyl butanal, 3-methyl butanal, 2-methyl propanal) were also more abundant and are products of Strecker degradation/Maillard reaction. Ethyl benzaldehyde (sweet) was also more abundant but although not a direct product of Strecker degradation, its base substrate, benzaldehyde, is, with the ethanol component derived from yeast metabolism. Nonadecane, an alkane formed via thermal lipid degradation was also abundant but is unlikely to impact the sensory perception of the IP sample.

The volatile profile of the remaining samples were more similar to each other and were characterised by the fact that they all contained Irish Pea Roasted (IP+R), indicating that compounds formed during roasting were the primary discriminating factor in these samples in comparison to the W, CP and IP bread samples. Both the IP+R and IP+US+R bread samples were the most similar to each other and were the least similar to the W bread sample and the CP and IP samples. The volatile profile of IP+R was dominated by a single abundant compound, carvone (mint, basil, fennel), a terpene directly from the pea, with many other volatiles present at relatively low abundance compared to the CP and IP bread samples. This suggests that many volatiles are lost during the roasting process. The IP+US+R bread sample exhibited higher abundances of 2,4-nonadienal (watermelon), 2-nonenal-E (fatty, green, cucumber, aldehydic, citrus), 2,4-heptadienal-E,E (nutty, fatty), 3-octen-2-one (nutty, earthy), and 2-ethyl-furan (chemical, beany, ethereal, cocoa, bready, malty, coffee, nutty), all products of thermal lipid oxidation. This indicates that the inclusion of ultrasound (US) either enhanced lipid oxidation or reduced inhibition to lipid oxidation. The bread samples IP+US+MW+R and IP+MW+R were similar, but the heatmap suggests that inclusion of microwave (MW) treatment had a greater impact on the volatile profile than the inclusion of US treatment, as the MW treated samples were less similar to the IP+R bread sample than those treated with US. The IP+MW+R bread sample had a higher abundance of more volatiles than all the other samples. This was particularly evident in relation to enhanced formation of compounds

associated with the Maillard reaction: ethyl pyrazine (peanut butter, wood), 2-ethenyl-6-methyl-pyrazine (hazelnut, nutty), 2,3-dimethyl pyrazine (nut, peanut butter, cocoa, meat), 3,6-cocoa pyrazine (potato, cocoa, roasted, nutty), 3,5-cocoa pyrazine (burnt almond, roasted, nutty, coffee), 2,5-dimethyl-pyrazine (cocoa, roasted nut, roast beef, medicinal), maltol (caramel), 4-methylpyrimidine (no referenced odour), 1-acetyl-1H-pyrrole (nutty, walnut, bread), and 2-furanmethanol (burnt). The IP+MW+R bread sample also contained significant abundance of volatiles derived from yeast metabolism, such as pentanoic acid (sweat), nonanoic acid (green, fatty), and ethyl nonanoate (fruity, rose, waxy, rummy, winey, tropical) and thermal lipid oxidation; γ-nonalactone (coconut, peach), 2-tridecanone (fatty, waxy, dairy, milk, coconut, nutty, herbal, earthy), and nonanal (fatty, citrus, green). The IP+MW+R bread sample also contained a high abundance of benzaldehyde (almond, burnt sugar) a Strecker degradation product, benzoic acid (balsamic, urine) likely directly from the pea, p-vinylguaiacol (clove, curry), likely a derivative of ferulic acid in the pea and capronitrile (no referenced odour) likely from cyanogenic compounds in the pea and/or thermal decarboxylation of amino acids during baking. The IP+US+MW+R bread sample shared many volatiles with the IP+MW+R sample but lower levels of many Maillard reactions compounds, suggesting that the inclusion of US changed the impact of MW alone. The IP+US+MW+R bread sample had a greater abundance of the lipid oxidation compounds; 1-heptanol (chemical, green) and Z-4-decen-1-ol (waxy, fatty, fruity), and the yeast metabolism compounds, phenylethyl alcohol (honey, spice, rose, lilac) and ethyl octanoate (fruity, fatty). This IP+US+MW+R bread sample also exhibited the highest abundance of 2-octene-E (no odour) of no aromatic importance and geranyl acetone, a terpenoid ketone likely originating directly from the pea.

In summary, the volatile compounds reflect contributions from pea-derived components, yeast metabolism, Strecker/Maillard reactions, and lipid oxidation primarily driven by thermal processes. The inclusion of the two types of pea flour altered the volatile

profile from the wheat sample. The volatile profile of the breads produced from Irish pea flour was further modified by roasting of the pea flour, and also by the incorporation of US and MW treatments and with interactive effects of these treatments.

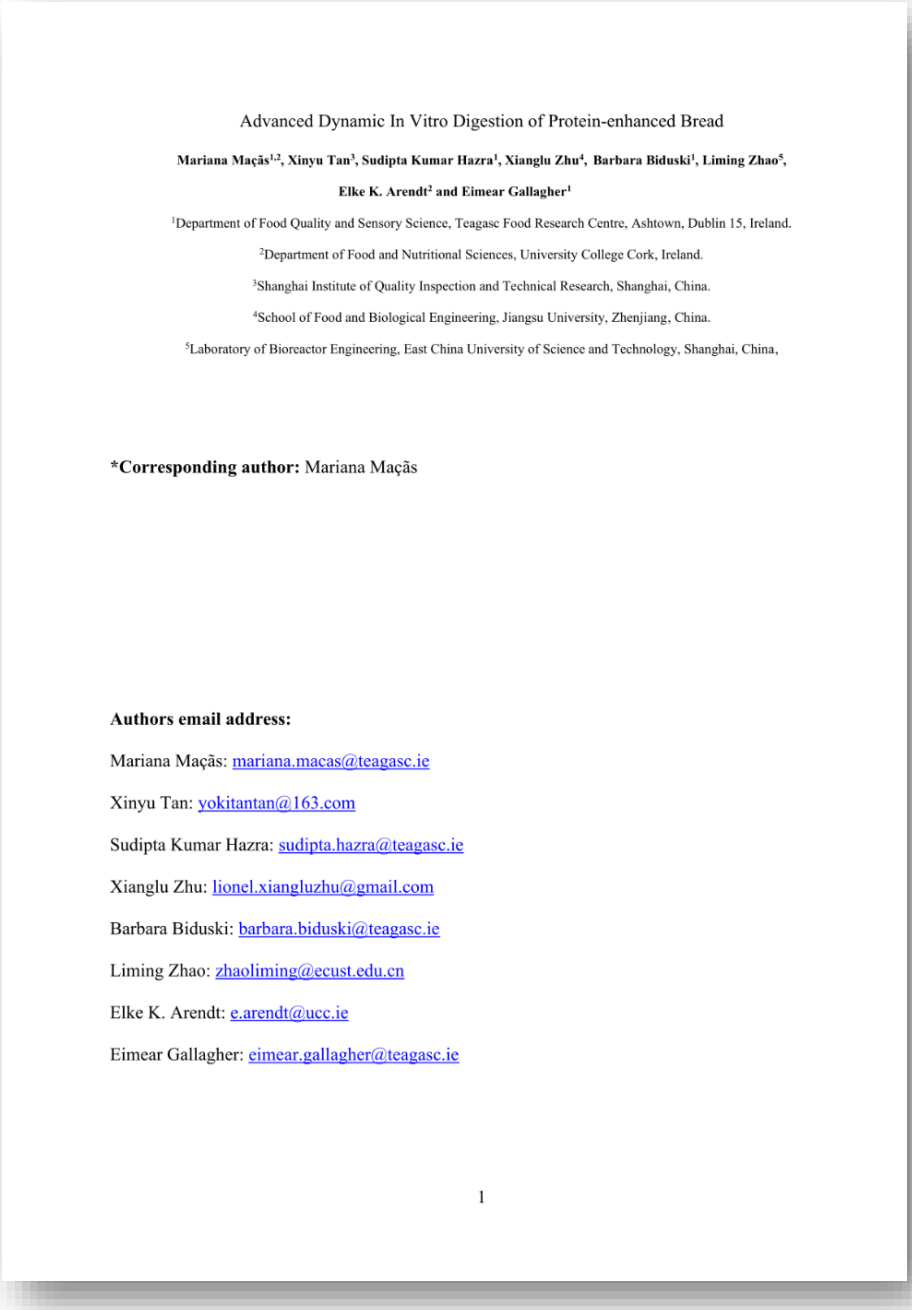
5.6 Conclusion

This study demonstrates that incorporating 35% pea flour into wheat-based bread formulations represents a nutritionally valuable enrichment level, significantly improving the protein and fibre content of the final product. However, the use of pea flour also brought challenges for bread quality, especially in terms of texture, volume, and aroma attributes. Breads made with pea flour displayed a denser structure, harder texture, and lower loaf volume. The inclusion of pea flour altered the volatile profile, with the source of and treatment of the pea flour also having an impact. In terms of the volatile profile the greatest impact in terms of treatment of the pea flour was roasting which reduced the abundance of many volatiles, however the addition of microwave and ultrasound with roasting, further altered the volatile profile. Roasting pea flour with microwave enhanced many Maillard reaction products, as well as some lipid oxidation or yeast metabolism compounds, but this impact was less pronounced if ultrasound was included with these treatments.

The application of roasting had a clear positive impact on these limitations, enhancing both texture and aroma by reducing the intensity of undesirable notes. When roasting was combined with technologies such as ultrasound (US) and microwave (MW), the results varied. While dual combinations (e.g. IP+US+R or IP+MW+R) did not further improve structure, the triple combination (IP+US+MW+R) proved effective. This treatment resulted in breads with higher specific volume, softer texture, and more complex, balanced aroma profiles that masked the off-notes typically associated with legume-based products, making them more closely resemble traditional wheat bread.

These findings suggest that certain processing technologies can offer a promising strategy to tailor the structural and sensory properties of legume-enriched breads. The use of combined treatments such roasting, ultrasound and microwave open a novel field of opportunity. By optimising exposure times and intensities, it may be possible to unlock the full potential of legume flours, transforming their functional and nutritional qualities in ways that meet both technological and consumer demands. Further exploration in this direction could pave the way for the development of high-protein, fibre-rich baked products without compromising sensory and physical appeal.

6 Advanced dynamic in vitro digestion of protein-enhanced bread



Advanced Dynamic In Vitro Digestion of Protein-enhanced Bread

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Under review:

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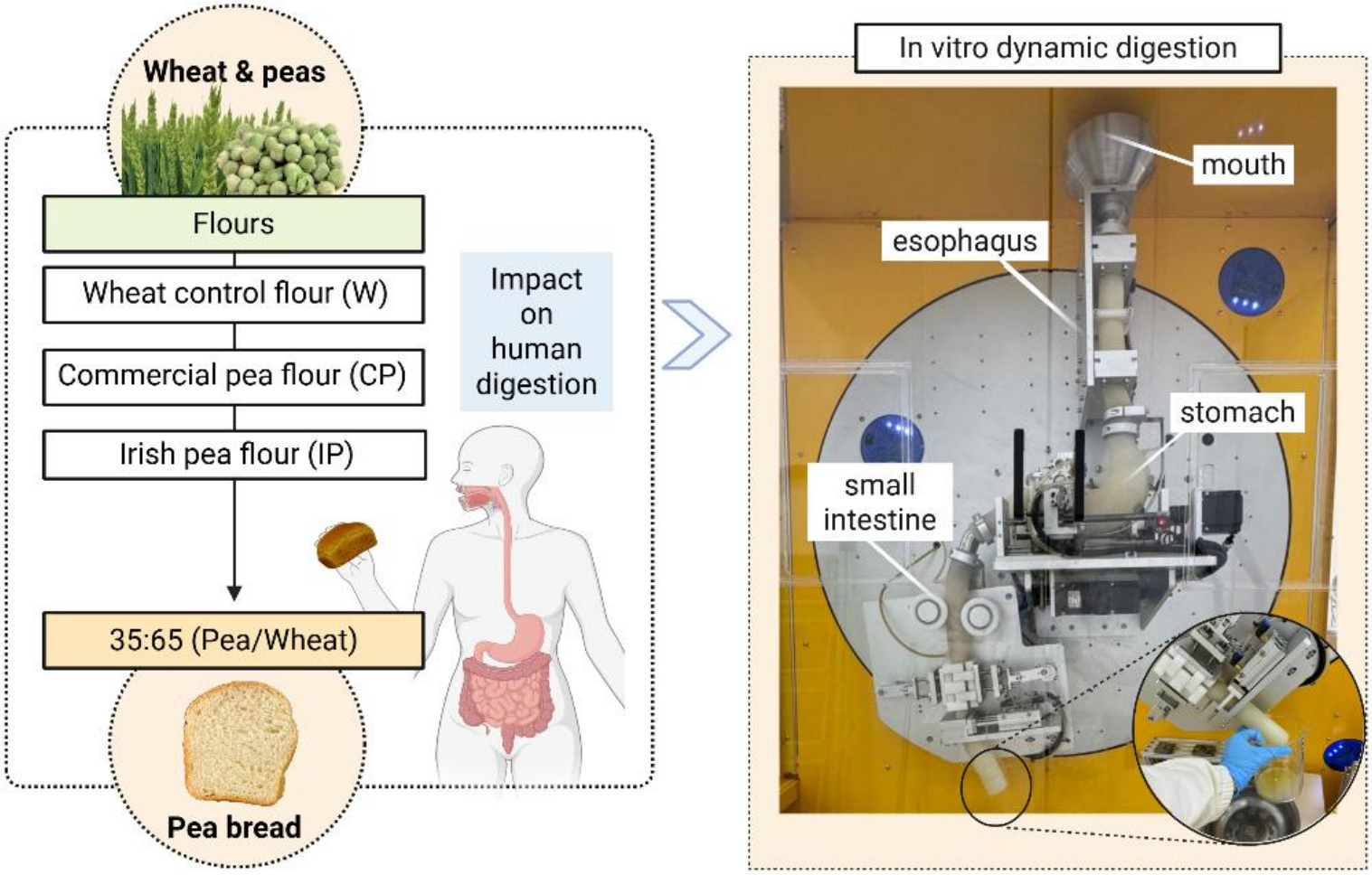
6.1 Graphical abstract



6.2 Abstract

Pea flours are valuable ingredients for improving the nutritional quality and sustainability of bakery products, yet their impact on digestion remains underexplored. This study aimed to evaluate how the inclusion and composition of pea flour affect the starch and protein digestibility of wheat and composite pea-wheat breads (35:65%). Using the Dynamic In Vitro Human Stomach System (DIVHS-IV), starch and protein digestibility were monitored over a 120 minute digestion period. Pea-enriched breads exhibited lower reducing sugar release and a reduced estimated glycaemic index compared with wheat bread, indicating reduced starch digestibility. Protein and free amino acid analyses revealed higher protein hydrolysis for the bread containing Irish pea (IP) relative to the breads containing commercial pea and only wheat (W), supported by differences in protein family distribution and matrix structure. Overall, the inclusion of Irish pea flour improved bread protein digestibility and lowered glycaemic potential, supporting its application in nutritionally enhanced and sustainable bakery formulations.

6.3 Introduction

Bread is one of the most widely consumed staple foods globally. Wheat bread dominates the market due to its favourable viscoelastic properties, which enable the production of loaves with desirable texture, volume, and sensory qualities (Dong & Karboune, 2021). The heavy reliance on wheat, not only heightens supply vulnerabilities and faces challenges from unsuitable climate conditions for its cultivation, but also brings environmental and health drawbacks, prompting a shift toward plant-based alternative ingredients (Biduski et al., 2023; Y. Wang & C. Jian, 2022). In addition, wheat has a high starch digestibility and can lead to elevated postprandial glycaemic responses (Giuberti et al., 2016). As interest continues to grow in improving the nutritional profile of staple foods, incorporating legume flours in food formulations has gained increasing attention (Chinma et al., 2025; Maçãs et al., 2024a).

Legumes, such as peas (*Pisum sativum L.*), are nutrient-dense ingredients rich in protein, dietary fibre, minerals and bioactive compounds (Binti Mohd Zaini et al., 2021). Incorporating peas into baked goods can support the local economy and sustainable practices through environmental benefits such as nitrogen fixation, which reduces reliance on synthetic fertilizers (Cusworth et al., 2021; Uebersax et al., 2023).

When incorporated into bread, pea flour has been shown to increase protein content, enhance amino acid balance, and reduce the starch digestibility (Chinma et al., 2025; Maçãs et al., 2025; O. Moreno-Araiza et al., 2023). However, the addition of pea flour also modifies dough rheology and bread structure, which can affect consumer acceptance and potentially influence nutrient bio accessibility (K. A. Millar et al., 2019; Young et al., 2019).

The digestibility of starch and protein in bread is influenced by multiple factors, including starch-protein interactions, degree of starch gelatinization, protein denaturation, and the presence of anti-nutritional factors, such as trypsin inhibitors and hemagglutinins in legumes, tannins in both legumes and cereals and phytates in cereals and oilseeds (Gilani et al., 2005;

Jenkins et al., 1987; Li et al., 2023; Samtiya et al., 2020). The growing conditions of peas, including soil type, climate and agricultural practices coupled with milling can markedly influence their chemical composition, particle size and damaged starch which in turn affects their functional characteristics and the rate and extent of digestion (Soncin Alfaro et al., 2025; Tibaldi et al., 2025).

While several studies have examined the techno-functional and sensory aspects of legume-enriched breads, there is limited documented research assessing comparing their nutritional performance, particularly in terms of in vitro starch and protein digestibility. In this study, the digestion process of wheat/pea breads was simulated using the Dynamic Human Stomach System (DIVHS-IV). This advanced in vitro digestion model simulates the physiological conditions of the human upper gastrointestinal tract with precise control over parameters such as temperature, gastric secretions and peristaltic movements (Li et al., 2020; Zhou et al., 2025). While static models such as INFOGEST are widely used and highly valued for their standardized, well-established protocols and accessibility, they naturally do not capture the full complexity of the human body, and some dynamic aspects of digestion may be missed. The DIVHS-IV allows dynamic monitoring of nutrient release over time, providing a more physiologically accurate representation of the human digestion process (Zhou et al., 2025). Applying this system to compare wheat breads with those containing pea inclusions provides a novel approach to evaluate how compositional variances and particle size differences can affect human digestion. The present study investigated and compared the starch and protein digestibility of three bread types: a standard wheat bread (W), bread supplemented with 35 % commercially sourced pea flour (CP) and bread containing 35% Irish-grown pea flour (IP).

6.4 Materials and methods

6.4.1 Raw material, grain processing and bread-making

Bread preparation was carried out using strong wheat flour (Shakleton's Milling, Ireland), a commercially available pea flour (of Hungarian origin) (Mills & Folks, UK), and Irish-grown pea grains (variety Carrington) supplied by Wynnes Ltd (Kildare, Ireland).

The whole Irish peas were ground using a hammer mill (Labmill 3100, Perten®, Sweden), following the procedure optimised by (Maçãs et al., 2023). The flours were blended in a 35:65 ratio of pea to wheat flour for bread-making. Wheat bread was used as a control sample.

The breads were produced using either 100% wheat flour (control formulation) or composite flours containing 35% pea flour and 65% wheat flour (Table 6.1). The formulations also included salt (2%), unsalted butter (1%), bread improver (1%), yeast (1.5%), and water, the latter adjusted according to the absorption capacity determined by Mixolab© analysis. The bread preparation process and equipment followed the methodology described by (Maçãs et al., 2025).

Table 6.1. Abbreviations of bread formulations used in this study.

Abbreviation	Bread Formulations
W	Control strong wheat flour
CP	Commercial pea flour: wheat flour (35:65)
IP	Irish pea flour: wheat flour (35:65)

6.4.2 In vitro dynamic digestion of bread

Digestive solution preparation:

Simulated digestive fluids were prepared in advance by dissolving the components described in Table 6.2 in 1L of distilled water. Prior to digestion the required enzymes (Tables

6.3 and 6.4) were freshly incorporated before each digestion and sonicated for 10 minutes to ensure full dissolution.

Table 6.2. Enzyme concentrations and respective manufacturer.

Experimental Material	Manufacturer (Country)
a-Amylase (35 U/mg)	Macklin, Shanghai, China
Pepsin (3000 U/mg)	Aladdin Scientific, China
Pancreatin from porcine pancreas (4*USP)	Sigma Corporation, USA
Lipase (30-90 U/mg)	Sigma Corporation, USA
Ox Bile Powder (40%)	Yuanye Bio-technology, Shanghai

Table 6.3. Enzyme addition to the simulated digestive electrolyte solutions

Simulated Fluid	Enzyme(s) Added	Volume of Electrolyte Solution
Saliva	0.285 α-amylase	100 mL
Gastric Juice	0.0417 g pepsin	100 mL
Intestinal Juice	1.75 g pancreatin + 0.30 g lipase	100 mL
Bile	6.00 g bile salts	100 mL

Table 6.4. Preparation of simulated digestive electrolyte solutions

Electrolyte	Saliva	Gastric Juice	Intestinal Juice	Bile
NaCl (g)	0.116	5.504	14.024	10.518
KCl (g)	0.148	1.648	1.128	0.752
$NaHCO_3$ (g)	2.100		6.776	11.57
NaH_2PO_4 (g)		0.532		
$CaCl_2$ (g)		0.604		
NH_4Cl (g)		0.612		
KH_2PO_4 (g)			0.160	
$MgCl_2$ (g)			0.100	
HCl (mL)	0.300	2.800	0.100	
pH	6.800	1.600	7.800	8.200

Digestion simulation:

Digestion was simulated using the esophagus, stomach and duodenum compartments of the Dynamic In Vitro Human Stomach System (DIVHS-IV) (Xiao Dong Pro-health Instrumentation Co., Suzhou, China), equipment settings are provided on Appendix (Table 6.A1). A schematic representation of the system can be found in J. Wang et al. (2022). The equipment was operated at a constant temperature of 37 °C, and peristaltic movement was

driven by compressed air. Bread samples (12 g) were first manually ground using a mortar and pestle to replicate chewing and then combined with simulated saliva in a beaker, followed by incubation in a 37 °C water bath for 5 minutes to mimic oral digestion. During the digestion, gastric and intestinal fluids were delivered at a flow rate of 0.29 mL/min, while bile was introduced at 0.19 mL/min. The "orally" digested mixture was then transferred into the dynamic digestion model. The digestion process continued for a total of 120 minutes. Digestion lasted 2 hours, with samples taken from the duodenum at 45 minutes and every 15 minutes thereafter. Digestion time was determined based on methodologies previously applied (Capriles et al., 2009; Ye et al., 2025; H. Zhang et al., 2022). Samples for each analysis were collected from the duodenum compartment at the 45 minute mark and subsequently every 15 minutes until the end of the 120 minute digestion period. The collected digesta were immediately centrifuged at 4000 rpm for 4 minutes at -4 °C. The resulting supernatant and pellet were separated, rapidly frozen in liquid nitrogen to inactivate enzymes, and stored at -20 °C until further analysis. All experimental replicates were conducted in triplicate.

6.4.3 Scanning electron microscopy (SEM) of bread and digesta

To investigate the microstructure of both the undigested bread and the digested sediment, samples were initially freeze-dried. Morphological analysis was performed using a Hitachi S3400–N scanning electron microscope (SEM; Hitachi, Japan) operated under low vacuum at 15 kV. For each bread formulation, three independent samples were analysed. The microstructure of undigested bread and digested residues (digesta) was examined after freeze-drying to remove moisture and preserve structural integrity. The dried samples were fractured to expose internal surfaces, mounted on aluminium stubs with conductive carbon tape, and coated with a thin (8–10 nm) gold–palladium layer using a sputter coater (Quorum Q150R, UK). Observations were performed with a Hitachi S-3400N scanning electron microscope (Hitachi, Japan) operating under low vacuum at 15 kV. Images were captured at magnifications

between ×500 and ×2000. Three replicates of each bread formulation were analysed, and representative micrographs were selected from different sample areas to account for structural variability.

6.4.4 Reducing sugars and estimated glycemic index

Reducing sugar concentration was determined using the 3,5-dinitrosalicylic acid (DNS) colorimetric method based on Miller (1959). Samples were diluted 1:10 (v/v) with deionized water prior to analysis. Diluted samples (1 mL) were combined with 1 mL of DNS reagent and heated in a boiling water bath at 100 °C for 7 minutes. After cooling, absorbance was measured at 540 nm using a microplate reader (Infinite M Nano, Tecan, **S**witzerland). A standard curve was generated using glucose solutions ranging from 0 to 1 mg/ml. Final concentrations were calculated using the standard curve and corrected for the dilution factor.

The result of total reducing sugar released at after 45 minutes of digestion and every 15 minutes thereafter until the end point at 120 minutes was obtained by multiplying the reducing sugar concentration (mg/ml) by the digesta volume (ml). Cumulative release was calculated by adding the total reducing sugar of each time point to the cumulative value from the previous time point (Woolnough et al., 2010).

The kinetics of reducing sugar released, the hydrolyses index (HI) and the estimated glycaemic index (eGI) were calculated according to Graça et al. (2020). The hydrolysis index (HI) was calculated (Equation 4) by dividing the area under the hydrolysis curve (AUC 0–120 min) of the bread samples by that of the reference food, which was white wheat bread (W).

$$(4) \quad HI = \frac{AUC\ of\ product}{AUC\ Reference\ food} \times\ 100$$

The estimated glycemic index (eGI) was determined using Equation (5).

$$(5) \quad eGI = (0.549 \times HI) + 39.71$$

6.4.5 Protein digestibility

The protein concentration in the supernatant digesta was determined using the Pierce BCA Protein Assay Kit (Cat. No. 23225, Thermo Scientific), following the instructions provided by the manufacturer, with bovine serum albumin (BSA) employed as the standard. Protein digestibility was evaluated by measuring the amount of protein released into the digesta at each time point.

6.4.6 Protein identification

The digested samples were reconstituted in 100 µL of 0.1% formic acid and chromatographically separated (5 µL) using a Thermo Scientific Ultimate 3000 UHPLC system coupled with a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, USA). Protein identification was performed using Protein Discoverer 2.0 software (Thermo Fisher Scientific, USA). The MS/MS data were searched against an in silico tryptic digest of Poaceae proteins from the UniProt database (version 2018/02; 2,891,190 sequences), supplemented with the common repository of adventitious proteins (cRAP). Search results were manually inspected, and protein identifications were filtered using a 1% global false discovery rate (FDR). The resulting dataset was subsequently examined to identify proteins of interest. For each identified protein, the corresponding family classification was obtained from the UniProt database (https://www.uniprot.org/) based on sequence similarity. Pie charts were then created to represent the relative proportions of the different protein families detected in each bread sample. In addition, temporal plots of the main protein families were constructed to show changes in the number of identified proteins over the digestion period, providing insight into variations in protein availability throughout the process.

6.4.7 Free amino acids

Quantification of free amino acids was carried out using an ACQUITY UPLC iClass system (Waters, Milford, MA, USA) coupled with a 6500+ Qtrap tandem mass spectrometer (AB Sciex) equipped with a Turbo Spray ion source. Chromatographic separation was achieved on a Glycan BEH Amide column (1.7 µm, 2.1 × 100 mm) maintained at 35 °C, with a flow rate of 0.3 mL/min and a 1 µL injection volume.

The mobile phase consisted of:

A) water containing 20 mM ammonium formate and 0.35% formic acid

B) 90% acetonitrile with 20 mM ammonium formate and 1.15% formic acid.

The gradient was expressed as the percentage of phase A), with B) making up the remainder to 100% at each step: 5% A) (0–2 min), 20% A) (2–7 min), 60% A) (7–10 min), held at 60% A)(10–12 min), and re-equilibrated to 5% A) (12.1–15 min).

Detection was conducted in both positive and negative ionization modes using multiple reaction monitoring (MRM), with optimized Q1→Q3 transitions specific to each amino acid (e.g., alanine 90.1→44.1). Instrument source conditions included curtain gas at 40 psi, GS1 at 55 psi, GS2 at 50 psi, a source temperature of 650 °C, and an ion spray voltage of ±3000 V. Data processing was done using Analyst software version 1.7.3, and quantification was based on external calibration curves, with results expressed as ng/mL of extract.

6.5 Results and discussion

6.5.1 Visual and microscopic analysis of bread digesta

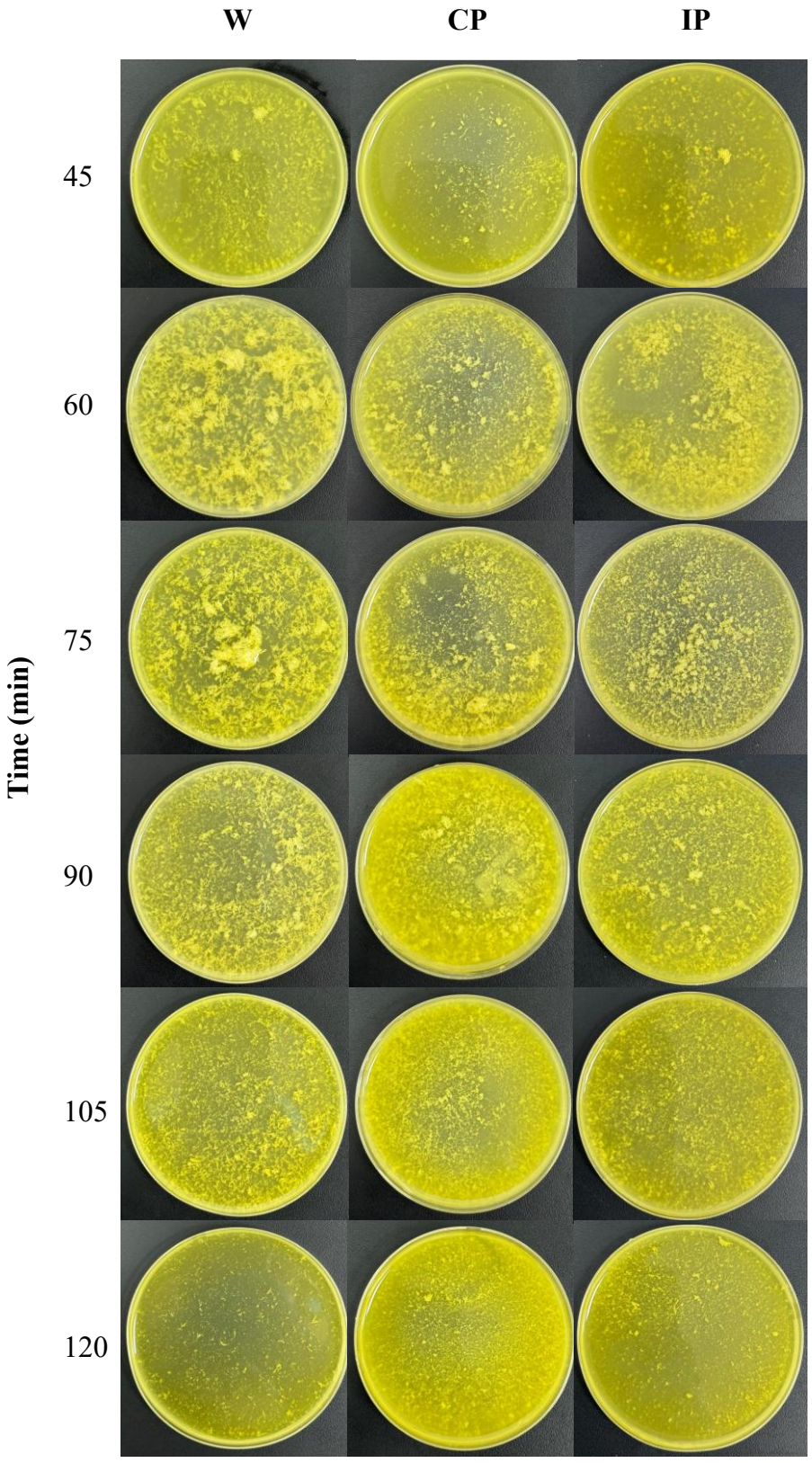


Figure 6.1. Visual appearance of digesta from wheat breads and breads produced with 35%:65% (pea:wheat), during in vitro digestion (45-120 min).

Figure 6.1 presents the visual changes and progressive reduction in particle size in the bread digesta over the digestion period. Throughout the digestion process, wheat bread (W) and the pea-containing breads (IP and CP) showed visible differences, highlighting how matrix composition influences disintegration behaviour. In the early to mid-stages of digestion (up to ~75 minutes), wheat bread consistently showed larger visible particles. This may be attributed to the lighter, more aerated structure of wheat bread, which allows for rapid water absorption and swelling but results in bulkier fragments. In contrast, both IP and CP bread samples produced digesta more uniformly dispersed particles from the beginning of the process. Pea-containing bread has previously been reported to have a dense structure (Kim Adrienne Millar et al., 2019) The higher fibre and protein content of pea flour (**Chapter 4** Table 4.1) may limit water penetration, leading to a more compact yet consistently fragmented appearance during digestion. At 90 minutes, and more clearly at 120 minutes, the wheat bread showed a marked reduction in particle size. At 120 is the sample showing the fewer and smaller fragments remaining. This suggests that the more aerated matrix of wheat bread broke down faster and more completely over time. The sustained compactness of the pea-enriched samples may indicate slower or more resistant nutrient release.

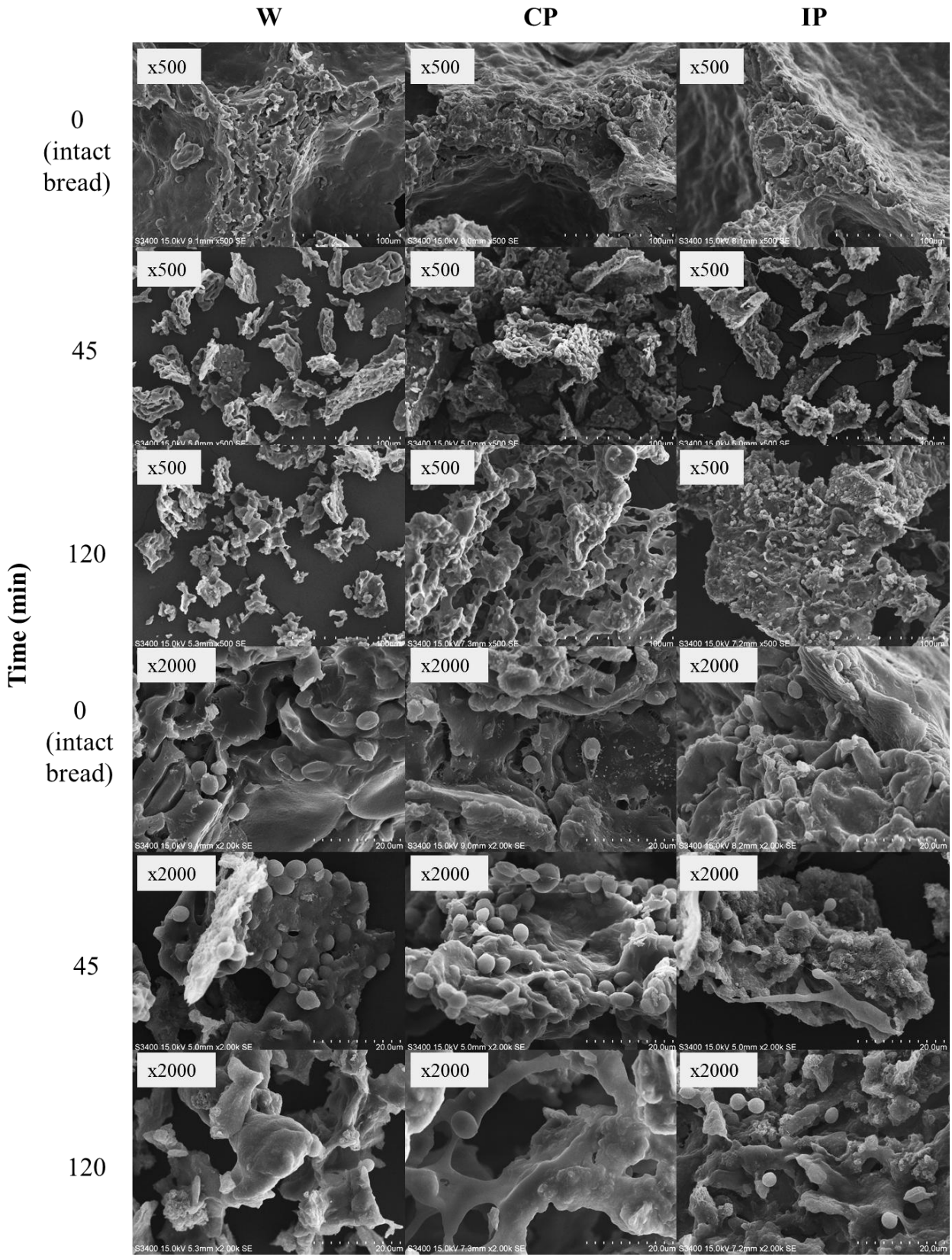


Figure 6.2. SEM micrographs of bread digesta from 35%:65% (pea:wheat), at 0 min (intact bread), 45 min and 120 min of in vitro digestion, at magnifications of ×500 and ×2,000.

SEM micrographs are presented in Figure 6.2. No visible differences were observed at 0 minutes (intact bread) or after 45 minutes of digestion among all the formulations. At 120 minutes, however, images taken at higher magnification (2000×) revealed that starch granules appeared to be fully digested in the wheat bread digesta, as no intact granules were visible. In contrast, starch granules were still observed in CP bread, with a higher number remaining intact in IP bread. The faster digestion of wheat bread is likely due to its lower protein and fibre content. In pea-enriched breads, the higher levels of protein and fibre can form a more compact matrix and stronger protein-starch interactions, reducing enzyme accessibility and slowing digestion (Do et al., 2022; H. Zhang et al., 2022). The greater resistance of the bread sample containing Irish pea flour to enzymatic breakdown highlights its potential role in reduced GI formulations (Table 6.5). These findings also support the idea that compositional variances and particle size differences of pea samples can influence bread digestibility.

6.5.2 Starch digestibility: reducing sugars and estimated glycemic index

Starch digestion was assessed in the bread digesta. The release of reducing sugars (mg) during the in vitro digestion process was monitored over 120 minutes. The area under the hydrolysis curve (AUC) was used to calculate the hydrolysis index (HI) and estimate the glycaemic index (eGI) of each bread. These analyses provide a comprehensive understanding of how the use of different pea flour samples influence starch digestibility patterns and the potential glycaemic response.

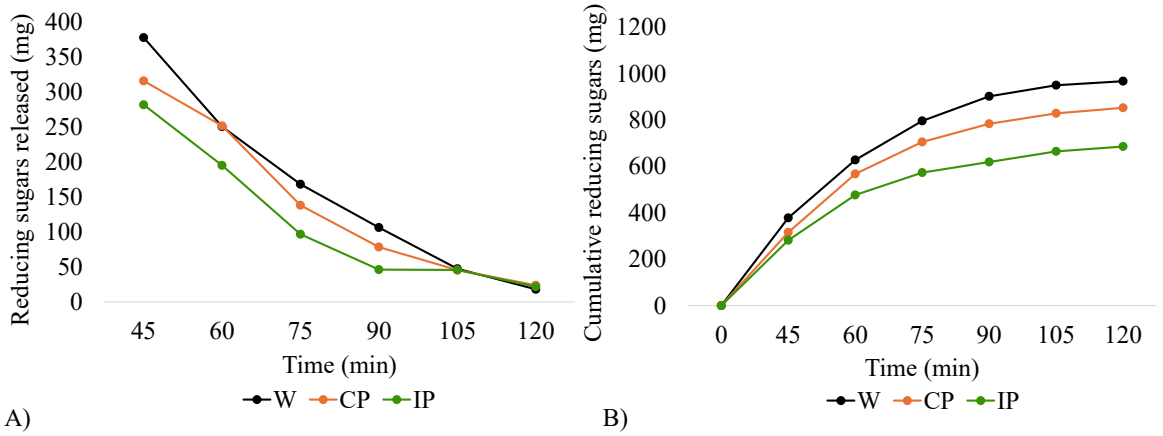


Figure 6.3. A) Reducing sugars released (mg) and B) cumulative reducing sugars (mg) during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

Table 6.5. Hydrolysis index (HI) and estimated glycemic index (eGI) of breads formulated with 35%:65% (pea:wheat).

Bread*	HI	eGI
W	100^{a}	94.6^{a}
CP	87.3^{ab}	87.7^{ab}
IP	71.7^{b}	79.1^{b}

*W: Wheat bread; CP: breads containing commercial pea flour; IP: breads containing Irish pea flour. Data presented as means ± standard deviation. Values with a different letter (a-b) are significantly different ($p < 0.05$) along the column.

Measuring reducing sugar release at each time point during the in vitro digestion process provides valuable insight into starch breakdown and sugar availability throughout the process. As shown in Figure 6.3 A, the release of reducing sugars was highest at 45 minutes and gradually declined over time for all samples. The lowest sugar release was observed in the bread containing Irish pea flour (IP). This can be explained by its lower starch content compared with the bread made using commercial pea flour (CP) (Table 4.1, **Chapter 4**). The lower starch content in IP bread results in a lower proportion of digestible carbohydrates.

The cumulative reducing sugar profile is shown in Figure 6.3 B. and Table 6.5. Particularly at 120 minutes, the difference in cumulative sugar release between digested breads is noticeable (Figure 6.3 B), with the wheat control (W) exhibiting the highest values, followed by breads containing commercial pea flour (CP), and finally those made with Irish pea flour (IP). This may be attributed to the reduced starch content combined with higher levels of dietary

fibre and protein in the bread samples which had pea flour incorporations (**Chapter 4 Table 4.1**), and the strong starch-protein interactions in pea that delay enzymatic hydrolysis (Johnston et al., 2023; Lu et al., 2018). Similar reductions in starch digestibility have also been reported in other studies on legume-enriched breads (Angioloni & Collar, 2012; Collar et al., 2014; Jordge et al., 2022; Lu et al., 2018).

Starch digestibility is closely linked to the hydrolysis index (HI) and glycemic index (GI). The GI serves as a measure of the postprandial blood glucose response following the consumption of carbohydrate-rich foods (Magallanes-Cruz et al., 2017). The estimated GI results were aligned with the sugar release results, where the wheat bread control exhibited the highest value (94.6), followed by the CP breads (87.7) and the IP samples (79.1). Similar trend was found for the hydrolysis index results. The HI and eGI of W bread was significantly higher ($p < 0.05$) than that of IP sample, indicating that the incorporation of pea flour can effectively reduce starch digestibility and, consequently, the potential glycaemic response of the bread.

6.5.3 Protein digestibility: proteins and free amino acids

Protein digestion from bread formulations was evaluated using a protein quantification kit (BCA Protein Assay Kit) which, on digesta, primarily detects larger peptides, typically above ~3 kDa according to the manufacturer of the kit (Scientific, 2022). The proteins released during digestion, were identified and subsequently grouped according to their respective families. Amino acid quantification and characterization was carried out. Protein and free amino acid contents were analysed in the supernatant of the digesta after centrifugation. These analyses collectively revealed how ingredient source affects protein breakdown dynamics over digestion time.

Total protein and amino acid concentration over digestion time

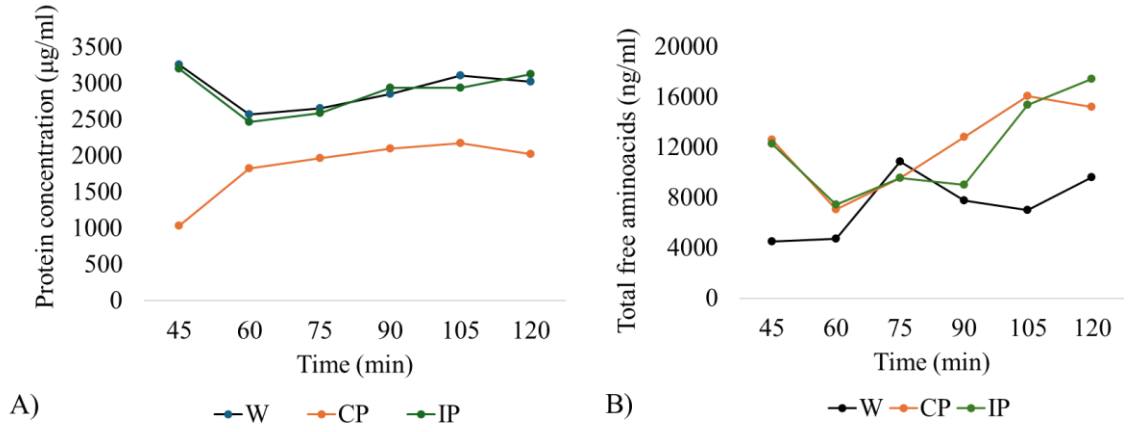


Figure 6.4. A) Protein concentration released (ug/ml) and B) total free amino acids released (ng/mL) during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

Both protein concentration and total free amino acid profiles were measured in parallel over the 120 minutes digestion process to evaluate protein digestion **Figure 6.4 A and B**. Protein concentration indicates the presence of intermediate hydrolysis products, whereas free amino acid levels reflect the extent of breakdown into individual amino acids.

All bread digesta displayed distinct digestion behaviours. The bread containing Irish pea flour (IP) showed a protein release similar to that of the wheat bread (W) but higher than that of the commercial pea bread (CP). Moreover, the free amino acid concentrations in the IP sample were consistently higher than in the other breads across most time points, while the W sample exhibited the lowest levels. These findings indicate that the proteins in the Irish pea bread (IP) were more accessible to digestive enzymes and more efficiently hydrolysed compared with those in the wheat (W) and commercial pea containing (CP) breads. The lower protein concentration observed at all digestion time points in the bread containing commercial pea flour (CP), compared with that containing Irish pea flour (IP), may be attributed to differences composition (**Chapter 4** **Table 4.1**), particle size distribution (**Chapter 4** **Figure 4.1** and Appendix **Table 4.A2**) and distribution of protein families (discussed in Protein profiling section). The commercial pea flour had a significantly lower protein content when compared to the Irish pea flour, which likely contributed to the reduced protein release observed during digestion. In addition, variations in protease inhibitors (available in the

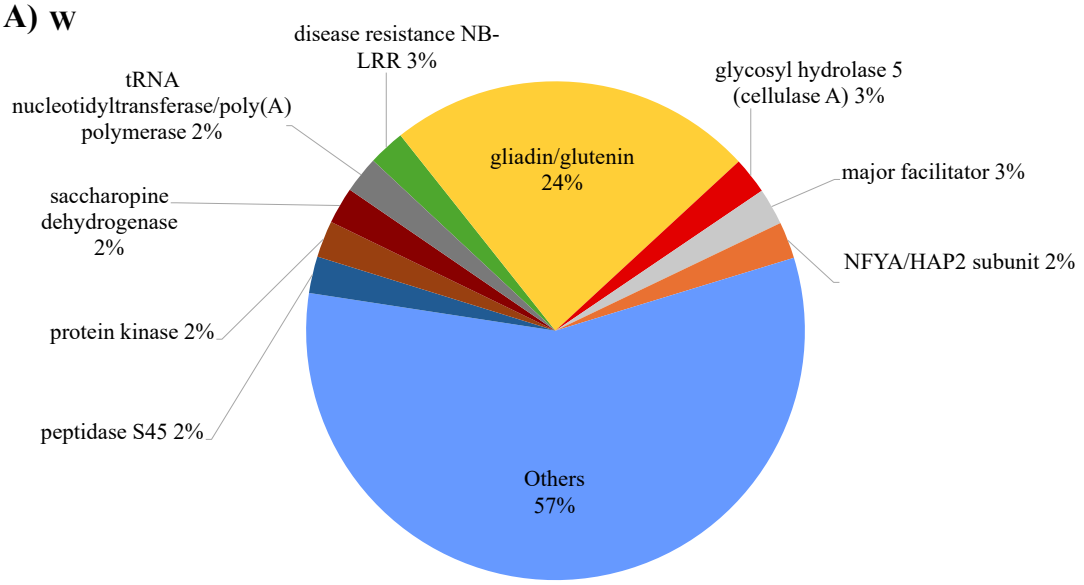
albumin fraction), dietary fibres, phytic acid, and phenolic compounds present in peas may influence their overall protein digestibility (Yang et al., 2023). The different particle size distributions between CP and IP samples can impact the dough matrix structure and, consequently, bread quality. In **chapter 4** it was demonstrated that the bread containing commercial pea (CP) exhibited a firmer texture compared to the bread containing Irish pea (IP) (results available in **Table 4.2**). Such structural differences may reduce or delay protein accessibility to digestive enzymes, thereby contributing to its lower protein concentrations (Byars et al., 2021; Olakanmi et al., 2024).

All bread samples showed distinct patterns in amino acid release over time. For wheat bread (W), amino acid concentrations exhibited a peak at 75 min, followed by another marked increase at 120 min. In contrast, breads containing pea flour (CP and IP) showed a substantial release of amino acids at 45 min, indicating faster initial protein hydrolysis, which likely enhances protein bio accessibility. After this early peak, concentrations decreased slightly but rose again from 90 min onwards, continuing to increase until 120 min. The varying rates of protein digestion reflect differences in molecular conformation and amino acid composition which affects enzyme accessibility between wheat and pea derived proteins.

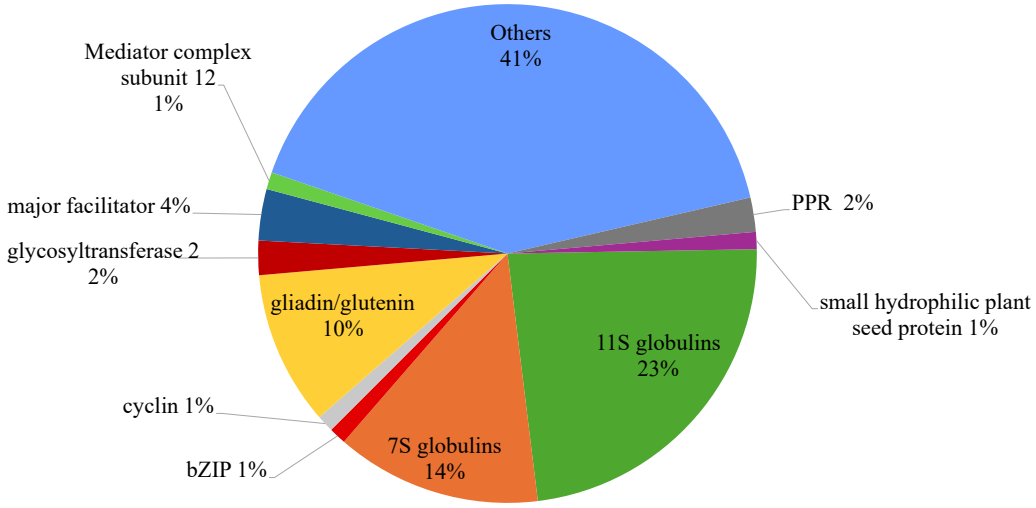
From the consistently high protein concentrations observed across all digestion time points, particularly at 120 min, it is evident that protein digestion is far from completion, confirming ongoing protein hydrolysis. Studies on legumes and wheat have demonstrated that protein digestion can continue for up to 200 minutes (Berrazaga et al., 2020; Khrisanapant et al., 2021). This study provides valuable insight into the rate of protein hydrolysis during digestion; however, extending the digestion time would allow a more complete assessment of total protein availability in these breads.

Protein profiling

A) W


disease resistance NB-LRR 3%
tRNA nucleotidyltransferase/poly(A) polymerase 2%
glycosyl hydrolase 5 (cellulase A) 3%
gliadin/glutenin 24%
major facilitator 3%
saccharopine dehydrogenase 2%
NFYA/HAP2 subunit 2%
protein kinase 2%
peptidase S45 2%
Others 57%

B) CP


Others 41%
Mediator complex subunit 12 1%
major facilitator 4%
PPR 2%
glycosyltransferase 2 2%
small hydrophilic plant seed protein 1%
gliadin/glutenin 10%
11S globulins 23%
cyclin 1%
7S globulins 14%
bZIP 1%

C) IP

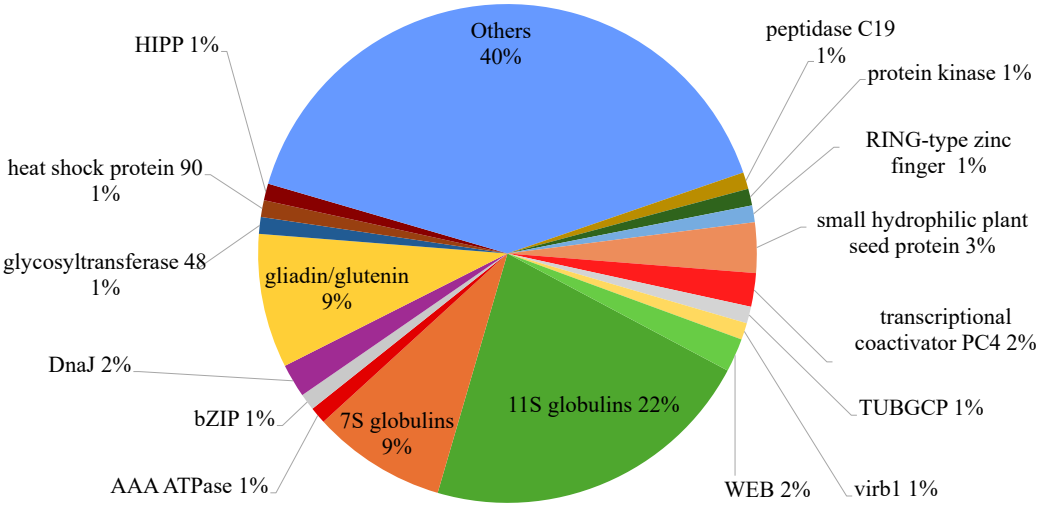

Others 40%
HIPP 1%
peptidase C19 1%
protein kinase 1%
RING-type zinc finger 1%
heat shock protein 90 1%
small hydrophilic plant seed protein 3%
glycosyltransferase 48 1%
gliadin/glutenin 9%
transcriptional coactivator PC4 2%
DnaJ 2%
TUBGCP 1%
11S globulins 22%
bZIP 1%
7S globulins 9%
WEB 2%
virb1 1%
AAA ATPase 1%

Figure 6.5. Distribution of parent proteins identified in the digested bread samples.

The distribution of the parent proteins identified in the digested bread samples is represented in Figure 6.5. The dominant protein family detected in the wheat bread was gliadin/glutenin, whereas in the breads containing pea flour, the main families were gliadin/glutenin, 7S globulins, and 11S globulins. Gliadin and glutenin are the main gluten proteins in wheat. They are responsible for the dough viscoelasticity, which gives the bread its aerated matrix and overall structural integrity (Buehler, 2021). The main protein constituents in peas are 7S vicilin, 7S convicilin and 11S legumin, belonging to the 7S and 11S globulin families, respectively (Yang et al., 2023). These storage proteins represent about 70–80% of the total pea protein content and are crucial in defining the functional and nutritional characteristics of legume-derived foods (Kimura et al., 2008).

Between the two pea formulations, the proportion of identified 7S globulin proteins was notably higher in the bread containing commercial pea flour (CP) than in that made with Irish pea flour (IP). This difference is likely associated with variations in the intrinsic composition of the grains. The ratio between 11S and 7S globulins plays a key role in determining the functional properties and nutritional quality of pea proteins and consequently influences their digestion behaviour (Liang & Tang, 2013; Yang et al., 2023). Glutenin and glisdin,

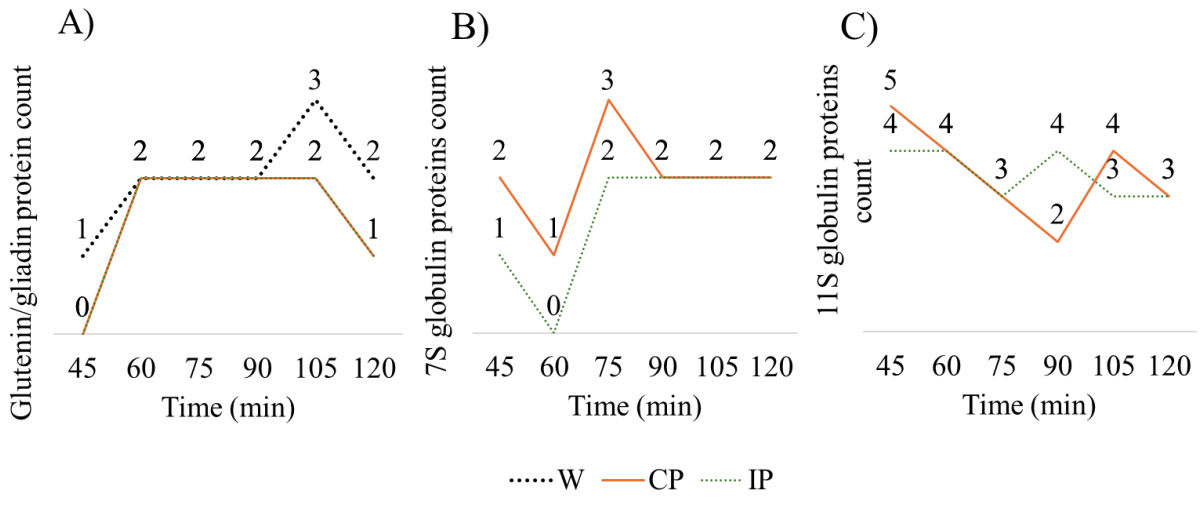


Figure 6.6. Protein counts (1-4) of major protein families identified during in vitro digestion of breads containing 35% pea flour and 65% wheat flour over 120 minutes: A) gliadin/glutenin, B) 7S globulin and C) 11S globulin families.

Figure 6.6 shows the main parent protein profiles and their respective protein counts, representing the number of proteins identified in each protein family at each time point. For example, a value of 4 indicates that four proteins were identified within that family. The samples exhibited fluctuations in protein counts at different time points, reflecting the dynamic nature of digestion and indicating that proteins are hydrolysed at varying rates depending on the protein type and the surrounding food matrix, which influence structural organization and enzyme accessibility (Chen et al., 2023; Ogawa et al., 2018).

Regarding the glutenin and gliadin families, fewer proteins were identified over time in the samples containing pea flour compared with the wheat control. This reduction may be attributed to the partial dilution of wheat proteins in the composite formulations and to potential interactions between wheat proteins and pea-derived enzyme inhibitors, which can modify protein detectability and slow enzymatic accessibility during digestion (Ogawa et al., 2018). Although the presence of pea flour may reduce wheat protein digestibility, its inclusion greatly increases total protein content and improves the amino acid balance, making the overall nutritional value of the bread considerably higher.

As shown in the parent protein distribution (Figure 6.5), the CP sample presented a higher number of identified 7S globulins. The 11S globulin family showed fluctuations in protein counts during digestion between the pea samples, suggesting gradual unfolding and stepwise hydrolysis of these proteins. The observed variations indicate compositional differences between the two pea flours, which influence pea protein functionality and interactions within the bread matrix (Husband et al., 2024; Ogawa et al., 2018). These differences may affect the number of proteins detected, as well as the rate and overall pattern of protein degradation during digestion.

Amino acid profiling

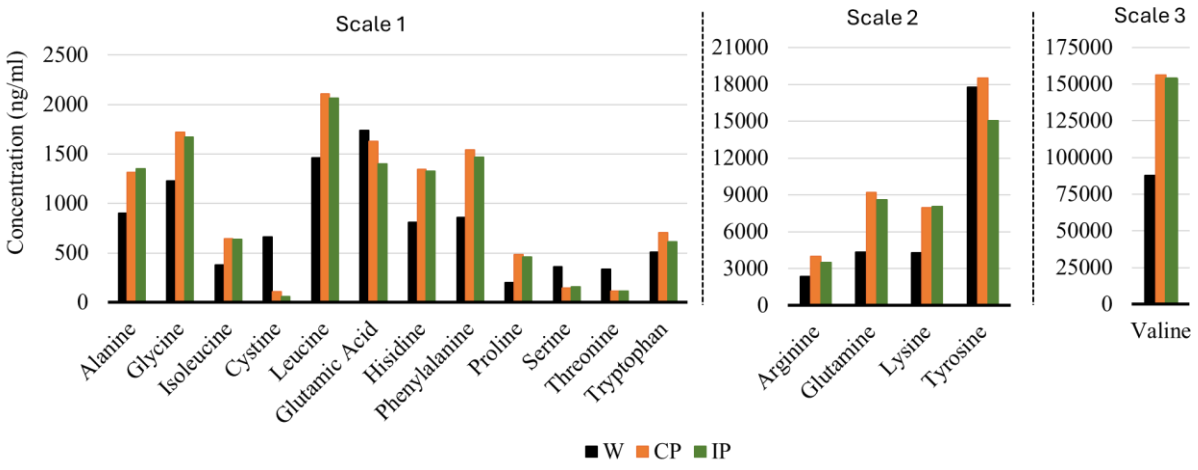


Figure 6.7. Free amino acid concentration (ng/mL) during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

The amino acids detected in the highest concentrations in all the samples were valine and tyrosine, while other amino acids such as lysine, leucine, and glutamine were also among the most represented amino acids. This pattern is consistent with earlier studies showing that branched-chain amino acids (such as valine and leucine) and aromatic amino acids (including tyrosine, glutamine, arginine, and tryptophan) are typically predominant in the free amino acid profiles of bread and legume-based products (Igual et al., 2021; Omoba & Isah, 2018; Würtz et al., 2013).

Figure 6.7 showed that the incorporation of pea flour (CP and IP) led to a clear increase in the concentration of most of the amino acids when compared to wheat bread (W), in particular valine, glutamine, lysine and leucine. This can be attributed to the higher protein content of pea flour and its complementary amino acid profile (Monika Wójcik et al., 2021). These findings align with previous research showing that bread enriched with pea protein has higher levels of free amino acids after in vitro digestion (Igual et al., 2021; Monika Wójcik et al., 2021; M. Wójcik et al., 2021). Igual et al. (2021) reported a significant ($p < 0.05$) increase in valine, lysine, tyrosine, and glutamine with pea protein addition, consistent with the results of this present study. In addition, cereals commonly lack sufficient levels of lysine and leucine

(Kowalski et al., 2022). Therefore, combining cereals with pulses improved the overall protein quality by balancing their complementary amino acid profiles, creating a more nutritionally complete composition.

6.6 Conclusion

The present study provides new insights into how the inclusion and composition of pea flour influence the nutritional and digestive behaviour of wheat and composite breads. Visual observations and SEM imaging revealed marked structural differences among formulations, with pea-enriched breads (containing commercial (CP) and Irish pea (IP) flours) exhibiting denser matrices that disintegrated more slowly during digestion compared with wheat bread. In addition, SEM micrographs revealed a more pronounced reduction of starch granules over time in the breads containing pea flour (CP and IP). The incorporation of pea flour, particularly the Irish pea flour, markedly reduced starch digestibility, as evidenced by lower cumulative reducing sugar release, hydrolysis index, and estimated glycaemic index values compared with wheat bread. Protein digestion patterns also varied depending on the type of pea flour incorporated. The bread formulated with Irish pea flour (IP) exhibited higher protein and free amino acid concentrations throughout digestion than the bread sample which contained commercial pea flour (CP), indicating higher protein hydrolysis efficiency. These effects were associated with differences in flour composition, particle size, and protein family distribution, particularly the 7S globulin. Overall, the findings demonstrate that the compositional characteristics and particle size of pea flours significantly modulate both starch and protein digestibility. Incorporating locally produced flour has been shown to enhance the nutritional quality of bread by improving protein digestibility and reducing glycaemic potential, while also contributing to more sustainable food production

7 Advanced dynamic in vitro digestion of pea-wheat composite bread: influence of roasting, ultrasound, and microwave processing on pea flour

Advanced Dynamic In Vitro Digestion of Pea–Wheat Composite Bread: Influence of Roasting, Ultrasound, and Microwave Processing on Pea Flour

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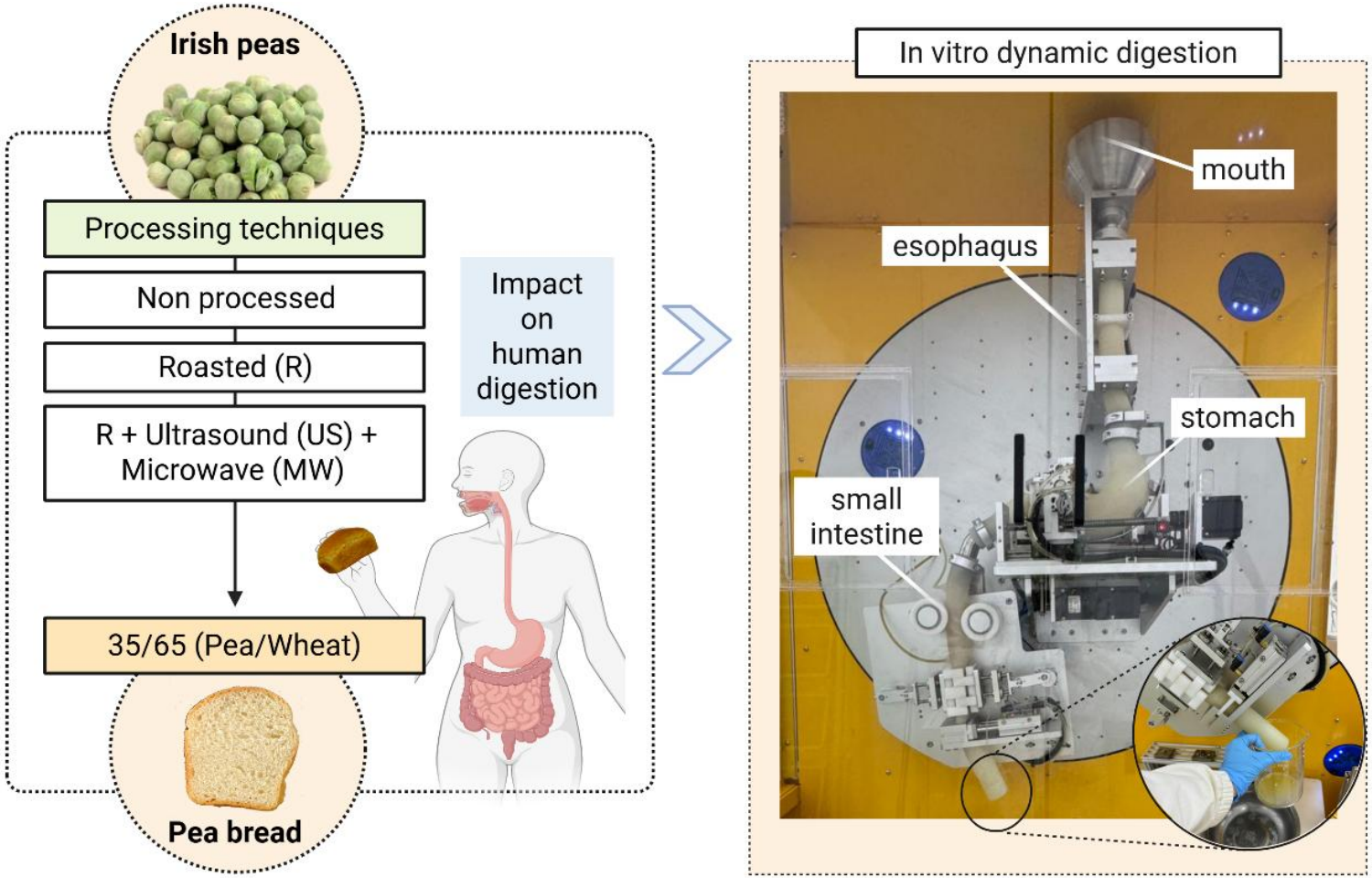
7.1 Graphical abstract



7.2 Abstract

Bread enriched with pea flour offers nutritional advantages while promoting the use of sustainable ingredients. Processing techniques can enhance the techno-functional properties and flavour of legumes, yet their impact on digestion remains underexplored. This study evaluated how roasting, ultrasound, and microwave treatments applied to Irish pea flour influence the structural and digestive properties of composite pea–wheat breads (35:65, pea:wheat). Three bread formulations were prepared, blending wheat flour with untreated Irish pea flour (IP bread), with roasted Irish pea flour (IP+R bread), and with a combined roasted-ultrasound–microwave treated Irish pea flour (IP+US+MW+R bread). These breads were digested in a dynamic in vitro digestive system (DIVHS-IV). Visual and microscopic analyses revealed progressive matrix disintegration, with the IP+US+MW+R bread samples displaying a more disrupted structure. This bread exhibited a slower release of reducing sugars and lower estimated glycaemic index values, while the IP bread showed higher protein and free amino acid concentrations, indicating greater protein digestibility. Overall, pea flour which was subjected to combined processing treatments resulted in modulated starch and protein digestion kinetics, supporting their potential for nutritionally improved, lower-glycaemic breads.

7.3 Introduction

As a staple food consumed globally, bread plays a central role in many diets. Wheat bread, in particular, dominates the western market largely due to its unique dough viscoelastic properties, which facilitate the production of loaves with optimal texture, volume, and sensory appeal (Dong & Karboune, 2021). A strong reliance on wheat can increase the vulnerability of the food supply chain, particularly given that climatic conditions are not always optimal for its cultivation. Intensive wheat farming may also have negative effects on the environment and soil health, encouraging the search for alternative crops (Biduski et al., 2023; Y. Wang & C. Jian, 2022). Additionally, wheat starch is highly digestible, which can result in increased postprandial glycaemic responses (Giuberti et al., 2016). With increasing emphasis on improving the nutritional quality of staple foods, the use of legume flours in bread formulations has gained significant interest as a promising approach to enhance nutritional profile and bioactive compounds (Chinma et al., 2025; Foschia et al., 2017; Maçãs et al., 2024a).

Peas (*Pisum sativum L.*), a legume species well-suited to the temperate climate of Ireland, represent an important source of high-quality nutrients. They are rich in protein, dietary fibre, essential minerals and a variety of bioactive compounds including polyphenols and antioxidants (Binti Mohd Zaini et al., 2021). From an environmental perspective, peas contribute to sustainable agriculture practices through soil nitrogen fixation. This naturally enhances soil fertility, which can reduce the need for synthetic fertilizers (Cusworth et al., 2021; Uebersax et al., 2023). Pea flour incorporation in bread products offers promising nutritional advantages, such as enhanced protein and a more complete amino acid profile, alongside a decrease in rapidly digestible starch fractions (Kali Kotsiou et al., 2021; Maçãs et al., 2025; O. Moreno-Araiza et al., 2023). However, these benefits may be accompanied with technical and acceptability challenges, as the presence of pea flour can alter dough viscoelastic

properties and crumb structure, texture, flavour and potentially influence nutrient release and absorption during digestion (K. A. Millar et al., 2019; Young et al., 2019).

Processing technologies applied to ingredients can play a crucial role in modifying the structural characteristics of the resulting food products. For example techno-functional properties and flavour profiles of the breads may be enhanced, which can lead to greater consumer acceptability (Bourré et al., 2019; Guldiken et al., 2022; K. A. Millar et al., 2019; Nilufer-Erdil et al., 2012). Dry heating during the roasting process is a common technique applied to cereals and grains. It, can result in a number of biochemical alterations: these include the onset of Maillard reactions, a reduction in antinutritional compounds, protein denaturation, and starch gelatinization (van Rooyen et al., 2022; Young et al., 2019). While these changes may improve nutrient availability, they may also lead to the formation of protein-starch complexes or Maillard reaction products that hinder enzymatic digestion (C. Yang et al., 2019).

Processing ingredients using ultrasound (US) technology involves the application of high-intensity, low-frequency sound waves, which generate cavitation, the rapid formation and collapse of microscopic bubbles (Amanda Gomes Almeida Sá et al., 2022; Singh et al., 2025). Microwave (MW) treatment uses non-ionizing electromagnetic waves, producing heat primarily through mechanisms such as dipolar rotation and ionic conduction (Rivera et al., 2025; Amanda Gomes Almeida Sá et al., 2022). Ultrasound and microwave treatments have shown evidence of improving the physicochemical properties of pea flour and other pulse-based ingredients, including molecular weight, particle size, and zeta potential (Amanda Gomes Almeida Sá et al., 2022; Sun et al., 2020). These treatments can also enhance the techno functional properties of flours and ingredients, such as pasting behaviour, water solubility, and foaming and emulsifying capacity (Deng et al., 2022a; Rivera et al., 2025; Amanda Gomes Almeida Sá et al., 2022). Such effects occur due to the action of US and MW inducing microstructural disruptions and alter molecules conformation, leading to changes in macromolecular structure, consequently, influencing digestion by affecting nutrient

accessibility and enzymatic breakdown during gastrointestinal processing (Deng et al., 2022a; Rivera et al., 2025; Wang et al., 2023). When ultrasound and microwave treatments are combined with roasting, they can cause more pronounced structural changes in both proteins and starch (Wang et al., 2020). These modifications may alter how susceptible these macronutrients are to digestive enzymes, potentially affecting the rate and extent of nutrient release during digestion. However, the exact consequences of these combined treatments remain unclear, as their effects depend heavily on the specific properties of the material and processing conditions. To date, there is limited research investigating the impact of combined processing treatments, and no results specifically on roasting, ultrasound and microwave on pea-containing products digestion, highlighting a gap in knowledge.

The Dynamic Human Stomach System (DIVHS-IV) used in this study is an advanced in vitro method that mimics the physiological environment of the human upper gastrointestinal tract through controlled temperature, secretion dynamics, pH variation, and simulated peristaltic activity (Li et al., 2020; Zhou et al., 2025). The INFOGEST static digestion model is highly valued and widely adopted for its practicality and reliability, yet it does not mimic the intricate physiological conditions of the human gastrointestinal tract. The DIVHS-IV system allows for dynamic monitoring of nutrient release over time, providing a more physiologically accurate representation of human digestion. Therefore, the present work aimed to compare the digestibility of three bread types, where 35% of wheat flour in a yeasted wheat bread formulation was replaced with 35% of untreated Irish pea flour (IP), 35% of roasted Irish pea flour (IP+R) and 35% of ultrasound, microwaved and roasted treated Irish pea flour (IP+US+MW+R).

7.4 Materials and methods

7.4.1 Raw material, grain processing and bread-making

Irish-grown pea grains (variety Carrington), supplied by Wynnes Ltd (Kildare, Ireland), and strong wheat flour obtained from Shakleton's Milling (Ireland) were used to prepare the pea–wheat composite bread. These grains were divided into three groups: Untreated, treatment via roasting, and combined ultrasound-microwave and roasting treatments.

For the roasting process, pea samples (1 kg) were roasted at 170 °C for 16 minutes in a deck oven (MacPan, Thienne, Italy). Before roasting, the Irish pea grains were soaked in water for 27 minutes to standardise their moisture content. This pre-soaking step ensured comparable hydration levels with the samples used in the ultrasound and microwave treatments, which were also conducted in aqueous conditions.

For the combined treatments, the pea samples were initially processed using a combined ultrasound-microwave treatment with an E200 extractor (Idco SAS, Marseille, France), equipped with a cooling system to maintain the sample temperature below 30 °C. Given the limited literature available on this topic, several preliminary trials (data not shown) were performed to determine the optimal operating conditions. Irish pea grains (1kg) were mixed with tap water (2 L) and treated in the E200 system. The microwave operated at 1000 W, while ultrasound was applied at full amplitude (200 W) and a frequency of 25 kHz. The combined treatment lasted 27 minutes in total, consisting of four 3-minute treatment cycles separated by 5-minute cooling intervals to prevent overheating. After treatment, the samples were roasted at 170 °C for 16 minutes in a deck oven (MacPan, Thienne, Italy).

Untreated and treated whole peas were ground using a hammer mill (Labmill 3100, Perten®, Sweden), following the optimized procedure described by Maçãs et al. (2023).

Bread formulations consisted of a composite flour containing 35% pea flour and 65% strong wheat flour (Table 7.1). Additional ingredients included salt (2%), unsalted butter (1%),

improver (1 %), yeast (1.5%), and water, the quantity of which was adjusted based on Mixolab water absorption values. The ingredients, baking process and equipment employed were consistent with those reported by (Maçãs et al., 2025).

Table 7.1. Abbreviations of bread formulations (produced with 35% pea flour:65% wheat flour) used in this study.

Abbreviation	Bread Formulations
IP	Irish pea flour untreated: wheat flour (35:65)
IP+R	Irish pea flour + roasting: wheat flour (35:65)
IP+US+MW+R	Irish pea flour + ultrasound+ microwave + roasting: wheat flour (35:65)

7.4.2 In vitro dynamic digestion of bread

Digestive solution preparation:

Simulated digestive electrolyte solutions were prepared in advance by dissolving the listed components (Table 7.2) in 1L of distilled water. Prior to digestion, enzymes were freshly added (Tables 7.3 and 7.4) before each digestion and sonicated for 10 minutes after their addition to ensure complete dissolution.

Table 7.2. Enzyme concentrations and respective manufacturer.

Experimental Material	Manufacturer (Country)
a-Amylase (35 U/mg)	Macklin (China)
Pepsin (3000 U/mg)	Aladdin Scientific (China)
Pancreatin from porcine pancreas (4*USP)	Sigma Corporation (USA)
Lipase (30-90 U/mg)	Sigma Corporation (USA)
Ox Bile Powder (40%)	Yanaye Bio-technology (China)

Table 7.3. Enzyme addition to the simulated digestive electrolyte solutions

Simulated Fluid	Enzyme(s) Added	Volume of Electrolyte Solution
Saliva	0.285 α-amylase	100 mL
Gastric Juice	0.0417 g pepsin	100 mL
Intestinal Juice	1.75 g pancreatin + 0.30 g lipase	100 mL
Bile	6.00 g bile salts	100 mL

Table 7.4. Preparation of simulated digestive electrolyte solutions

Electrolyte	Saliva	Gastric Juice	Intestinal Juice	Bile
NaCl (g)	0.116	5.504	14.024	10.518
KCl (g)	0.148	1.648	1.128	0.752
$NaHCO_3$ (g)	2.100		6.776	11.57
NaH_2PO_4 (g)		0.532		
$CaCl_2$ (g)		0.604		
NH_4Cl (g)		0.612		
KH_2PO_4 (g)			0.160	
$MgCl_2$ (g)			0.100	
HCl (mL, reference)	0.300	2.800	0.100	
pH	6.800	1.600	7.800	8.200

Digestion simulation:

This study used the esophagus, stomach and duodenum compartments of the DIVHS-IV dynamic in vitro digestion system (Xiao Dong Pro-health Instrumentation Co., Suzhou, China) to simulate digestion, following the conditions described in the Appendix (**Table 6.A1**). A labelled diagram of the system is available in J. Wang et al. (2022). The equipment was maintained at 37 °C, with peristaltic movement controlled by compressed air. Bread samples (12 g) were ground with a mortar and pestle to simulate oral mastication, then mixed with simulated saliva in a beaker and incubated in a 37 °C water bath for 5 minutes. During the digestion process, gastric fluid was released at a rate of 0.29 mL/min, intestinal fluid at a rate of 0.29 mL/min, and bile at a rate of 0.19 mL/min. The sample obtained after the oral digestion phase was then transferred into the dynamic digestion model. Digestion lasted 2 hours, with samples taken from the duodenum at 45 minutes and every 15 minutes thereafter until 120 minutes. Digestion time was determined based on methodologies previously applied (Capriles et al., 2009; Ye et al., 2025; H. Zhang et al., 2022).

The 120-minute digestion duration was chosen in accordance with previous in vitro digestion studies on bakery products. Digesta were promptly collected, centrifuged at 4000 rpm for 4 minutes at −4 °C. Supernatant and sediment were separated, flash-frozen in liquid

nitrogen to stop enzymatic activity, and stored at−20 °C for analysis. Each sample was prepared in triplicate.

7.4.3 Scanning electron microscopy (SEM) of bread and digesta

To examine the microstructural characteristics of both undigested bread and the digested residues (digesta), samples were initially freeze-dried to remove moisture and stabilize the structure. The freeze-dried materials were carefully fractured to expose internal surfaces and mounted on aluminium stubs using double-sided conductive carbon tape. To improve surface conductivity and minimize charging under the electron beam, the mounted samples were sputter-coated with a thin layer of gold–palladium (approximately 8–10 nm) using a sputter coater (e.g., Quorum Q150R, UK). Morphological observations were conducted using a Hitachi S-3400N scanning electron microscope (Hitachi, Japan) operated in low-vacuum mode at an accelerating voltage of 15 kV. Imaging was performed at multiple magnifications (ranging from ×50 to ×2000) to visualize both the overall porous structure and fine structural details of the bread matrix and digesta particles. For each bread formulation, three independent samples were prepared and analysed, and representative micrographs were collected from different regions of the specimen to account for heterogeneity.

7.4.4 Reducing sugars and Estimated Glycemic Index

The concentration of reducing sugars was measured using the 3,5-dinitrosalicylic acid (DNS) colorimetric assay, following the method originally described by Miller (1959). Prior to analysis, samples were diluted tenfold (1:10, v/v) with deionized water. For the assay, 1 mL of the diluted sample was mixed with 1 mL of DNS reagent and incubated in a boiling water bath at 100 °C for 7 minutes. After cooling to room temperature, absorbance was recorded at 540 nm using a microplate reader (Infinite M Nano, Tecan, **S**witzerland). Glucose solutions ranging from 0 to 1 mg/mL were used to generate a calibration curve. Final sugar

concentrations were calculated from the standard curve and adjusted according to the dilution factor. Analysis was performed in triplicate.

At each sampling point, the total amount of reducing sugars released was calculated by multiplying the measured concentration (mg/mL) by the corresponding volume of digesta (mL). The cumulative release over time was determined by summing the total reducing sugar from each interval with the accumulated value from the preceding time points (Woolnough et al., 2010).

The kinetics of reducing sugar release, along with the estimation of the Hydrolysis Index (HI) and Glycaemic Index (eGI), were evaluated following the method described by Graça et al. (2020). The HI was calculated (Equation 6) by dividing the area under the hydrolysis curve (AUC 0–120 min) of the samples bread by that of the reference food, white wheat bread (W).

$$(6) \quad HI = \frac{AUC\ of\ product}{AUC\ Reference\ food} \times\ 100$$

The estimated eGI was determined using Equation (7).

$$(7) \quad eGI = (0.549 \times HI) + 39.71$$

7.4.5 Protein digestibility

The protein concentration in the supernatant digesta was measured using the Pierce BCA Protein Assay Kit (Cat. No. 23225, Thermo Scientific). The analysis was performed in triplicate, following the manufacturer's protocol, with bovine serum albumin (BSA) as the calibration standard. Protein digestibility was then estimated by comparing the amount of protein released into the digesta for each time point.

7.4.6 Protein identification

Digested samples were resuspended in 100 µL of 0.1% formic acid, and 5 µL of each sample was injected for chromatographic separation using a Thermon Scientific Ultimate 3000

UHPLC system, coupled to a Q-Ex active Plus mass spectrometer (Thermon Fisher Scientific, USA). Protein identification was carried out using Protein Discoverer software version 2.0 (Thermon Fisher Scientific). The resulting MS/MS spectra were searched against a database comprising an in silico tryptic digest of Pinaceae protein sequences obtained from Uniports (release 2018/02; 2,891,190 entries), along with the crape database to account for common contaminant proteins. Identified proteins results were filtered using a global false discovery rate (FDR) threshold of 1%. The final dataset was analysed to determine proteins of particular interest.

For each identified protein, protein family classifications based on sequence similarity were retrieved from the UnIPRot database (https://www.UnIPRot.org/). Pie charts were subsequently generated based on protein family assignments corresponding to the unique set of identified proteins from each bread sample, illustrating the relative proportions of different protein families within each sample. Plots of the major protein families were generated to depict the number of identified proteins across digestion time, allowing evaluation of temporal trends in protein availability throughout the digestion process.

7.4.7 Free amino acids

Free amino acids were quantified using an ACQUITY UPLC class system (Waters, Milford, MA, USA) coupled to a tandem mass spectrometer equipped with a Turbo Spray ion source (6500+Qtrap, AB Scitex). Separation was performed on a Glycan BEH Amide column (1.7 am, 2.1 × 100 mm) at 35 °C, with a flow rate of 0.3 mL/min and an injection volume of 1 all.

The mobile phases:

A) water containing 20 mM ammonium formate and 0.35% formic acid

B) 90% acetonitrile with 20 mM ammonium formate and 1.15% formic acid.

The gradient was described in terms of the proportion of mobile phase A), with phase B) constituting the balance to reach 100% at each stage: 5% A) (0–2 min), 20% A) (2–7 min), 60% A) (7–10 min), maintained at 60% A) (10–12 min), and then returned to 5% A) (12.1–15 min) for re-equilibration.

Analyses were performed in positive or negative ionization modes using multiple reaction monitoring (MRM) with optimized Q1→Q3 transitions for each amino acid (e.g., alanine 90.1→44.1). Source parameters were curtain gas 40 psi, GS1 55 psi, GS2 50 psi, source temperature 650 °C and ion spray voltage ±3000 V. Data acquisition and integration were performed in Analyst 1.7.3, and concentrations were calculated from external calibration curves and expressed as ng/mL of extract.

7.5 Results and discussion

7.5.1 Visual and microscopic analysis of bread digesta

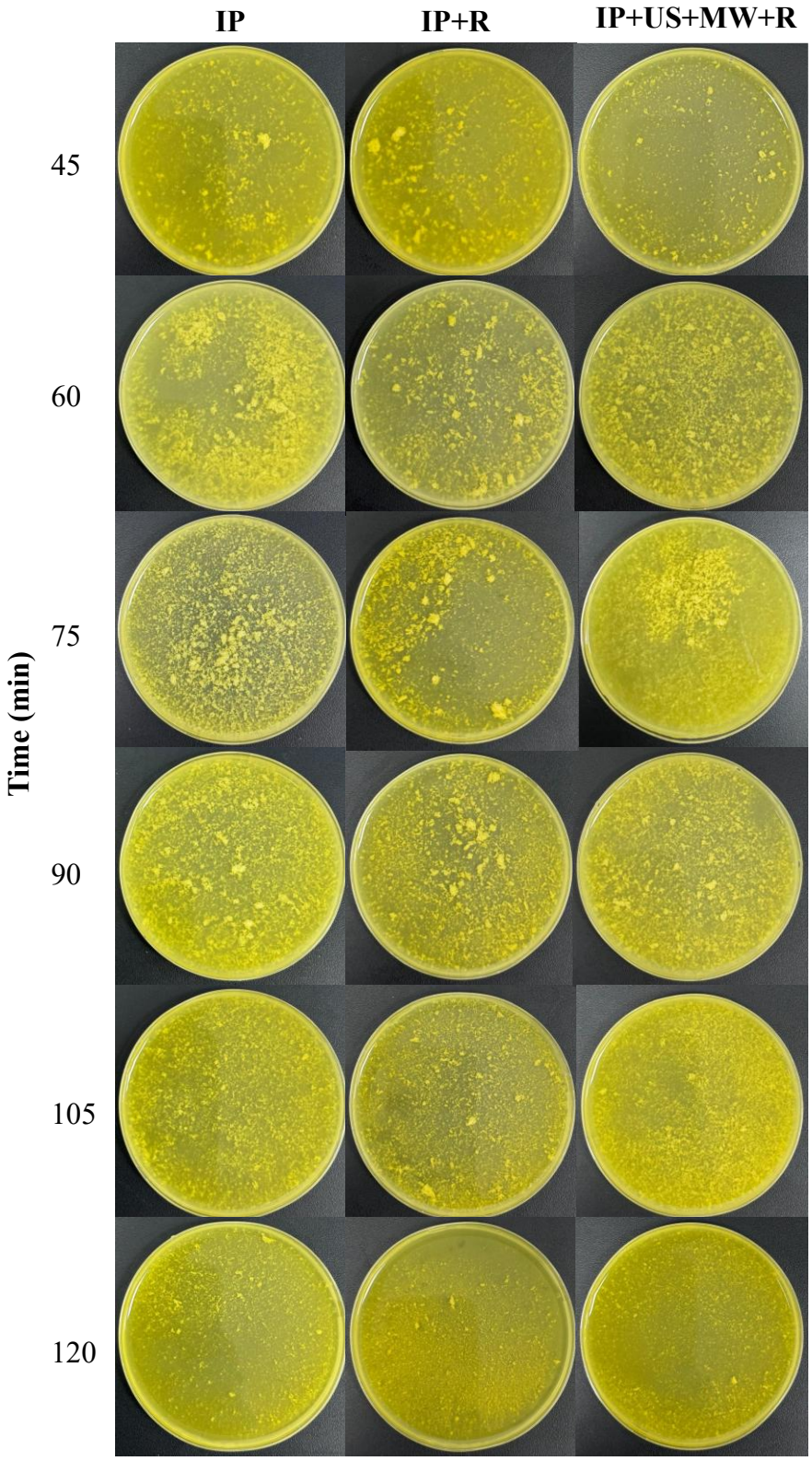


Figure 7.1. Visual appearance of digesta from breads produced with 35%:65% (pea:wheat), during in vitro digestion (45–120 min).

Figure 7.1 presents the visual changes in the bread digesta over the digestion period. Images of the digesta showed progressive disintegration, with larger fragments at 45 min and a clear reduction in particle size and sedimentation by 120 min. Up to 90 minutes of digestion, untreated Irish pea and roasted Irish pea bread digesta (IP and IP+R) showed more sedimentation and denser aggregates, indicating less physical disintegration through digestion. In contrast, the ultrasound + microwaved + roasted Irish pea bread digesta (IP+US+MW+R) remained more turbid and evenly dispersed, showing finer particle breakdown. Microwave and ultrasound treatments can promote starch–protein matrix modification and partial cell wall disruption, resulting in greater porosity and structural disintegration. These findings are also confirmed in the SEM images, highlighted with yellow arrows in Figure 7.2, at magnification 2000x, 0 and 120 minutes. Such disintegration may enhance enzyme accessibility and potentially accelerate nutrient release during digestion. A study on chestnut starch reported that combined ultrasound–microwave treatment significantly damaged granule surfaces, reduced crystallinity and weakened structural integrity, supporting the idea of matrix disruption and increased enzyme access (Wang et al., 2020).

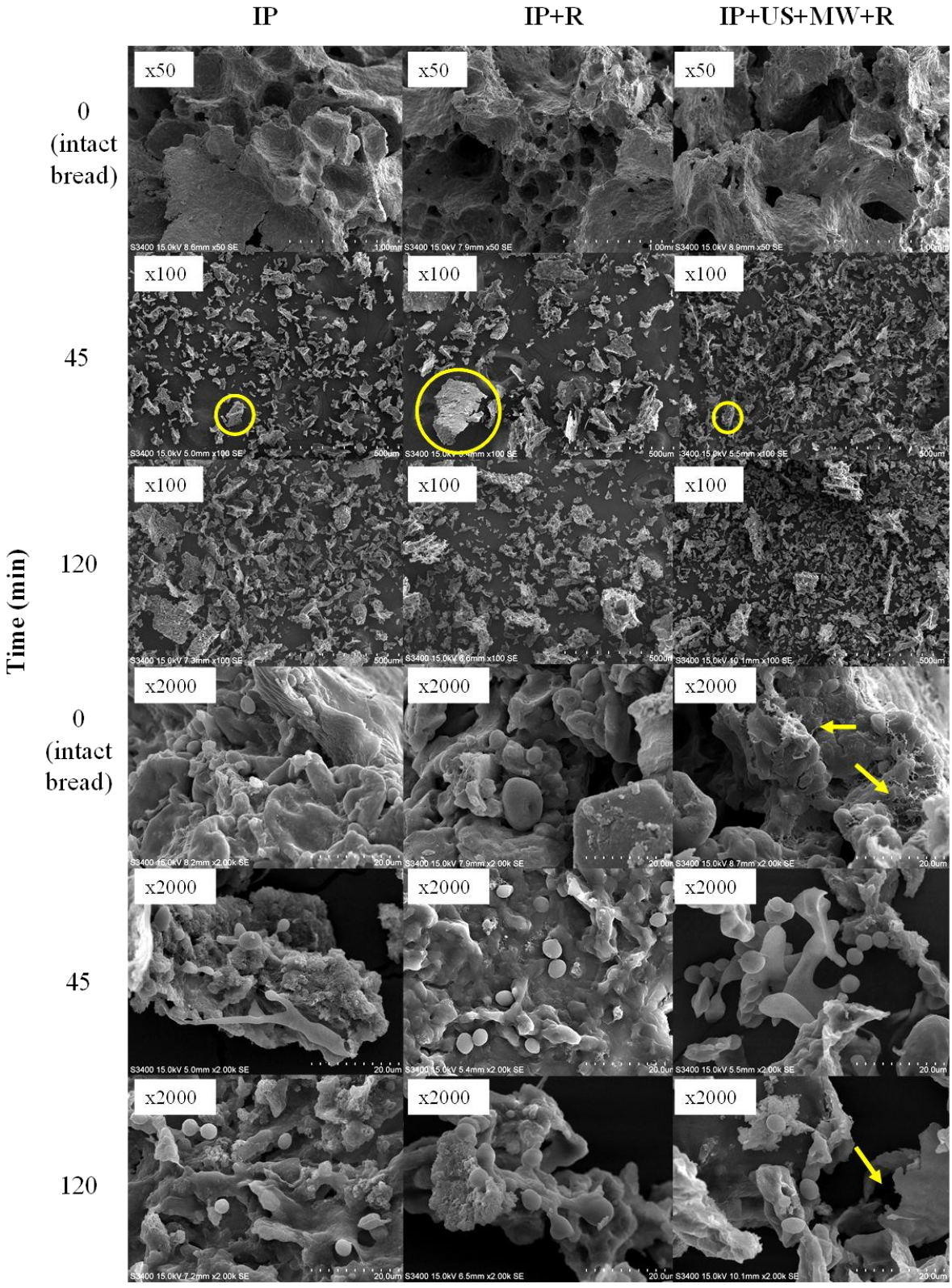


Figure 7.2. SEM micrographs showing the bread digesta from 35%:65% (pea:wheat), at 0 min (intact bread), 45 min, and 120 min of in vitro digestion.

SEM images of breads are presented in **Figure 7.2** revealed structural differences in response to digestion. At 45 minutes of digestion, the IP+R bread digesta displayed larger, more compact, and irregular particles, with differences in particle size highlighted by yellow circles at 100× magnification. This behaviour can be attributed to the roasting treatment, which induces starch pre-gelatinization and protein denaturation (Young et al., 2020), which can lead to a tighter starch–protein network in certain regions, thereby delaying enzymatic access during the initial digestion phase. IP+US+MW+R and IP bread samples appeared more fragmented and dispersed.

Bread digesta containing roasted pea flour treated with ultrasound, microwaving, and roasting (IP+US+MW+R) exhibiting a markedly more disrupted starch–protein matrix compared to bread digesta containing untreated pea and roasted pea (IP and IP+R), particularly evident at 2000x magnification at 0 and 120 minutes, as indicated by yellow arrows. These structural changes are consistent with the effects of ultrasound and microwave treatment, which have been shown to induce surface damage, pore formation and loss of crystallinity in food matrices and likely influence digestibility (Farrokhi et al., 2025; Sadowska et al., 2003; Solaesa et al., 2022).

All samples displayed clear evidence of digestion, with visible matrix disintegration and granule erosion confirming active enzymatic action throughout the digestion period. At 2000× magnification, starch granules were abundant at 45 minutes but appeared progressively fewer by 120 minutes of digestion

7.5.2 Starch digestibility: reducing sugars and eGI

The composition and processing of bakery products strongly influence gastrointestinal digestion by affecting the release and absorption of nutrients and bioactive compounds such as starch, proteins, fibres, vitamins, minerals, and phytochemicals (Fernández-Tomé, 2024). Starch, the main carbohydrate in baked goods, shows variable digestibility depending on its

type, crystallinity, and interactions within the food matrix, as well as gelatinisation during baking and retrogradation during storage (Udenigwe & Fogliano, 2017; Zhu, 2015b). In addition, dietary fibre, present in considerable amounts in peas, contributes to health benefits, including a reduced glycaemic response (Dhingra et al., 2012). Starch digestion dynamics were examined in each pea-wheat bread sample (IP, IP+R and IP+US+MW+R). The reducing sugars released (mg) during in vitro digestion was quantified over the 120-minute digestion period. The area under the hydrolysis curve (AUC) was used to calculate the hydrolysis index (HI) and estimate the glycaemic index (eGI) of each bread.

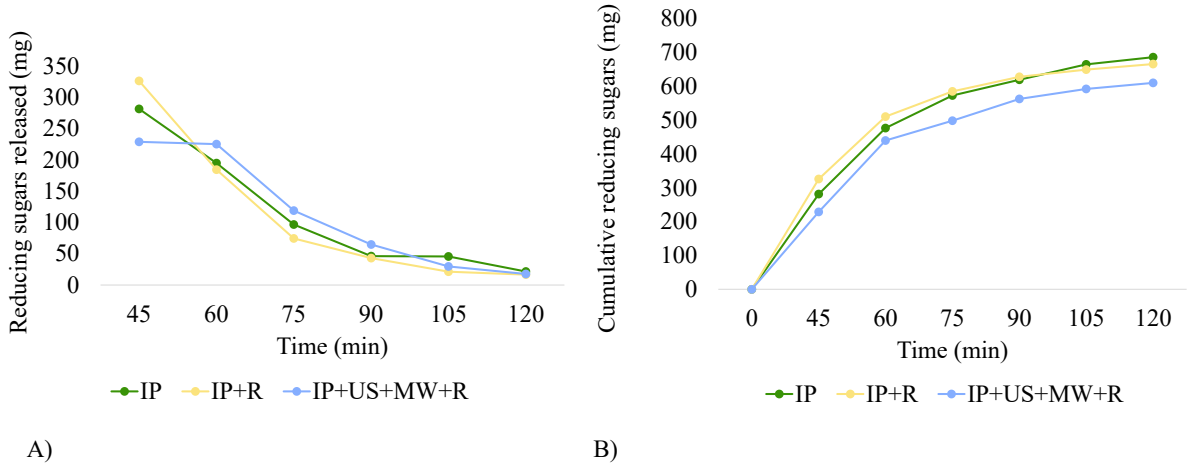


Figure 7.3. A) Reducing sugars released (mg) and B) cumulative reducing sugars (mg) during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

Table 7.5. Hydrolysis index (HI) and estimated glycemic index (eGI) of breads from 35%:65% (pea:wheat).

Sample*	HI(%)	eGI
IP	71.7[a]	79.1[a]
IP+R	74.4[a]	80.5[a]
IP+US+MW+R	67.0[a]	76.5[a]

*IP: breads containing untreated Irish pea flour; IP+R: breads containing roasted Irish pea flour; IP+US+MW+R: breads containing ultrasound, microwave and roasted Irish pea flour. Values with a different letter are significantly different ($p < 0.05$) along the column.

In Figure 3 A, a progressive decrease in reducing sugars is observed for bread digesta containing untreated pea and roasted pea (IP and IP+R). The bread digesta containing pea flour

treated with ultrasound, microwaving, and roasting (IP+US+MW+R) exhibited a constant release up to 60 minutes, followed by a subsequent decrease in rate. Initially, the IP+US+MW+R bread sample exhibited the lowest values throughout most of the digestion, suggesting a more gradual sugar release. These results indicate that ultrasound and microwave treatments effectively reduced sugar availability, suggesting a potential to modulate sugar release with low or null impact on the bread proximate composition, as can be observed in the **Chapter 5** Table 5.2 . Ultrasound induces cavitation, which can disrupt granule crystallinity and depolymerize starch chains (Flores-Silva et al., 2017). Microwave induces structural modifications in starch, including the formation of resistant starch through starch recombination at high power, along with changes in molecular vibrations and crystallinity, which can reduce enzymatic accessibility (Deng et al., 2022a). Similar results were found by Zhao et al. (2019), were ultrasound and microwave treated lotus seed starch green tea treated samples showed lower digestion efficiency compared to the untreated sample. In another study, microwave and ultrasound process and toughening treatments were used to modify the physical properties of wheat starch. The combination of all treatments produced the most effective results, showing the slowest starch digestibility (K. Zhang et al., 2021).

The **Figure 7.3 B** present the cumulative reducing sugars, that is the total accumulated amount of reducing sugars produced over time. Although IP and IP+R show comparable trends, IP+US+MW+R exhibits a more gradual and reduced sugar release.

Glycemic index (GI) reflects how quickly carbohydrates in food are broken down and absorbed, affecting blood glucose levels after eating. As starch digestibility plays a major role in this process, eGI is often used to characterize the nutritional profile of starchy foods (Magallanes-Cruz et al., 2017). **Table 7.5** shows that both the hydrolysis index (HI) and the estimated glycaemic index (eGI calculated from HI) were lowest for the IP+US+MW+R sample (eGI = 76.5) compared with the IP (eGI = 79.10) and IP+R (eGI = 80.5) breads. Although these differences were not statistically significant ($p > 0.05$), the trend aligns with

the slower reducing sugar release observed in Figure 7.3, suggesting that the structural modifications induced by ultrasound and microwave treatments may contribute to a reduced glycaemic potential. These treatments may have altered molecular conformation, leading to the formation of a denser matrix with stronger agglomerates that limit enzyme accessibility during digestion. The bread digesta containing roasted pea flour (IP+R) presented similar starch digestibility to bread digesta with untreated pea flour (IP) which was unexpected, as thermal treatments such as roasting are generally associated with reduced starch digestibility, due to starch pregelatinizing and the formation of more compact or less enzyme-accessible structures (Simsek et al., 2016; Urooj & Puttaraj, 1994). However, when roasted pea flour is incorporated into a bread matrix, characterized by a high-water content and exposure to elevated baking temperatures the effects of roasting on starch structure and digestibility may be diminished (Lu et al., 2018). A similar trend was reported by Lu et al. (2018) , where roasting slow digestive starch and resistant starch in pea flour where both found to increase, however these fractions were lost during bread baking.

White wheat bread was used as the reference food to calculate the HI, and by definition, its HI value was assigned as 100% (Goñi et al., 1997). This value is considerably higher than those obtained for all pea-containing breads, where the maximum HI recorded was 74.4% for the IP+R breads, and the lowest HI of 67% was obtained for the IP+US+MW+R bread samples, highlighting the significant reduction in glycaemic potential resulting from pea flour incorporation.

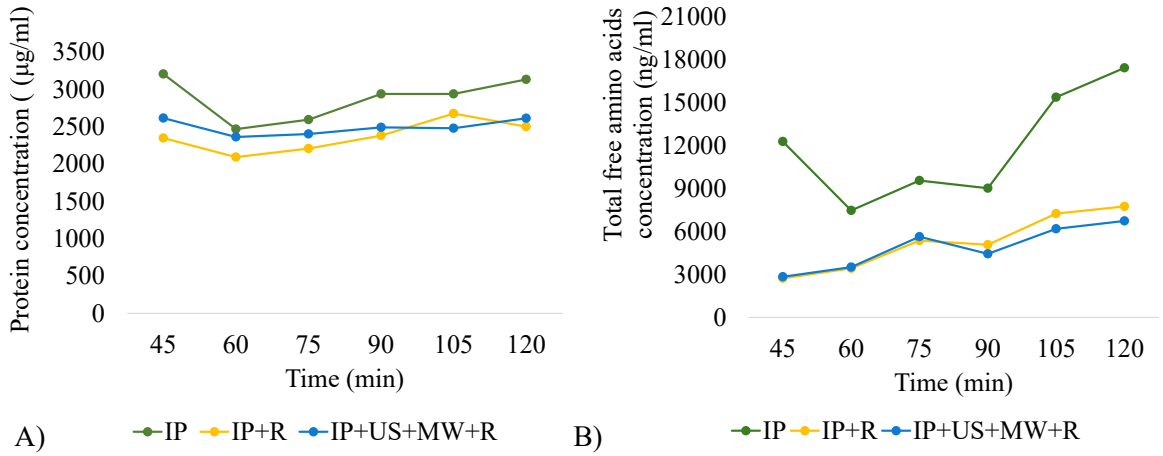
7.5.3 Protein digestibility: proteins and free amino acids

During digestion, proteases and peptidases break down dietary proteins into smaller molecules such as amino acids, dipeptides, and tripeptides within the gastrointestinal tract (Wu, 2010). These molecules may exhibit bioactive properties, including antioxidant, antihypertensive, hypolipidaemic, antidiabetic, and anticancer effects (Udenigwe & Fogliano,

2017). The rate and extent of protein release during digestion are largely determined by enzyme accessibility, which refers to how effectively digestive enzymes can reach and act on their target substrates within the food matrix. This process can be hindered by factors such as protein folding, aggregation, crosslinking, or interactions with other macromolecules like starch, fibre and antinutritional compounds which restrict enzyme penetration (Sá et al., 2020; Cuihong Yang et al., 2019). Processing techniques can change raw material characteristics such as the porosity, particle size, protein conformation and matrix interactions (Amanda Gomes Almeida Sá et al., 2022; Sun et al., 2020). These transformations can improve or, in some cases, reduce the efficiency of enzymatic hydrolysis during digestion (Sá et al., 2020).

Protein digestibility was assessed using a combination of complementary analytical methods. The quantification of protein concentration was carried out using the BCA Protein Assay Kit, which, primarily detects larger peptides, typically above ~3 kDa (Scientific, 2022). The proteins released during digestion were identified. These proteins were further classified into their respective families. In parallel, free amino acid profiling was conducted to quantify and identify the smallest protein digestion products. Together, these methods offer a comprehensive assessment of how processing treatments influence the extent and progression of protein digestion over time.

Total protein and amino acids concentration over digestion time



A) B)

Figure 7.4. A) Protein concentration (ug/ml) and B) total free amino acids released during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

Protein and total free amino concentrations are presented on **Figure 7.4 A and B**. While protein concentration reflects the presence of partially hydrolysed proteins, free amino acid analysis shows how much the protein has been broken down into its smallest building blocks. Protein and free amino acid concentrations were consistently higher for the bread containing untreated Irish pea (IP) compared to the bread samples containing roasted, ultrasound and microwave pea flour (IP+R and IP+US+MW+R). This is particularly evident in the free amino acid results (**Figure 7.4 B**), indicating that a higher protein content has been fully hydrolysed into absorbable form.

The lower protein and free amino acid concentrations observed in the treated breads (IP+R and IP+US+MW+R) may be attributed to structural modifications induced during processing. Roasting, ultrasound, and microwave treatments can promote protein aggregation, crosslinking, and interactions with other macromolecules such as starch, fibre, and polyphenols. These molecular rearrangements can lead to the formation of more compact or complex matrices that restrict enzyme accessibility. during digestion. While mild heat treatment is known to enhance digestibility by disrupting peptide cross-links and inactivating natural protease inhibitors, more intense thermal processing, such as roasting, promote the formation of protein structures that resist enzymatic hydrolysis (Bhutta et al., 2013). In particular, the Maillard reaction, can lead to covalent cross-linking between sugars and lysine side chains, potentially rendering up to 30% of lysine nutritionally unavailable (Bhutta et al., 2013; Kohli & Singha, 2024). According to previous studies, roasting, microwave, and ultrasound treatments have been reported to both enhance and reduce protein bioavailability (Donata Drulyte & Vibeke Orlien, 2019; Kayembe & van Rensburg, 2013; King et al., 2024). The overall effect depends on the nature of the material and the specific processing parameters applied (Maçãs et al., 2024a). While processing can improve certain functional and sensory

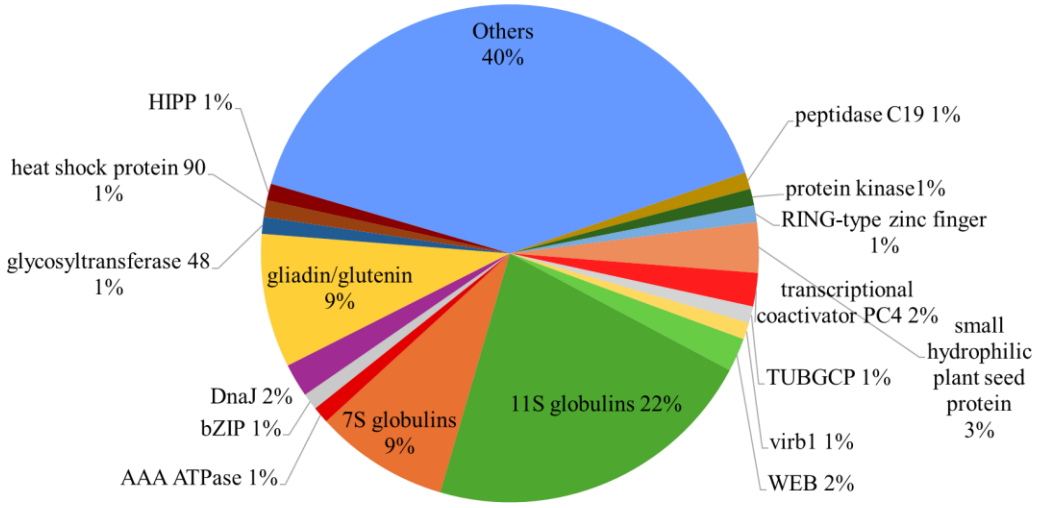
attributes, it may simultaneously alter protein structure in ways that hinder enzymatic hydrolysis and nutrient bio accessibility.

In addition, the fluctuations observed in free amino acid concentration over time suggest that proteins are digested at different rates, likely influenced by their molecular structure, amino acid composition, and accessibility to enzymatic cleavage. The bread samples contain both wheat and pea proteins, each with distinct structural and functional properties. As a result, the timing of free amino acid release is influenced by how each protein source responds to the applied treatments and its susceptibility to enzymatic breakdown.

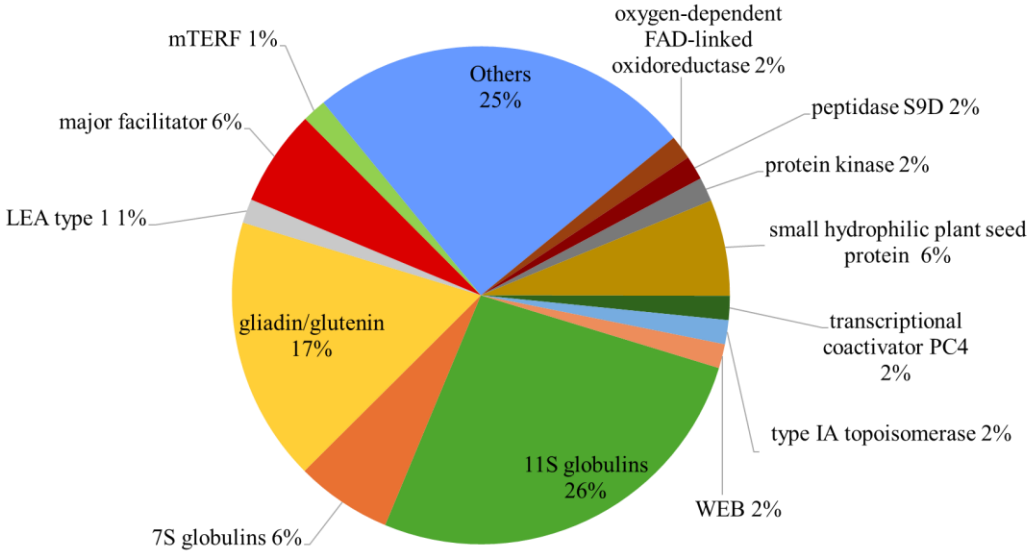
The continuous high levels of protein observed throughout the digestion period, especially at 120 minutes, suggest that protein hydrolysis is still actively occurring and far from complete. This highlights the need for extended digestion durations to better evaluate the extent of protein breakdown. Previous research on legumes and wheat has shown that protein digestion can continue for as long as 200 minutes (Berrazaga et al., 2020; Khrisanapant et al., 2021).

Protein profiling

A) IP



B) IP+R



C) IP+US+MW+R

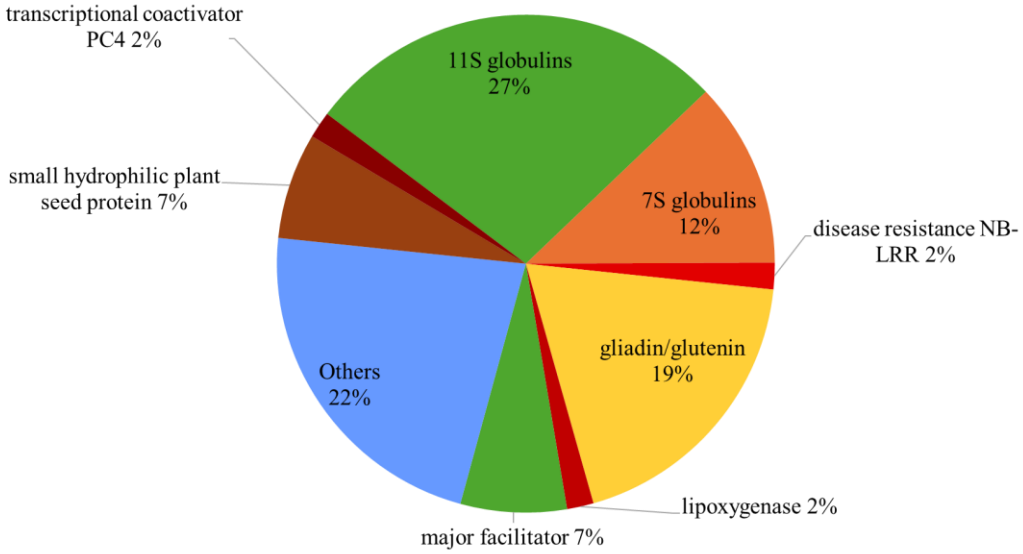


Figure 7.5. Distribution of parent proteins identified in the digested bread samples.

The distribution of the parent proteins identified in the digested bread samples is represented in **Figure 7.5**. As expected, the dominant protein families detected across all samples were gliadin/glutenin, 7S globulin proteins, and 11S globulin proteins. Gliadin and glutenin, are gluten proteins and the most abundant proteins in wheat. They are mainly responsible for bread dough elasticity and structure due to their viscoelastic properties (Buehler, 2021). The major protein components in pea are 7S vicilin, 7S convicilin and 11S legumin. These belong to 7S and 11S globulins parent proteins respectively and are the primary storage proteins, accounting for approximately 70%-80% of the total protein content in peas (Kimura et al., 2008; Yang et al., 2023). These globulins play a key role in determining the functional and nutritional properties of legume-based foods, therefore, their presence is important for defining the structural, textural, and emulsifying properties of products as well as their protein quality and digestibility.(Kimura et al., 2008).

The bread samples containing processed peas (IP+R and IP+US+MW+R) showed a higher relative abundance of gliadin/glutenin and 11S globulin proteins, indicating that roasting, ultrasound, and microwave treatments altered the distribution of these protein families. Processing influences protein digestibility through both endogenous and exogenous factors. Endogenous factors relate to the protein structural characteristics and how processing alters its conformation, while exogenous factors involve interactions within the food matrix, such as with carbohydrates, lipids, and anti-nutritional compounds (D. Drulyte & V. Orlien, 2019; Ohanenye et al., 2022).

However, despite the lower protein diversity observed in the bread containing untreated Irish pea (IP), it exhibited greater overall protein digestibility, as indicated by higher protein and free amino acid concentrations. This suggests that, while processing may enhance protein diversity, it can also induce aggregation or conformational rearrangements that reduce enzyme accessibility and consequently slow down overall protein digestion.

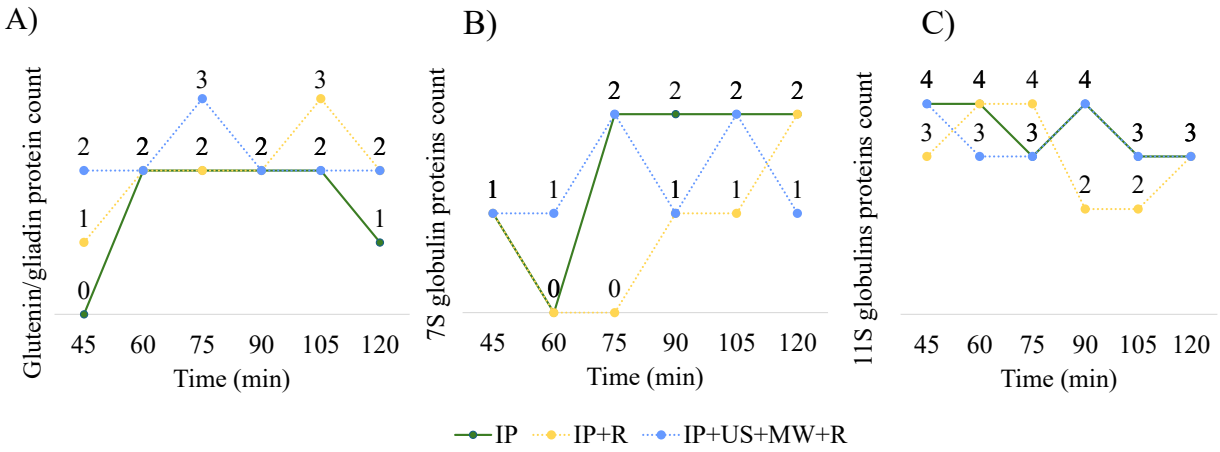


Figure 7.6. Protein counts (1-4) of major protein families identified during in vitro digestion of breads containing 35% pea flour and 65% wheat flour over 120 minutes: A) gliadin/glutenin, B) 7S globulin and C) 11S globulin families.

Figure 7.6 presents the main parent protein profiles along with their corresponding protein counts, indicating how many proteins were detected in each family at the different digestion time points. For instance, a count of four means that four distinct proteins were identified within that specific protein family. All samples exhibited fluctuations in protein counts at different time points, reflecting the dynamic nature of digestion and indicating that proteins are hydrolysed at varying rates depending on their structural properties and accessibility. All bread samples contained the same ingredients; however, the pea flour underwent different processing treatments. These variations likely result in differences in protein unfolding and aggregation induced by the processing treatments (roasting, ultrasound, and microwave), which can modify enzyme-molecules interactions (D. Drulyte & V. Orlien, 2019; Ohanenye et al., 2022). Overall, these findings align with the protein concentration data and provide deeper insight into how ingredient processing influences protein release patterns.

Amino acid profiling

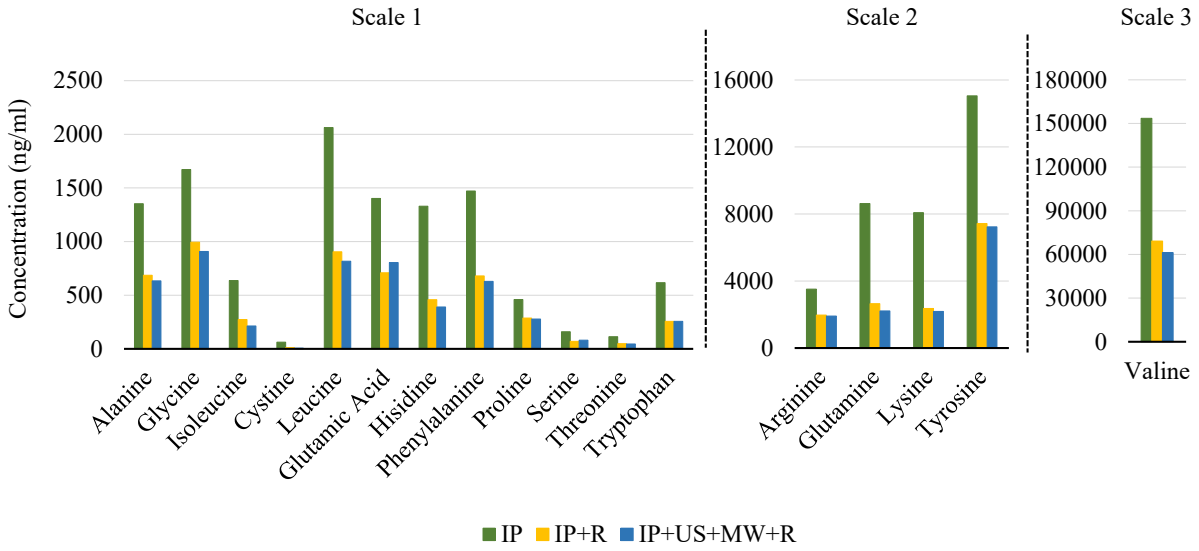


Figure 7.7. Free amino acids concentration during in vitro digestion of breads from 35%:65% (pea:wheat) over 120 minutes.

Cereals commonly lack sufficient levels of certain essential amino acids, with lysine, threonine, and leucine being the most frequently limiting (Kowalski et al., 2022). Therefore, combining cereals with pulses improves the overall protein quality by balancing their complementary amino acid profiles, creating a more nutritionally complete composition (Maçãs et al., 2023).

In the free amino acid profile (**Figure 7.7**) of the digested bread samples, valine, tyrosine, glutamine, and lysine were present at relatively high concentrations, highlighting their potential nutritional relevance. Valine, an essential branched-chain amino acid, contributes to skeletal muscle protein synthesis, tissue repair and energy metabolism (Sharma et al., 2023). Tyrosine, classified as a conditionally essential amino acid, functions as a biochemical precursor for catecholamine neurotransmitters (dopamine, norepinephrine, epinephrine), which can influence cognitive performance, mood regulation and the physiological response to stress (Jongkees et al., 2015). Glutamine, although typically considered a non-essential amino acid, assumes conditional essentiality during periods of metabolic stress. Under such conditions, it supports cellular repair processes and contributes to the attenuation of tissue damage and inflammation, thereby enhancing recovery efficiency (Newsholme et al., 2023). Lysine, an

essential amino acid, is indispensable for protein synthesis (Tomé & Bos, 2007). The predominance of these amino acids aligns with previous findings that branched-chain (e.g. valine and leucine**)** and aromatic amino acids (e.g. tyrosine**,** glutamine, arginine and tryptophan) dominate the free amino acid profiles of bread and legume-enriched products due to their high representation in storage proteins and the release promoted during proteolysis (Igual et al., 2021; Omoba & Isah, 2018; Würtz et al., 2013).

The **Figure 7.7** showed that the overall reduction in total free amino acids observed in **Figure 7.4 B** for the bread digesta containing pea flour treated with ultrasound, microwaving and roasting (IP+R and IP+US+MW+R) is also evident at the level of individual amino acids when compared to the bread digesta containing untreated Irish pea (IP). This decrease is likely related to protein aggregation induced by processing, which can hinder enzyme accessibility during digestion, as previously discussed.

7.6 Conclusion

Combined ultrasound, microwave and roasting treatments applied to Irish pea flour significantly influenced the structural and digestive behaviour of composite pea-wheat breads. In particular, when compared with untreated pea flour (IP), processing induced distinct modifications evident in the visual appearance of the digesta, SEM micrographs, and in both starch and protein digestibility. The digesta and SEM images revealed progressive matrix disintegration and particle fragmentation, with the processed flours (IP+US+MW+R) micrographs showing a more disrupted starch-protein matrix, suggesting increased structural breakdown. Although the processed samples showed greater matrix disruption, suggesting that could potentially easier enzyme access, this was not confirmed. The processing induced new structural molecular and matrix rearrangements that hindered enzymatic hydrolysis of proteins. Consequently, the sample containing the combined treated pea flour (IP+US+MW+R) exhibited a slower release of reducing sugars, which was also reflected in lower estimated

glycaemic index values. The protein release of this sample was also lower when compared to the bread containing untreated pea flour. Untreated pea bread (IP) exhibited higher protein and free amino acid concentrations, suggesting faster protein digestibility. Protein profiling further revealed that processing altered the detectable distribution of major protein families, likely due to conformational rearrangements. These findings emphasize that both endogenous factors (molecular structure) and exogenous factors (matrix interactions) govern protein and starch digestibility in complex food systems. Overall, the results highlight that combined ultrasound-microwave-roasting treatments can modulate starch and protein digestion kinetics, contributing to the development of legume-enriched breads with reduced glycaemic potential.

8 Research findings and future directions

8.1 General discussion

The increasing demand for plant-based foods, driven by health, environmental and ethical concerns, continues to transform the bakery sector. Bread, as one of the most widely consumed food products globally, represents a suitable product for the incorporation of highly nutritious plant-based ingredients. Among these, pulses are of growing interest due to their high protein and fibre content, as well as their potential to improve the sustainability of food systems. However, the use of pulses such as peas remains limited by their impact on dough viscoelasticity, loaf volume, crumb texture and flavour attributes. This thesis addressed these challenges through a multi-level approach: evaluating conventional and novel processing techniques for Irish-grown peas, determining their effects on grains, flour, dough, and bread quality, applying spectroscopic tools for quality assessment and classification, and finally investigating their influence on digestibility using a dynamic in vitro model. Irish-grown peas were specifically selected because they represent a sustainable, locally available protein source that can strengthen national agri-food systems while reducing reliance on imports, making their optimisation particularly relevant for both environmental and economic goals.

The literature review, **Chapter 1**, established the current state of knowledge, demonstrating that processing methods can mitigate some of the negative effects of pulse inclusion in breadmaking and providing evidence to prioritise the most relevant and promising techniques for further study. It also highlighted the opportunity for rapid, non-destructive tools such as NIR spectroscopy to better characterise and monitor flour and bread quality changes.

Milling strongly influences flour functionality and bread quality, yet limited scientific research and commercial knowledge on pea milling made it a key experimental focus. In **Chapter 2**, different milling techniques were applied to pea grains, to characterise the resulting

flour functionality and to assess the rheological properties of composite doughs containing 15% pea and 85% wheat flour. All pea flours showed an increase in protein content compared with wheat flour. Flours produced using the hammer mill and cutting mill exhibited higher fibre levels than those obtained with the roller mill. Hammer milling provided the highest processing efficiency, yielding 93.9% flour. Cutting milling, by contrast, produced flours with a lower yield, coarser particles and more compact starch-protein aggregates, as confirmed by scanning electron microscopy. Roller milling generated flours with the highest pasting viscosity and gel hardness, a behaviour likely linked to its higher starch concentration and lower levels of insoluble dietary fibre.

Following flour characterisation (**Chapter 2**), in **Chapter 3**, breads were prepared using a 15% substitution of wheat flour with pea flour obtained from the different milling techniques. This allowed the direct evaluation of how milling influenced not only flour properties and dough performance but also final bread quality. The inclusion of pea flour improved the nutritional profile of breads, particularly by increasing protein and insoluble dietary fibre levels. The highest fibre contents were observed in breads prepared with cutting-milled and hammer-milled flours, confirming the influence of milling on nutritional outcomes. In contrast, breads produced with pea flour from the roller mill showed a significant reduction in crumb cell number relative to wheat bread, indicating a denser structure and, in general, lower baking quality indicators, suggesting that roller milling is less suitable for bread applications. Hammer-milled breads were the most promising, as they exhibited the slowest rate of crumb firming over storage, indicating better textural stability. In addition, the inclusion of pea flour increased the total amino acid content of bread by approximately 15%, demonstrating its value as a functional ingredient for nutritional enrichment. Due to the higher flour yield and favourable bread quality parameters obtained with hammer milling, as confirmed by statistical analysis, this process was selected for the subsequent research in this thesis.

Near Infra-red spectroscopy (NIR) studies, also included in **Chapters 2** and **3**, examined whether these wheat/pea bread composited (with pea flour produced with the different milling processes) could be distinguished. The use of NIR successfully mirrored results from proximate composition analyses and, through principal component analysis (PCA), clearly discriminated between flour types (roller, hammer and cutting milled). More importantly, NIR could also distinguish the composite breads, despite the relatively low level of pea inclusion (15%). Beyond classification, NIR demonstrated the ability to track bread staling over storage time, grouping samples according to freshness. These results underline the potential of NIR as a practical tool for real-time, non-destructive quality monitoring, particularly in industrial contexts where rapid decisions are important. This finding was particularly noteworthy because the discrimination was achieved without narrowing the dataset to only wheat or only pea breads. Previous studies had already demonstrated that NIR can differentiate within a single product category; however, in this case, breads with inherently different physical and chemical characteristics (wheat breads and pea-enriched breads) were successfully distinguished within the same dataset. This highlights the potential of Near Infra-red spectroscopy in capturing complex compositional and structural differences in bread products. More importantly, it opens new opportunities for practical applications, such as real-time monitoring of freshness and quality across diverse bread types in retail environments.

Beyond milling, in **Chapter 4**, additional treatments (roasting, ultrasound, and microwave) were selected based on insights from the literature and the meta-analysis presented in **Chapter 1.** Emerging technologies such as, ultrasound and microwave are considered energy-efficient and environmentally friendly, and their growing adoption in the food industry reflects their technological feasibility and alignment with sustainable processing trends.

These processes were applied to peas, both individually and in combination. Scanning electron microscopy revealed a more extensive microstructural breakdown in the Irish pea grains treated with ultrasound, microwave and roasting processes (IP+US+MW+R). For the

resulting pea flours, FTIR analysis demonstrated that roasting led to a decrease in starch crystallinity, along with a transition in protein secondary structure from α-helices and β-sheets towards more disordered random-coil conformations. In addition, roasting altered starch gelatinisation and protein structure, consistent with previous literature. Roasted Irish pea flour (IP+R) and flours subjected to the combined treatments (IP+US+MW+R) exhibited higher gel hardness and final viscosity, resembling more closely the pasting profile of wheat flour and indicating improved water retention and structural stability. The study also demonstrated that using ultrasound and microwave separately produced different outcomes compared with their combined application, particularly in terms of microstructure and pasting properties.

The findings from this chapter showed that processing can be strategically tailored to modulate flour behaviour, with roasting providing stability and combined treatments offering greater structural modification for potential use in breadmaking.

Numerous studies in the literature have indicated that incorporating over 20% pulse flour as a substitute for wheat flour in bread is difficult, with few studies reaching 30% substitution levels. To bridge this gap, preliminary trials using roasting, ultrasound, and microwave treated peas established a level of 35% pea flour to be an optimum inclusion level to enhance nutritional value while maintaining acceptable bread quality. **Chapter 5** demonstrated that breads a 35% substitution of wheat flour with treated pea flour enhanced bread protein and fibre levels compared to those made with wheat flour alone. Breads made with untreated Irish pea flour showed poor quality, particularly higher crumb hardness when compared to the wheat control. The application of processing treatments effectively mitigated these drawbacks. The roasting process improved water absorption and moderated undesirable beany flavours, while the combined application of roasting, ultrasound, and microwave (IP+US+MW+R) treatments produced the most favourable results in terms of greater loaf specific volume, soft crumb texture, and a more complex flavour profile, demonstrating that combined processing can offset

the technological and sensory limitations typically associated with high levels of pulse incorporation, including reduced dough viscoelasticity and the presence of off-flavours.

Beyond technological performance, the research presented in this thesis also examined how pea incorporation and processing treatments influence nutrient release and absorption from breads during digestion. While product structure and texture determine consumer acceptance, nutritional quality, particularly protein absorption and glycaemic response is a central driver of innovation in plant-based foods. To delve deeper into this area, digestion studies were carried out using the Dynamic Human Stomach System (DIVHS-IV), which more closely replicates physiological conditions than static in vitro models by simulating gastric secretions, peristaltic movements and dynamic release of nutrients over time. This advanced system allowed for a more realistic assessment of how differences in cereals and pulses (wheat vs. pea), flour characteristics (commercially available pea vs. Irish pea), and processing techniques (roasting, ultrasound and microwave) influence bread digestion, helping to bridge the gap between laboratory findings and expected in vivo outcomes.

In **Chapter 6**, the digestibility of three bread types, control wheat bread (W), bread supplemented with 35% commercial pea flour (CP) and bread containing 35% Irish pea flour (IP), was compared. The study demonstrated that the inclusion of pea flour substantially influenced starch and protein digestion kinetics. Pea-enriched breads exhibited a lower rate of starch release and a reduced eGI compared with wheat bread, confirming the potential of legume incorporation to attenuate postprandial glycaemic response. Among the composite formulations, the bread containing Irish pea flour (IP) showed slower starch digestion yet faster protein release than the commercial pea formulation (CP), suggesting that compositional and structural differences between pea types can affect the digestion behave.

Chapter 7 extended this work further by examining how the pea processing treatments (roasting, ultrasound, microwave) influence digestion of the subsequent pea-enriched breads. Breads containing untreated Irish pea flour (IP), roasted pea flour (IP+R) and flour processed

with roasting, ultrasound and microwave (IP+US+MW+R) were evaluated using the dynamic in vitro digestion model. The results showed that processing markedly influenced nutrient release during digestion. The combined treatments (IP+US+MW+R) promoted a more gradual and sustained release of nutrients, resulting in the lowest estimated glycaemic index. Protein digestibility also varied across treatments, reflecting the structural and matrix modifications induced by processing, with the fastest protein release observed for the untreated Irish pea bread (IP). Overall, the findings demonstrated that advanced processing technologies can be used to modulate starch and protein digestibility, supporting the design of legume-enriched breads with improved nutritional functionality.

8.2 Directions for future research and applications

The findings of this thesis open several promising directions for advancing pulse application and their integration into sustainable food systems. To build on this research, future studies could address key remaining knowledge gaps and technical challenges.

- Hammer milling emerged as the most suitable milling technique for peas: future work could investigate how industrial scale hammer milling will influence the flour performance. Extending this approach to other locally grown pulses would also help to broaden the findings and applications and identify wider opportunities for sustainable food and bakery innovation.
- Roasting, ultrasound, and microwave showed strong potential, particularly in combination. Scaling these treatments to pilot and industrial levels will be critical to evaluate energy efficiency, cost-effectiveness, and practical feasibility, ensuring that technological gains translate to real-world applications.
- Near Infra-Red spectroscopy demonstrated value for classification and staling assessment under laboratory conditions. The next step would be to build robust calibration models across diverse bread formulations and test real-time, inline

applications. Coupled with machine learning, spectroscopy could be embedded in production lines to monitor bread quality continuously, reduce waste and support consistent and timely product standardization.

- Life cycle assessment (LCA) could quantify the environmental impacts of pea production and processing. Such insights would ensure that future innovations in food innovation align with broader goals of reducing greenhouse gas emissions, conserving resources and supporting local agricultural economies.
- From a formulation perspective, particular attention should be given to sensory performance, taste remains a decisive factor for consumer acceptance of pulse-enriched breads. Additional strategies such as enzymatic treatments, hydrocolloids, or sourdough fermentation could also be tested to further improve overall sensory appeal.
- Regarding the impact of pea-enriched breads on health, further studies could investigate how their consumption influences the gut microbiome. As dietary fibre, resistant starch and bioactive compounds present in peas can modulate microbial composition and activity, this represents a promising area to link bread formulation with long-term health outcomes. Such studies could explore shifts in microbial diversity, production of short-chain fatty acids and potential benefits for metabolic health.

By following this roadmap, locally grown peas and pulses could be transformed into high-quality bakery products that are nutritionally valuable, technologically feasible and environmentally sustainable.

8.3 Research impact

This research has advanced both scientific understanding and practical applications for the incorporation of Irish-grown peas into bread, generating outcomes that extend across health, added value products, agriculture, sustainability and the economy. Irish peas represent

an underutilised crop, with limited prior research on their functional properties, processing optimisation and consumer acceptance. By developing milling, processing, analytical and nutritional strategies, this thesis demonstrates how Irish peas can be transformed into a valuable food ingredient for a nutritionally enhanced and technologically optimised bread product.

A key impact of this work lies in its contribution to enhance sustainability and food system resilience. The use of locally grown peas reduces dependency on imported protein sources, shortens supply chains, and lowers the carbon footprint of bread production. Furthermore, peas contribute to improved soil health and nitrogen fixation, making them a valuable component of sustainable crop rotation. When integrated into staple food systems such as bread, these benefits extend from the farm to society level.

From an agricultural and economic perspective, this research plays an important role in supporting farmers by creating new added-value chains for pulse crops and contributing to a growing economy. Optimised milling protocols and processing techniques provide the industry with practical, data-driven strategies to valorise peas.

In terms of scientific innovation, the thesis has contributed to transforming science through pioneering research relating to use of advanced processing methods and new applications of traditional analytical tools in pea-enriched bread research. These processing technologies open new avenues to improve the functionality of other pulses, facilitating their incorporation into diverse food matrices. By demonstrating the potential of NIR spectroscopy for classifying flours and breads, tracking staling and characterising structural changes, this work has laid the foundation for real-time quality monitoring in industrial bakery production.

The broader societal impact of this work is reflected in improved human health. By showing that Irish-grown peas can be used to produce bread with enhanced protein and fibre content, lower eGI, improved flavour and quality parameters, this research demonstrates the potential to deliver healthier food products that meet consumer expectations while also promoting better dietary patterns.

This thesis contributes across multiple dimensions. Together, these outcomes highlight the role of academic research in driving real-world solutions that support both people and the planet.

8.4 Conclusion

The findings of this thesis demonstrate the interconnected role of processing, quality assessment, and digestion in enabling high levels of peas incorporation into bread. **Chapter 1**, the literature review, set the basis for determining the direction of the thesis by identifying the gaps in pulse-focused bakery research. **Chapters 2** and **3** established that milling is complex step, which influences flour functionality, dough rheology, and ultimately bread profiling. Hammer milling emerged as the most effective technique, combining high yield with favourable nutritional and functional properties for bakery applications.

Chapters 4 and **5** showed that additional processing methods, namely roasting, ultrasound and microwave, can further enhance the performance of pea flours, particularly when combined. This study is among the first to demonstrate that incorporating up to 35% pea flour is technically feasible when supported by advanced processing treatments, achieving improved structural baking attributes when compared with an untreated pea-wheat bread model. The roasting process improved water absorption and mitigated some off flavours associated with pea flour, while the combined application of roasting, ultrasound and microwave delivered the most favourable results in terms of loaf volume and crumb softness. These outcomes confirm that tailored processing can mitigate the technological and flavour challenges typically associated with pulse incorporation in bread.

Spectroscopy, integrated across several chapters, provided complementary insights. NIR was proven to be effective for rapid classification of flours and breads, even at low pea substitution levels, and successfully tracked bread staling of breads with diverse compositions. FTIR, in turn, revealed molecular-level changes in starch and protein structures mainly associated with the roasting processing. The integration of spectroscopy into this work illustrates its dual potential to deepen scientific understanding of structure-function relationships and to offer industry-appropriate tools for real-time staling monitoring.

Finally, the digestion studies (**Chapters 6** and **7**) connected pea flour processing and incorporation and with nutritional bio accessibility outcomes. Using a dynamic in vitro model, it was shown that pea-enriched breads slowed starch digestibility, suggesting a potential for a lower glycaemic response. Protein hydrolysis showed to be depended on pea composition and processing, with bread containing Irish pea (IP) exhibiting the fastest protein digestibility. This confirmed that flour processing techniques influence not only bread structure and texture but also the nutritional absorption of resulting breads.

By synthesising each element of each chapter, this thesis demonstrates a comprehensive study, from ingredient preparation (milling) to functionality optimisation (processing), product evaluation (bread quality and spectroscopy) and finally, to nutritional validation (digestion). Collectively, and on a local level, the thesis findings demonstrate that Irish-grown peas can serve as a high-value, sustainable ingredient, supporting local agriculture and reducing dependence on imported protein sources. Beyond their environmental and nutritional advantages, the results provide a framework for designing legume-enriched bakery products with tailored techno-functional and digestive benefits.

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10 Appendix

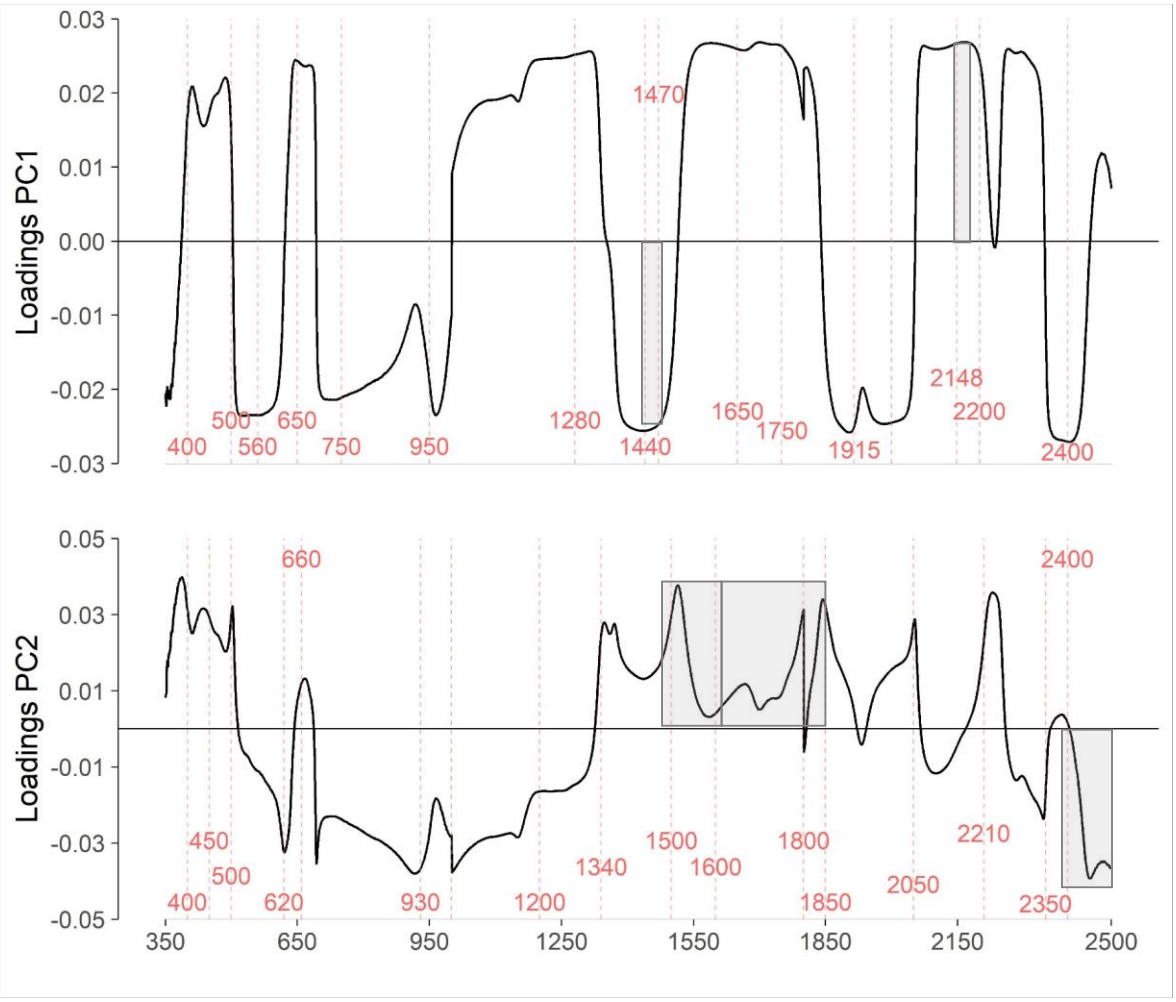


Figure 3.A1. SNVD Vis-NIR spectra loading plots of PC1 and PC2 of bread.

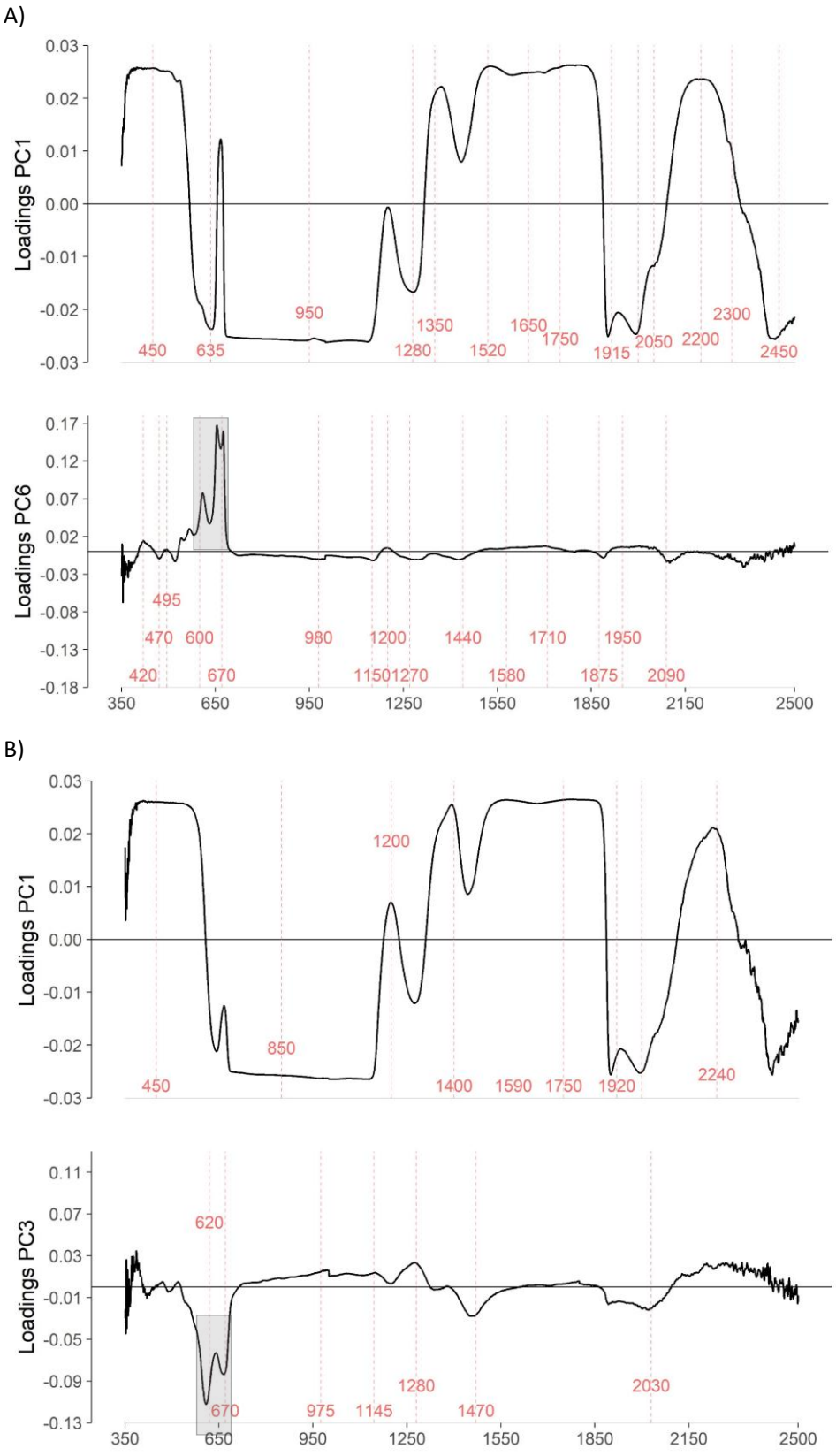


Figure 3.A2. A) SNVD Vis- spectra loading plots of PC1, PC6 of bread (15%:85% pea:wheat) on day 1, day 3 and day 7 post baking. B) SNVD Vis- spectra loading plots of PC1, PC3 of

bread (15%:85% pea:wheat) on day 7 post baking.

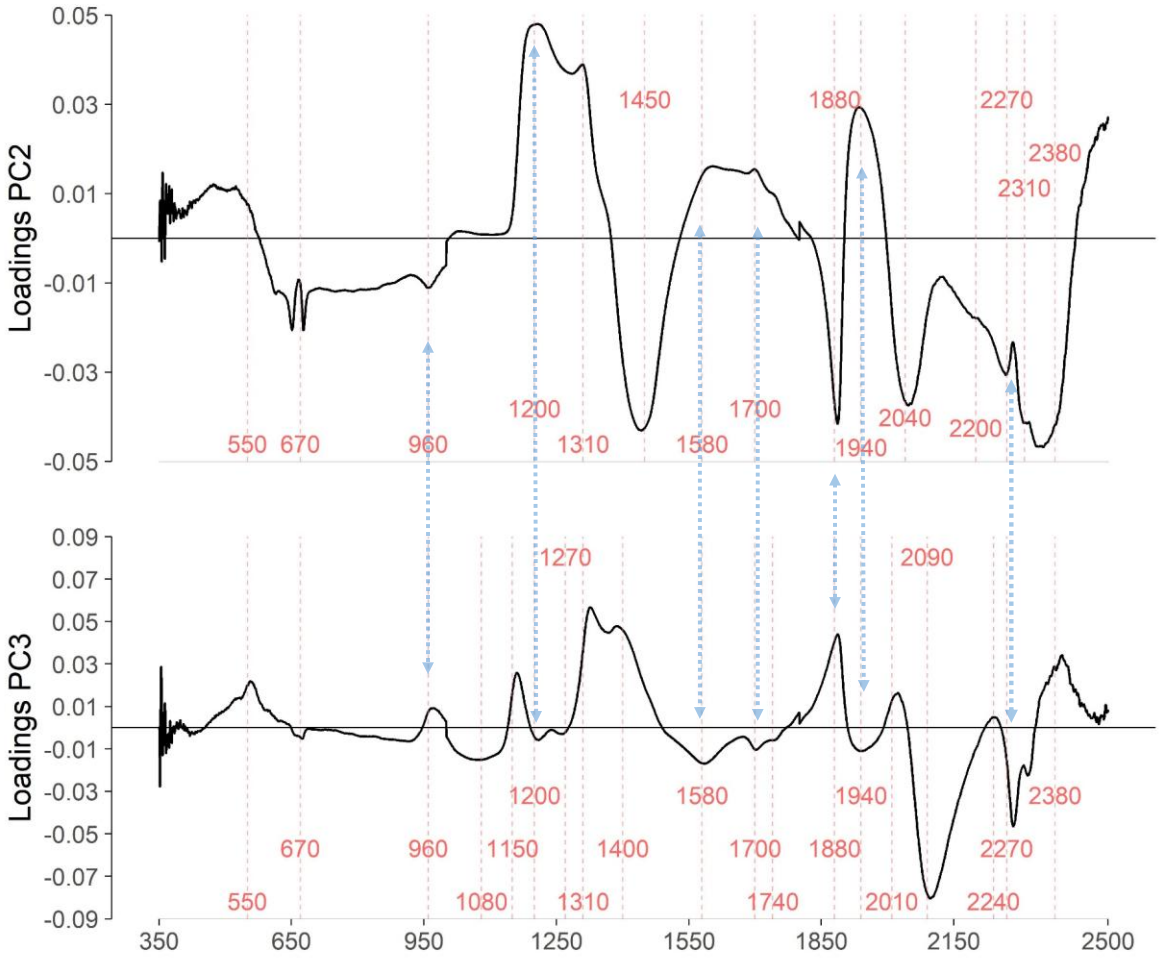


Figure 3.A3. SNVD Vis- spectra loading plots of PC2 and PC3 of all breads (15%:85% pea:wheat) on day 1, day 3 and day 7 post baking.

Table 4.A1. Abbreviations and descriptions of pea grains, flours, and composite flour blends (35:65) used in this study.

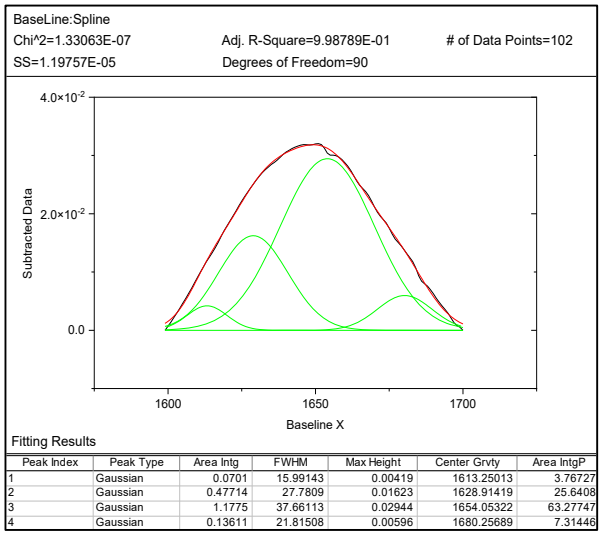
Abbreviation	Description
Pea grains	
IP	Irish pea grains
IP+R	Irish pea grains + roasting
IP+US+R	Irish pea grains + ultrasound + roasting
IP+MW+R	Irish pea grains + microwave + roasting
IP+US+MW+R	Irish pea grains + ultrasound + microwave + roasting
Flours	
W	Wheat commercial flour
CP	Commercial pea flour
IP	Irish pea flour
IP+R	Irish pea flour + roasting
IP+US+R	Irish pea flour + ultrasound + roasting
IP+MW+R	Irish pea flour + microwave + roasting
IP+US+MW+R	Irish pea flour + ultrasound + microwave + roasting
Composite flours	
CP 35%	Commercial pea:wheat flour (35:65)
IP 35%	Irish pea flour: wheat flour (35:65)
IP+R 35%	Irish pea flour + roasting: wheat flour (35:65)
IP+US+R 35%	Irish pea flour + ultrasound + roasting: wheat flour (35:65)
IP+MW+R 35%	Irish pea flour + microwave + roasting: wheat flour (35:65)
IP+US+MW+R 35%	Irish pea flour + microwave + roasting: wheat flour (35:65)

Table 4.A2. Particle size distribution curves of wheat flour and pea flours.

Flours*	Dx (10)	Dx (50)	Dx (90)	Dx (99)
W	13.22±3.75^{a}	85.43±10.18^{a}	194.67±12.10^{ab}	319.7±31.66^{b}
CP	5.63±0.70^{c}	35.3±1.92^{b}	161.0±6.25^{b}	255.67±5.69^{b}
IP	2.57±0.59^{c}	29.53±4.75^{b}	215.0±131.01^{ab}	421.0±267.86^{ab}
IP+R	7.44±0.50^{bc}	52.87±5.50^{b}	249.7±47.00^{ab}	538.0±200.61^{ab}
IP+US+R	6.72±0.24^{c}	46.23±2.47^{b}	218.7±43.66a^{b}	574.7±58.05^{ab}
IP+MW+R	11.95±2.88^{ab}	82.4±19.46^{a}	369.7±117.78^{a}	719.0±143.85^{a}
IP+US+MW+R	14.83±0.55^{a}	51.1±5.70^{b}	213.0±50.27^{ab}	421.7±76.58^{ab}

*W: Wheat commercial flour; CP: Commercial pea flour; IP: Irish pea flour non-treated; IP+R: Irish pea flour + roasting; IP+US+R: Irish pea flour + ultrasound + roasting; IP+MW+R: Irish pea flour + microwave + roasting; IP+US+MW+R: Irish pea flour + ultrasound + microwave+ roasting: wheat. Data presented as means ± standard deviation. Values with a different letter (a-c) are significantly different ($p < 0.05$) along the column..

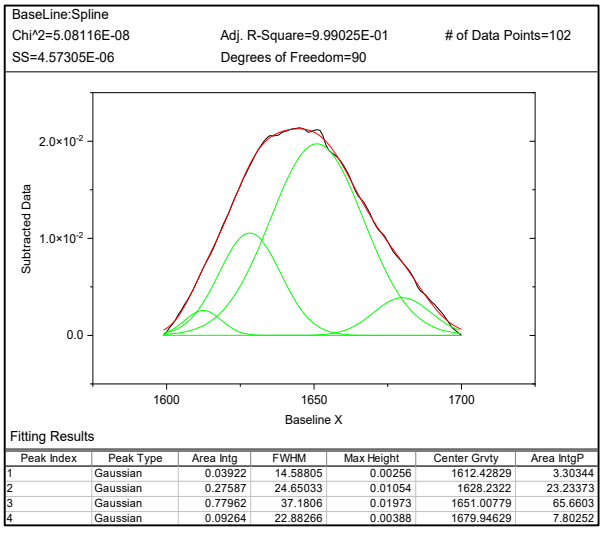
a) Wheat flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.0701	15.99143	0.00419	1613.25013	3.76727
2	Gaussian	0.47714	27.7809	0.01623	1628.91419	25.6408
3	Gaussian	1.1775	37.66113	0.02944	1654.05322	63.27747
4	Gaussian	0.13611	21.81508	0.00596	1680.25689	7.31446

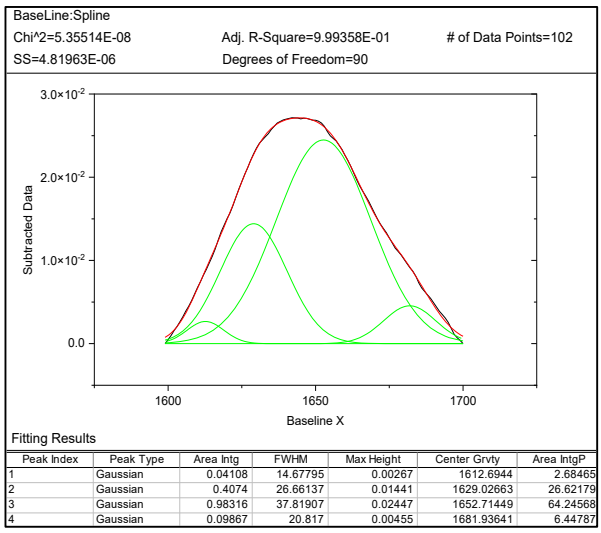
b) Commercial pea flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.03922	14.58805	0.00256	1612.42829	3.30344
2	Gaussian	0.27587	24.65033	0.01054	1628.2322	23.23373
3	Gaussian	0.77962	37.1806	0.01973	1651.00779	65.6603
4	Gaussian	0.09264	22.88266	0.00388	1679.94629	7.80252

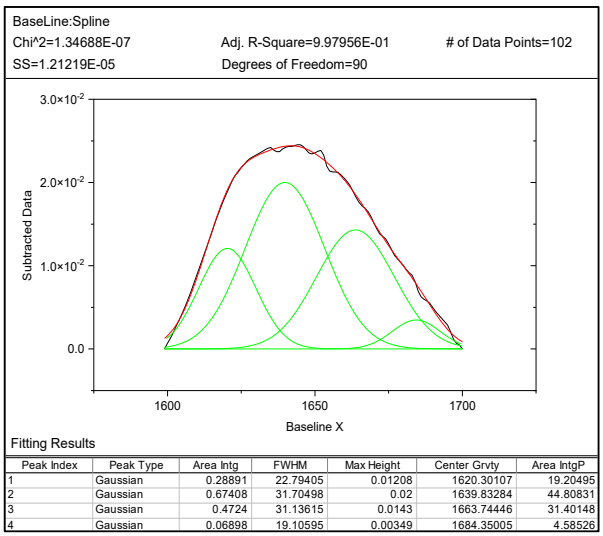
c) Irish pea flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.04108	14.67795	0.00267	1612.6944	2.68465
2	Gaussian	0.4074	26.66137	0.01441	1629.02663	26.62179
3	Gaussian	0.98316	37.81907	0.02447	1652.71449	64.24568
4	Gaussian	0.09867	20.817	0.00455	1681.93641	6.44787

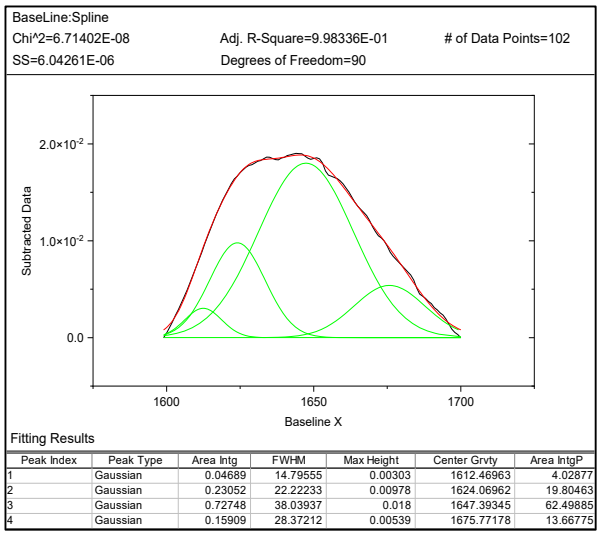
d) Irish pea roasted flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.28891	22.79405	0.01208	1620.30107	19.20495
2	Gaussian	0.67408	31.70498	0.02	1639.83284	44.80831
3	Gaussian	0.4724	31.13615	0.0143	1663.74446	31.40148
4	Gaussian	0.06898	19.10595	0.00349	1684.35005	4.58526

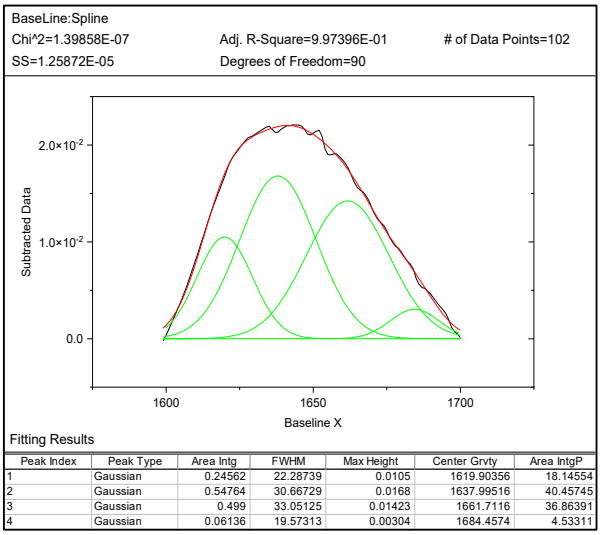
e) Irish pea ultrasound + roasted flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.04689	14.79555	0.00303	1612.46963	4.02877
2	Gaussian	0.23052	22.22233	0.00978	1624.06962	19.80463
3	Gaussian	0.72748	38.03937	0.018	1647.39345	62.49885
4	Gaussian	0.15909	28.37212	0.00539	1675.77178	13.66775

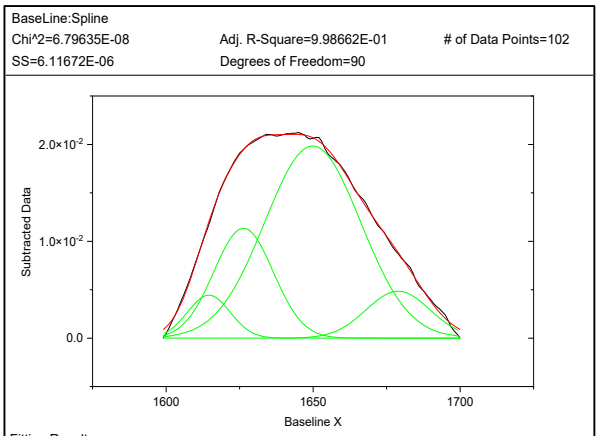
f) Irish pea microwave + roasted flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.24562	22.28739	0.0105	1619.90356	18.14554
2	Gaussian	0.54764	30.66729	0.0168	1637.99516	40.45745
3	Gaussian	0.499	33.05125	0.01423	1661.7116	36.86391
4	Gaussian	0.06136	19.57313	0.00304	1684.4574	4.53311

g) Irish pea ultrasound + microwave + roasted flour



Fitting Results

Peak Index	Peak Type	Area Intg	FWHM	Max Height	Center Grvty	Area IntgP
1	Gaussian	0.07923	17.08003	0.00443	1614.54623	6.09966
2	Gaussian	0.28518	23.70479	0.01134	1626.33035	21.95628
3	Gaussian	0.80644	38.27755	0.01983	1649.871	62.08817
4	Gaussian	0.12801	25.49846	0.00484	1678.90944	9.85589

Figure 4A1. Representative Gaussian deconvolution of the amide I band (1700–1600 cm^{-1}) for all flour samples. .

Table 4.A3. RVA of wheat flour, pea flours and flour blends 35:65 (pea:wheat)

Flours*	Peak viscosity (cP)	Trough 1 (cP)	Breakdown (cP)	Final Viscosity(cP)	Setback (cP)
W	276.1±0.78^{a}	149.74±1.64^{a}	126.36±1.42^{a}	325.28±3.53^{a}	175.54±1.91^{a}
CP	133.31±2.64^{f}	120.42±2.45^{b}	12.89±0.20^{h}	199.78±4.93^{c}	79.36±3.95^{ef}
IP	117.62±1.35^{g}	87.44±0.09^{f}	30.18±1.32^{g}	118.42±1.54^{g}	30.97±1.561^{h}
IP+R	65.41±2.75^{i}	53.75±2.53^{h}	11.66±0.31^{h}	100.78±3.34^{h}	47.04±0.96^{g}
IP+US+R	30.72±0.32^{j}	23.95±0.18^{i}	6.77±0.15^{d}	57.04±0.82^{i}	33.09±0.65^{h}
IP+MW+R	26.26±1.02^{j}	20.26±1.07^{i}	6.0±0.31^{i}	51.21±1.51^{i}	30.95±0.75^{h}
IP+MW+US+R	77.58±2.58^{h}	66.92±3.13^{g}	10.66±0.7^{e}	111.82±2.62^{g}	44.9±1.12^{g}
CP 35%	240.32±1.17^{b}	151.65±1.13^{a}	88.67±1.12^{b}	317.22±2.45^{a}	165.57±1.32^{b}
IP 35%	197.74±3.47^{c}	112.56±2.66^{c}	85.19±2.74^{c}	256.24±6.05^{b}	143.68±4.20^{c}
IP+R 35%	157.42±0.61^{d}	101.07±0.09^{d}	56.35±0.56^{h}	195.0±0.99^{cd}	93.93±1.05^{d}
IP+US+R 35%	147.57±3.25^{e}	95.3±1.60^{e}	52.27±1.69^{de}	177.29±2.47^{d}	81.99±1.18^{e}
IP+MW+R 35%	135.39±1.24^{f}	88.41±0.95^{f}	46.98±0.68^{f}	162.03±3.13^{e}	73.61±2.34^{f}
IP+US+MW+R 35%	155.2±1.02^{d}	100.99±0.82^{d}	54.21±0.20^{de}	189.77±2.96^{d}	88.78±2.24^{d}

***** W: Wheat commercial flour; CP: Commercial pea flour; IP: Irish pea flour non-treated; IP+R: Irish pea flour + roasting; IP+US+R: Irish pea flour + ultrasound + roasting; IP+MW+R: Irish pea flour + microwave + roasting; IP+US+MW+R: Irish pea flour + ultrasound + microwave+ roasting: wheat; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated: wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65). Data presented as means ± standard deviation. Values with a different letter (a-j) are significantly different ($p < 0.05$) along the column..

Table 4.A4. Dough properties Mixolab parameters of wheat flour and flour blends 35:65 (pea:wheat).

Flours*	Water absorption %	Stability (min)	DDT (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
W	62.0	11.69±0.14^{a}	8.74±0.09^{a}	0.55±0.01^{a}	1.95±0.015^{a}	1.84±0.05^{b}	2.88±0.1^{b}
CP 35%	56.9	6.2±0.09^{e}	5.06±0.15^{cd}	0.36±0.01^{d}	1.91±0.04^{ab}	2.15±0.03^{a}	3.14±0.10^{a}
IP 35%	60.5	3.33±0.19^{f}	4.02±0.05^{d}	0.36±0.01^{d}	1.88±0.02^{b}	1.38±0.03^{cd}	1.98±0.08^{c}
IP+R 35%	68.5	8.63±0.05^{c}	6.12±0.93^{bc}	0.45±0.01^{c}	1.56±0.00^{c}	1.4±0.01^{cd}	1.82±0.04^{c}
IP+US+R 35%	68.5	8.81±0.19^{c}	6.76±0.05^{b}	0.5±0.006^{b}	1.57±0.02^{c}	1.37±0.03^{cd}	1.8±0.08^{c}
IP+MW+R 35%	69.0	9.42±0.28^{b}	5.69±1.18^{bcd}	0.52±0.01^{ab}	1.57±0.02^{c}	1.34±0.03^{d}	1.8±0.05^{c}
IP+US+MW+R 35%	69.2	8.11±0.29^{d}	6.21±0.14^{bc}	0.47±0.03^{c}	1.6±0.02^{c}	1.44±0.03^{c}	1.9±0.04^{c}

* W: Wheat commercial flour; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated: wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65). Data presented as means ± standard deviation. Values with a different letter (a-f) are significantly different ($p < 0.05$) along the column.

Table 5.A1. Hardness staling rate (%) of breads containing different pea flour treatments over storage time.

Breads*	Day	Hardness staling rate (%)
W	1-3	44.2
W	3-7	16.3
W	1-7	35.3
CP	1-3	18.4
CP	3-7	12.7
CP	1-7	17.7
IP	1-3	17.3
IP	3-7	11.2
IP	1-7	15.9
IP+R	1-3	11.3
IP+R	3-7	11.1
IP+R	1-7	12.9
IP+US+R	1-3	14.3
IP+US+R	3-7	11.2
IP+US+R	1-7	14.4
IP+MW+R	1-3	18.3
IP+MW+R	3-7	8.9
IP+MW+R	1-7	14.2
IP+US+MW+R	1-3	19.7
IP+US+MW+R	3-7	9.9
IP+US+MW+R	1-7	15.84

*W: Wheat commercial flour; CP: Commercial pea flour; IP: Irish pea flour non-treated; IP+R: Irish pea flour + roasting; IP+US+R: Irish pea flour + ultrasound + roasting; IP+MW+R: Irish pea flour + microwave + roasting; IP+US+MW+R: Irish pea flour + ultrasound + microwave+ roasting: wheat; CP 35%: (commercial pea:wheat) (35:65); IP 35%: (Irish pea flour non-treated: wheat) (35:65); IP+R 35%: (Irish pea flour + roasting: wheat) (35:65); IP+US+R 35%: (Irish pea flour + ultrasound + roasting: wheat) (35:65); IP+MW+R 35%: (Irish pea flour + microwave + roasting: wheat) (35:65); IP+US+MW+R 35%: (Irish pea flour + ultrasound + microwave + roasting: wheat) (35:65).

Table 5.A2. Volatile compounds and their associated sensory descriptors.

Chemical Class	Compound (*common name*)	CAS	LRI	Ref LRI	Odour Descriptor	Odour Descriptor Reference
Alcohol	2-Methyl-1-propanol (*isobutyl alcohol*)	78-83-1	619	622	wine, solvent bitter	Flavornet.org
Alcohol	3-Methyl-1-butanol (*isoamyl alcohol*)	123-51-3	729	729	whiskey, malt, burnt	Flavornet.org
Alcohol	2-Methyl-1-butanol	137-32-6	733	733	wine, onion	Flavornet.org
Alcohol	1-Pentanol	71-41-0	760	760	balsamic	Flavornet.org
Alcohol	2-Penten-1-ol, (Z)-	1576-95-0	761	757	green, plastic, rubber	Flavornet.org
Alcohol	2,3-Butanediol, [S-(R*,R*)]-	19132-06-0	787		Fruity creamy buttery (isomer of this)	thegoodscentscompany.com
Alcohol	3-Hexen-1-ol, (Z)-	928-96-1	851	847	grass	Flavornet.org
Alcohol	1-Hexanol	111-27-3	863	862	resin, flower, green	Flavornet.org
Alcohol	1-Heptanol	111-70-6	966	963	chemical, green	Flavornet.org
Alcohol	1-Octen-3-ol	3391-86-4	977	975	mushroom	Flavornet.org
Alcohol	2-Ethyl-1-hexanol	104-76-7	1025	1027	rose, green	Flavornet.org
Alcohol	2-Octen-1-ol, (E)-	18409-17-1	1064	1063	soap, plastic	Flavornet.org
Alcohol	1-Octanol	111-87-5	1066	1066	chemical, metal, burnt	Flavornet.org
Alcohol	Phenylethyl alcohol	60-12-8	1117	1105	honey, spice, rose, lilac	Flavornet.org
Alcohol	(Z)-4-Decen-1-ol (*cis-4-decenol*)	57074-37-0	1254	1248	waxy, fatty, fruity	thegoodscentscompany.com
Aldehyde	2-Methyl-propanal (*isobutanal*)	78-84-2	552	551	pungent, malt, green	Flavornet.org
Aldehyde	3-Methyl-butanal (*isovaleraldehyde*)	590-86-3	649	646	malt	Flavornet.org
Aldehyde	2-Methyl-butanal	96-17-3	660	656	cocoa, almond	Flavornet.org
Aldehyde	Hexanal	66-25-1	798	790	grass, tallow, fat	Flavornet.org
Aldehyde	4-Heptenal, (Z)-	6728-31-0	894	896	biscuit, cream	Flavornet.org
Aldehyde	Heptanal	111-71-7	900	894	fat, citrus, rancid	Flavornet.org
Aldehyde	2,4-Hexadienal, (E,E)-	142-83-6	910	899	sweet, green, spicy, floral, citrus	Thegoodscentscompany.com
Aldehyde	2-Heptenal, (Z)-	57266-86-1	957	945	None	Thegoodscentscompany.com
Aldehyde	Octanal	124-13-0	1002	994	fat, soap, lemon, green	Flavornet.org
Aldehyde	2,4-Heptadenial (E,E)	4313-03-5	1011	1003	nut, fat	Flavornet.org

Aldehyde	Nonanal	124-19-6	1102	1097	fat, citrus, green	Flavornet.org
Aldehyde	2-Nonenal, (E)- (*trans-2-nonenal*)	18829-56-6	1159	1154	fatty, green, cucumber, aldehydic, citrus	Thegoodscentscompany.com
Aldehyde	2,4-Nonadienal	6750-03-4	1216	1213	watermelon	Flavornet.org
Aldehyde	2-Decenal, (E)-	3913-81-3	1262	1255	tallow	Flavornet.org
Aldehyde	2,4-Decadienal, (E,E)-	25152-84-5	1319	1309	fried, wax, fat	Flavornet.org
Alkane	2,4-Trimethyl-pentane	540-84-1	685	687	None	Thegoodscentscompany.com
Alkane	Heptane	142-82-5	699	700	alkane	Flavornet.org
Alkane	Nonane	111-84-2	897	900	alkane	Flavornet.org
Alkane	Decane	124-18-5	997	1000	alkane	Flavornet.org
Alkane	Undecane	1120-21-4	1112	1100	alkane	Flavornet.org
Alkane	Tridecane	629-50-5	1296	1300	alkane	Flavornet.org
Alkane	5-Methyl-tridecane	25117-31-1	1348	1355	None	Thegoodscentscompany.com
Alkane	Tetradecane	629-59-4	1395	1400	alkane	Flavornet.org
Alkane	Nonadecane	629-92-5	1597		alkane	Flavornet.org
Ketone	2,3-Butanedione (*diacetyl*)	431-03-8	582	593	butter	Flavornet.org
Ketone	1-Octen-3-one	4312-99-6	975	974	mushroom, metal	Flavornet.org
Ketone	3-Octen-2-one	1669-44-9	1036	1026	nut, crushed bug	Flavornet.org
Ketone	3,5-Octadien-2-one, (E,E)-	30086-02-3	1092	1075	fruity, green, grassy	Thegoodscentscompany.com
Ketone	2-Tridecanone	593-08-8	1491	1488	fatty, waxy, dairy, milk, coconut, nutty, herbal, earthy	Thegoodscentscompany.com
Furan	2-Ethyl-furan	3208-16-0	697		chemical, beany, ethereal, cocoa, bready, malty, coffee, nutty	thegoodscentscompany.com
Furan	Dihydro-2-methyl-3(2H)-furanone (*coffee furanone*)	3188-00-9	806	788	sweet, solvent, bready, butter, nutty	thegoodscentscompany.com
Furan	Furfural	98-01-1	829	822	bread, almond, sweet	Flavornet.org
Furan	2-Furanmethanol (*furfuryl alcohol*)	98-00-0	848	845	burnt	Flavornet.org
Furan	2-n-Butyl furan	4466-24-4	889	887	fruity, winey, sweet, spicy	thegoodscentscompany.com

Furan	1-(2-Furanyl)-ethanone (*2-acetyl furan*)	1192-62-7	908	896	Sweet, balsamic, almond, cocoa, caramellic, coffee	thegoodscentscompany.com
Furan	5-Methyl-2-furancarboxaldehyde (*5-methylfurfural*)	620-02-0	960	952	almond, caramel, burnt sugar	Flavornet.org
Furan	2-Pentyl-furan	3777-69-3	989	987	green bean, butter	Flavornet.org
Benzene	Toluene	108-88-3	766	759	paint	Flavornet.org
Benzene	m-Xylene	108-38-3	871	862	plastic	Flavornet.org
Benzene	1-Ethyl-2-methyl-benzene (*2-ethyltoluene*)	611-14-3	965	972	?	
Benzene	Benzaldehyde	100-52-7	967	952	almond, burnt sugar	Flavornet.org
Benzene	Benzeneacetaldehyde (*phenylacetaldehyde*)	122-78-1	1048	1037	hawthorne, honey, sweet	
Benzene	4-Ethyl-benzaldehyde	4748-78-1	1170	1163	sweet	Flavornet.org
Benzene	Benzoic acid	65-85-0	1157	1169	balsamic, urine	thegoodscentscompany.com
Pyrazine	2,5-Dimethyl-pyrazine	123-32-0	911	899	cocoa, roasted nut, roast beef, medicine	Flavornet.org
Pyrazine	Ethyl pyrazine	13925-00-3	916	905	peanut butter, wood	Flavornet.org
Pyrazine	2,3-Dimethyl-pyrazine	5910-89-4	917	905	nut, peanut butter, cocoa, meat	Flavornet.org
Pyrazine	2-Ethenyl-6-methyl-pyrazine (*2-ethyl-6-methylpyrazine*)	13925-09-2	1024	1013	hazelnut, nutty	thegoodscentscompany.com
Pyrazine	3-Ethyl-2,5-dimethyl-pyrazine (*3,6-cocoa pyrazine*)	13360-65-1	1076	1072	potato, cocoa, roasted, nutty	thegoodscentscompany.com
Pyrazine	2,3-Dimethyl-5-ethylpyrazine (*3,5-cocoa pyrazine*)	13925-07-0	1085	1075	burnt, almond, roasted, nutty, coffee	thegoodscentscompany.com
Acid	Acetic acid	64-19-7	642	622	sour	Flavornet.org
Acid	2-Methyl-butanoic acid	116-53-0	833	853	cheese, sweat	Flavornet.org
Acid	Pentanoic acid (*valeric acid*)	109-52-4	867	904	sweat	Flavornet.org
Acid	Nonanoic acid	112-05-0	1255	1275	green, fat	Flavornet.org
Alkene	1-Octene	111-66-0	787	790	gasoline	thegoodscentscompany.com
Alkene	2-Octene, (E)-	13389-42-9	811	808	None	thegoodscentscompany.com

Alkene	1,3-Octadiene	1002-33-1	823	826	None	thegoodscentscompany.com
Alkene	1,3-trans,5-cis-Octatriene	40087-61-4	878		?	
Hetrocyclic compound	Pyridine	110-86-1	771	736	rancid	Flavornet.org
Hetrocyclic compound	Indole	120-72-9	1300	1290	mothball, burnt	Flavornet.org
Hetrocyclic compound	4-Methylpyrimidine	3438-46-8	823	819	?	
Hetrocyclic compound	2-Acetyl-1H-pyrrole	1072-82-8	1062		nut, walnut, bread	Flavornet.org
Ester	Hexanoic acid, ethyl ester (*ethyl hexanoate*)	123-66-0	993	995	apple peel, fruit	Flavornet.org
Ester	Octanoic acid, ethyl ester (e*thyl octanoate*)	106-32-1	1190	1192	fruit, fat	Flavornet.org
Ester	Nonanoic acid, ethyl ester (*ethyl nonanoate*)	123-29-5	1288	1294	fruity, rose, waxy, rummy, winey, natural tropical	thegoodscentscompany.com
Terpene	a-Pinene	80-56-8	938	935	pine, turpentine	Flavornet.org
Terpene	b-Pinene	127-91-3	984	976	pine, resin, turpentine	Flavornet.org
Terpene	Carvone	99-49-0	1252	1230	mint, basil, fennel	Flavornet.org
Chorinated compound	1-Chloro-pentane	543-59-9	751	744	?	
Chorinated compound	1-Chloro-hexane	544-10-5	957	849	?	
Chorinated compound	1-Chloro-octane	111-85-3	1062		?	
Hyrdroxy Ketone	1-Hydroxy-2-propanone	116-09-6	708	655	pungent, sweet, caramellic, ethereal	thegoodscentscompany.com
Sulfur	Dimethyl disulfide	624-92-0	743	738	onion, cabbage, putrid	Flavornet.org
Nitrile	Hexanenitrile (*capronitrile*)	628-73-9	874	873	?	
Pyranone	Maltol	118-71-8	1113	1110	caramel	Flavornet.org

Phenol	2-Methoxy-4-vinylphenol (*p-vinylguaiacol*)	7786-61-0	1314	1307	clove, curry	Flavornet.org
Lactone	Dihydro-5-pentyl-2(3H)-furanone (*y-nonalactone*)	104-61-0	1363	1351	coconut, peach	Flavornet.org
Terpenoid ketone	5,9-Undecadien-2-one, 6,10-dimethyl-, (E)- (*geranyl acetone*)	3796-70-1	1446	1445	magnolia, green	Flavornet.org

Table 6.A1. Settings used in the DIVHS-IV dynamic in vitro digestion system.

Stages	**1**	**2**	**3**	**4**
Backup Syringe Pump Parameter Settings				
Speed (mL/min)	0.0	5.0	0.3	0.2
Time (min)	30	1	45	45
Esophagus Device Parameter Settings				
Open time of Upper Esophagus Clamp (min)	0.5	0	0	0
Close time of Lower Esophagus Clamp (min)	0.5	0	0	0
Shaking Rate of Esophagus (rpm)	400	0	0	0
Running Time (min)	10.0	0	0	0
Stomach Peristalsis Device Parameter Settings				
Forward Speed (mm/min)	500	0	0	0
Reset Speed (mm/min)	600	0	0	0
Running Time (min)	120	0	0	0
Pylorus Device Parameter Settings				
Releasing Speed (mm/min)	100	100	100	0
Releasing Time (min)	0	0.5	0.5	0
Clamping Speed (mm/min)	100	100	100	0
Clamping Time (min)	30	14.5	2.5	0
Running Time (min)	30	90	0	0
Duodenum Squeezing Device Parameter Settings				
Speed (rpm)	30	0	0	0
Time (min)	120	0	0	0
Duodenum Valve Parameter Settings				
Clamping Time (min)	44.5	14.5	0	0
Releasing Time (min)	0.5	0.5	0	0
Running Time (min)	45.0	75.0	0	0
Stomach Tilting Device Parameter Settings				
Speed (°/min)	-0.4	-0.3	-0.4	00.5
Time (min)	60.0	30.0	15.0	15.0