CATÓLICA
FACULTY OF BIOTECHNOLOGY
PORTO

INFLUENCE OF INTERCROPPING IN BREAD QUALITY AND PROCESSABILITY

by

Gabriel Filipe de Gonçalves Campos

October 2025

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Thesis presented to Escola Superior de Biotecnologia
of the Universidade Católica Portuguesa to fulfil the requirements of
Master of Science degree in Food Engineering

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Gabriel Filipe de Gonçalves Campos

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Abstract

The necessity toward more sustainable agricultural systems requires novel strategies to ease the transition from traditional practices, in order to reduce environmental impact, but without compromising food quality and processing. Intercropping, the simultaneous cultivation of two or more species, has emerged as a promising alternative to monoculture, particularly through cereal-legume combinations, as it provides a better synergy, e.g. greater nutritional bioavailability. This thesis evaluated the influence of wheat-legume intercropping on flour composition, hydration properties and rheological behaviour of the doughs, bread quality and extrusion performance.

Chemical analyses showed that pea flours contained more ash, fat and protein, while wheat flours were richer in starch. No major compositional differences were found between purecropped (PC) and intercropped (IC) wheat, apart from minor protein variations. Hydration and Rapid Visco Analysis analyses confirmed similar gelatinization and pasting profiles for IC and PC flours, both outperforming pea flours. Rheological tests highlighted stronger doughs in refined wheat (W700) compared to weaker wholegrain doughs, yet IC and PC behaved equivalently. Breadmaking and extrusion trials further demonstrated no significant differences between IC and PC.

Overall, the intercropping strategy showed to maintain wheat flour quality and technological performance while supporting a more sustainable cultivation practice. By delivering comparable bread quality and processability to monoculture wheat, cereal-legume intercropping represents a viable and environmentally advantageous strategy to align food production with the goals of resilient and sustainable agri-food systems.

Keywords: intercropping, cereal-legume combination, quality, processability, sustainable agriculture.

Resumo

A necessidade de sistemas agrícolas mais sustentáveis exige estratégias inovadoras que facilitem a transição a partir de práticas tradicionais, de modo a reduzir o impacto ambiental sem comprometer a qualidade e a processabilidade dos alimentos. O *intercropping*, que consiste no cultivo simultâneo de duas ou mais espécies, tem emergido como uma alternativa promissora à monocultura, sobretudo através das combinações de cereais com leguminosas, uma vez que favorece uma melhor biodisponibilidade nutricional. A presente dissertação avaliou a influência da sinergia trigo-leguminosa na composição das farinhas, nas propriedades de hidratação e no comportamento reológico das massas, bem como na qualidade do pão e no desempenho em extrusão.

As análises químicas mostraram que as farinhas de ervilha apresentaram maiores teores de cinzas, gordura e proteína, enquanto as farinhas de trigo foram mais ricas em amido. Não foram encontradas diferenças composicionais relevantes entre o trigo em monocultura (*purecropped*, PC) e em *intercropped* (IC), exceto por variações residuais no teor proteico. As análises de hidratação e *Rapid Visco Analysis* confirmaram perfis de gelatinização e de viscosidade semelhantes entre as farinhas de IC e PC, ambos superiores às farinhas de ervilha. Os ensaios reológicos evidenciaram massas mais fortes no trigo refinado (W700) em comparação com as massas integrais, mais quebradiças, embora as massas com farinhas de PC e IC tenham apresentado comportamentos equivalentes. Os ensaios de panificação e de extrusão confirmaram igualmente a ausência de diferenças significativas entre IC e PC.

Conclui-se, assim, que o *intercropping* mantém a qualidade da farinha de trigo e o seu desempenho tecnológico, ao mesmo tempo que promove práticas agrícolas mais sustentáveis. Ao assegurar uma qualidade do pão e uma processabilidade comparáveis às do trigo em monocultura, esta técnica de duas espécies mutuamente benéficas representa uma estratégia viável e ambientalmente vantajosa para alinhar a produção alimentar com os objetivos de sistemas agroalimentares resilientes e sustentáveis.

Palavras-chave: *intercropping*, combinação cereal-leguminosa, qualidade, processabilidade, agricultura sustentável.

Acknowledgments

O início desta tese não teria sido possível sem a experiência transformadora que o programa **ERASMUS** me proporcionou. Foi uma viagem mágica de crescimento pessoal, autoconhecimento e descoberta, que marcou profundamente o meu percurso académico e humano.

Em primeiro lugar, expresso a minha profunda gratidão à **Professora Cristina Silva** e ao **Professor Henry Jäger**, que desde o início me ajudaram a preparar esta jornada, garantindo que tudo acontecesse de forma fluida. Mesmo à distância, demonstraram sempre preocupação, orientação e apoio, pelos quais estarei eternamente agradecido.

Aos meus co-supervisores, **Julian Fischer** e **Eleonora Pichler**, agradeço de coração a forma calorosa e genuína com que me acolheram. A sua constante disponibilidade, boa disposição e rigor científico foram fundamentais para que eu me sentisse seguro e guiado em cada etapa.

À minha tutora, **Tânia Ribas**, manifesto o mais sincero reconhecimento. Desde o primeiro momento soube que era a escolha certa. A sua empatia, dedicação e humanidade tornaram este processo mais leve, sereno e enriquecedor.

Aos colegas e amigos que fiz dentro e fora do laboratório, deixo um enorme obrigado por cada atividade, viagem e gargalhada partilhada. Foram vocês que transformaram a minha experiência em algo inesquecível. Tornaram a despedida incrivelmente difícil e revelaram a genuinidade das nossas ligações. Uma menção especial aos meus amigos do coração – **Seymen Can Aydoğan, Aysu Erdim e Miriam Szepannek** – levo-vos comigo e prometo visitar-vos nos vossos países.

De Portugal, agradeço a todos os que viajaram para me visitar e fizeram com que me sentisse em casa, mesmo estando longe dela. Aos que partilharam comigo dias lindos e guardaram memórias preciosas, o meu mais profundo obrigado. Em especial à minha irmã **Cláudia**, ao seu companheiro **João** e à minha sobrinha **Leonor**, que largaram tudo para estarem ao meu lado. Aos meus **pais**, que não só me proporcionaram esta experiência única, como também cuidaram da minha **avó**, que, com coragem, superou o medo de voar para me poder abraçar em Viena. Às minhas melhores amigas e irmãs de coração, **Maria Francisca** e **Patrícia**, agradeço por nunca me deixarem sentir sozinho, pela presença

física e pelas incontáveis chamadas e mensagens que me sustentaram nos momentos em que mais precisei.

A todos os familiares e amigos que, mesmo não podendo estar presentes fisicamente, me deram apoio incondicional, deixo igualmente a minha gratidão. Cada gesto, palavra e carinho foram essenciais para que me sentisse realizado e pertencente.

Às minhas **Trinca-Espinhas**, este grupo peculiar que se tornou um marco no meu percurso académico e transbordaram para o meu percurso pessoal, sem elas, não me teria permitido a tantas conquistas.

Finalmente, a todas as **pessoas especiais** que conheci ao longo deste percurso académico e pessoal. Cada um de vocês deixou em mim uma marca, maior ou menor, que me ajudou a tornar-me numa versão melhor de mim mesmo. Levo comigo um pedaço de cada um, e é essa soma de presenças, aprendizagens e afetos que, para mim, define o verdadeiro significado do **amor**.

Esta dissertação é mais do que um trabalho académico, é um reflexo da minha maturidade, do meu crescimento e do amor que recebi ao longo desta caminhada. Obrigado por me fazerem sentir vivo e por me permitirem ser livre, à minha maneira.

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List of abbreviations and symbols

IC – Intercrop

PC – Purecrop

W700 – Refined wheat flour

W1600 PC – Wholegrain purecropped wheat flour

W1600 IC – **W**holegrain intercropped wheat flour

HI - Harvest Index

LER – Land Equivalent Ratio

FAO – Food and Agriculture Organization

ICC – International Association for Cereal Science and Technology

AACC – Cereals and Grains Association

GOPOD – Megazyme Glucose Determination Reagent

WAI – Water Absorption Index

WSI – Water Solubility Index

WA – Water Absorption

RVA – Raped Visco Analysis

DT – Development Time

CIELAB – International Commission on Illumination by three coordinates L*/a*/b*

CO_2 eq. per year - Annual greenhouse gas emissions, expressed as carbon dioxide equivalent, per year

BU - Brabender Units

FU – Farinograph Units

F_{max} - Maximum force in Newtons

F_{120} – Residual force after 120 seconds, in Newtons

kg/ha – Kilograms per hectare

rpm – Rotations per minute

cP - Centipoise

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1. Introduction

1.1. Study Framework

Although the exponential growth of the world population has already reached its inflexion point (after which the growth pace slowed down), it still finds itself in an accelerated cadence, being expected to reach a peak of 11.2 ± 1.5 billion people by the end of the 21st century (Taagepera & Nemčok, 2024; Timaeus et al., 2022). This ongoing increase translates to an immense pressure on food systems, which often follows intensive and expansive models to attend to the overall demand (Rehman et al., 2022).

According to FAO, the definition of a sustainable food system is defined as follows: "a food system that delivers food security and nutrition for all in such a way that the economic, social and environmental bases to generate food security and nutrition for future generations are not compromised" (*FAO*, n.d.). Thus, the adoption of sustainable agriculture plays a crucial role not only by enhancing greener outcomes but also in ensuring access to sufficient, safe and nutritious food that meets people's dietary needs and preferences in socially, economically and environmentally equitable ways.

Nevertheless, the undergoing agricultural regime, has been contributing to environmental degradation leading to soil erosion and malnutrition, inadequate land management, aquifer pollution, loss of biodiversity and reduction in overall crop resilience (Rehman et al., 2022). Among the current unsustainable patterns, the prevalence of monoculture is still a highly debated issue. Continuously cultivating the same crop on the same land can trigger greater susceptibility to environmental disturbances and extreme climate conditions, relying more on pesticides, herbicides and synthetic fertilizers (*European Commission*, n.d.).

This damaging repercussion directly contradicts the core principles of sustainability, which have become central to all sectors in recent years and emphasized in various international policies concerning the food chain and its related industries. One notable example is the Common Agricultural Policy (CAP) established by the European Commission, aligned with the Green Deals, for instance, the reduction of nutrient loss by at least 50% (without soil deterioration) and the decrease in use of 50% of all chemical and hazardous pesticides (European Commission - Green Deals, n.d.).

In the scope of these goals, alternative approaches such as intercropping have gained renewed attention, as it has potential to positively influence the management of natural resources in the crops as well as boost its resistance to the biotic and abiotic factors (Moreira et al., 2024).

1.2. Intercropping

Intercropping is an agricultural practice that involves growing on the same area of land, two or more crop species simultaneously, with sufficient overlap in their growth cycles to include their vegetative development stages (Gebru, n.d.).

The marginalization of intercropping throughout the 20th century was not solely the result of agronomic limitations or lack of scientific potential, but rather a consequence of dominant ideological and institutional priorities within agricultural science. As Harwood (2024) explains, the rise of monoculture-oriented research in industrialized nations was heavily influenced by a technocratic vision that favoured uniformity, mechanization and input-intensive models, traits that aligned poorly with the complexity and local specificity of intercropping systems. Scientifical and financial systems were shaped by this bias, which contributed to the neglect of mixed cropping approaches despite their proven benefits in small-scale contexts. This historical analysis highlights how ideological frameworks can constrain scientific inquiry and innovation and reinforces the importance of revisiting intercropping today not only as a practical agronomic tool, but also to challenge the structural biases embedded in modern agricultural systems (Harwood, 2024).

During the vegetative phase, plants develop non-reproductive lens such as leaves, stems and roots through physiological processes including cell division, elongation and differentiation. These processes contribute to biomass accumulation and lay the foundation for a successful reproductive phase, essential for an optimal yield.

Due to the accelerated metabolism activity, during this stage, plants have an increased need of water, light and nutrients. Under resource competition this can lead to excessive vegetative growth that leads to reduced maturation of reproductive structures, ultimately resulting in a decreased harvest index (HI) (*Plant Small RNA*, 2020).

As stated by Timaeus et al. (2022) in some studies when there is high density planting, often leads to increased vegetative investment instead of reproductive, reducing the HI. However, this does not necessarily imply intercropping as an unfavourable option. Studies on genetically diverse wheat populations have shown that, when the complementarity is strong, HI can indeed increase, making it a valuable indicator of interspecies mutual benefit.

In the context of intercropping, although different beings are in direct competition while sharing the same supplies, effective species selection and system design can promote positive plant-plant interactions. Different phonological characteristics, such as growth timing and development stages, can help reducing competition by allowing species to utilize resources in different timings (Nasar, 2019). When this complementarity between organisms are optimized, intercropping can improve resourcefulness and enhance grain yield (Demie et al., 2022).

The efficiency of the intercropping is usually measured through the Land Equivalent Ratio (LER), an index that indicates land productivity, comparing sole crops to the intercrop. If LER > 1, it states an advantage of intercropping compared to the monocropping, in terms of resource usage. Demie et al. (2022) concluded that 85% of the LER data studied presented a value greater than 1 for cereal/legume combination, highlighting the fact of the importance of genotype selection in this agricultural approach (Bedoussac et al., 2015; Demie et al., 2022).

1.3. Wheat-Legume Synergy in Intercropping

In Figure 1, it can be observed the symbiosis between cereals and legumes. This is of particular interest due to its ability to enhance nutrient bioavailability while providing multiple ecosystem services.

One of the most relevant forms of complementarity between these species lies in nitrogen dynamics: while wheat (*Triticum aestivum L.*) efficiently captures residual soil nitrogen from previous crops, the legume contributes by fixing atmospheric N_2, thus replenishing nitrogen levels with a cleaner, sustainable source that can also benefit the cereal partner (Mamine & Farès, 2020). The root system of the latter supports a rich rhizosphere, populated by diverse microorganisms capable of significantly improving microbial activity in the soil. This is largely driven by the plant's natural root exudates, which increase both the quantity and quality of microbial networks. These enhanced microbial dynamics promote more efficient nutrient cycling, support soil health and contribute to stronger root development and seedling establishment (Chamkhi et al., 2022).

Additionally, such systems contribute to carbon sequestration, a process in which atmospheric CO_2 is stored in the soil as soil organic carbon (SOC). This improves soil fertility and plays a critical role in mitigating climate change by enhancing ecosystem resilience and reducing greenhouse gas emissions (with potential reductions reaching up to 1400 Mt CO_2 eq. per year) (Rehman et al., 2022)

Moreover, in intercropping systems, phosphorus uptake can also benefit from interspecific facilitation (one species benefits, the other stands neutral). In this case, the legume plant increases soil P availability, allowing the companion crop to take advantage of it. This process can occur through direct mechanisms, such as the activity of root exudates, or indirectly, by altering the composition and function of the microbial communities within the rhizosphere. This is especially valuable in low-input systems, where phosphorus deficiency is a limiting factor (Y. Xue et al., 2016)

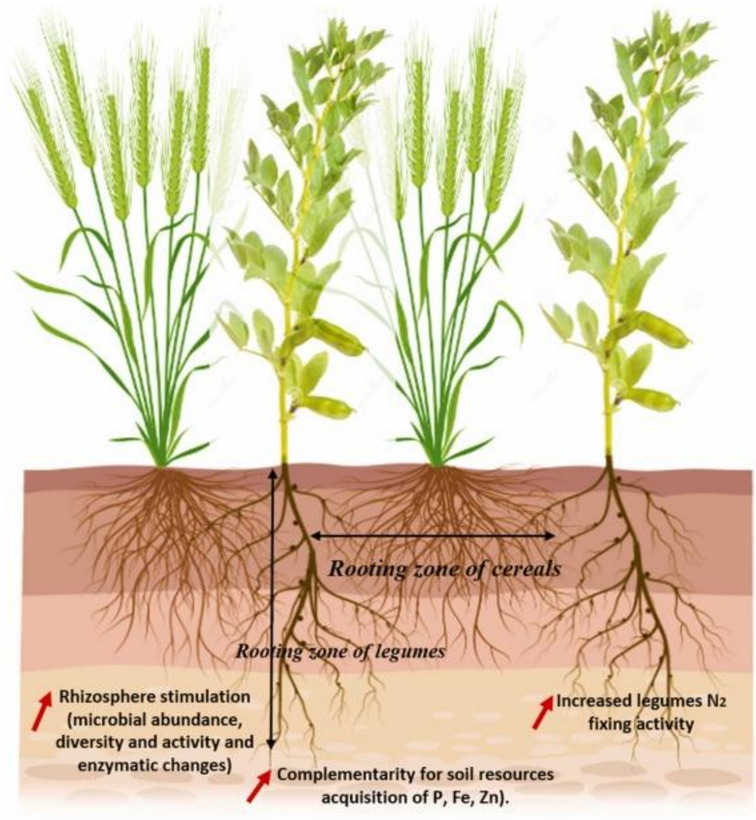


Figure 1- Root interactions in legume-cereal intercropping systems (Chamkhi et al., 2022)

This strong mutual reinforcement of nutrients enables plants to become more resistant to pests, diseases and weeds. In the same way, their biochemical interactions promote the production of secondary metabolites with potential allelopathic effects, providing them with self-sufficient natural herbicides and pesticides, against possible external biologic stresses (Rehman et al., 2022).

Even after harvest, such intercropping systems can enhance the nutritional quality of the raw materials and increase the value of derived products. Legumes play a key role in improving voluntary nutritional intake of the grains, particularly by increasing protein content, which helps meet the rising global demand for both food and feed proteins (Pankou et al., 2022).

1.4. Nutritional Impact of Cereals and Legumes

The cereal grains are configured into three main anatomical components (Figure 2): the bran, the outer layer, which is rich in dietary fibres, B-complex vitamins (such as riboflavin, thiamine, niacin), vitamin E (notably α-tocopherol) and minerals (Cu, Mg, Zn, Fe and P); the germ, the reproductive part, rich in essential oils, a small amount of protein and several micronutrients; and the endosperm which contains the majority of the starch and protein, constituting the base of the flour and largely influencing the quality of breadmaking (C. Xue et al., 2016)

Starch, the main component is composed of two major polysaccharides, which are amylose, mostly linear, and amylopectin, highly branched. In its native form, wheat starch exists as semicrystalline granules with organized regions formed by double helices of amylopectin chains. This matrix suffers reorganization and recrystallization during bread baking and aging, deeply affecting its firmness and texture (Nivelle et al., 2019).

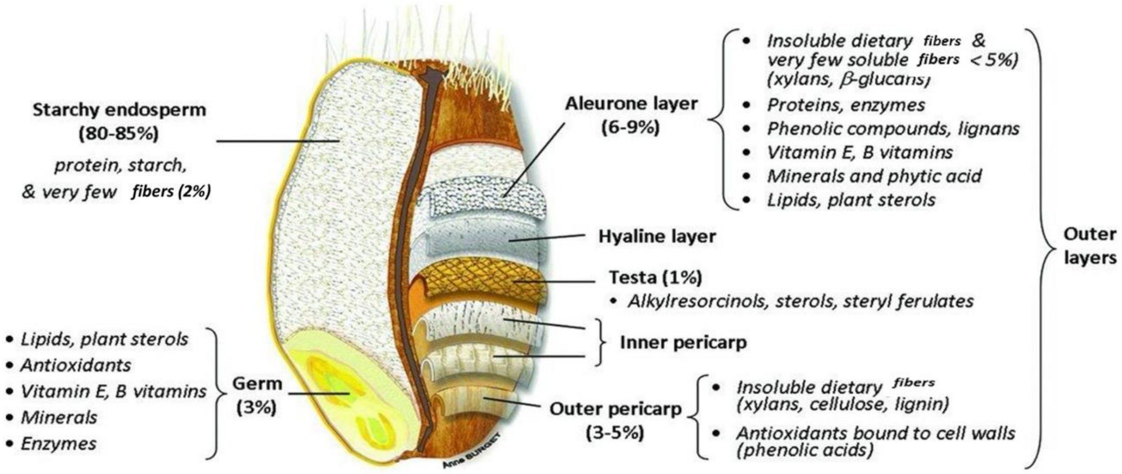


Figure 2- Anatomical structure and nutrient distribution in the wheat grain (Sztupecki et al., 2023).

Moreover, a key determinant is the relative proportion and molecular structure of the gluten protein fractions, which form a complex network primarily composed of intertwined gliadins and glutenins (Khalid et al., 2023)

Higher overall protein content has a significant impact not only on the mechanical performance of the dough, but can also modify certain organoleptic qualities, such as texture and mouthfeel, altering the consumer perception (Liu et al., 2025; C. Xue et al., 2016). Notably, numerous studies have shown that wheat grown in intercropping systems with legumes tends to exhibit increased protein concentration, likely due to improved nitrogen availability and resource complementarity (Liu et al., 2025).

Legumes themselves are packed with macro and micronutrients. In the case of peas, it typically contains 18-25% of proteins, slow-digesting carbohydrates, which reflects to a low glycaemic index, and dietary fibres, primarily located in the hulls, which supports gut health. They are also excellent sources of essential vitamins (e.g. folic acid and carotenoids) and minerals (e.g. calcium, iron and zinc), promoting the presence of bioactive compounds and the well-functioning of biological activities. Since most of the fibre is concentrated in the hulls, the decision to process peas with or without that external layer significantly affects the nutritional composition – especially the available fibre and protein content. The latter increases in dehulled peas, as proteins are not bound to the fibres (Mastromatteo et al., 2015; Wu et al., 2023).

1.5. Post-Harvest Processability and Breadmaking

Beyond their nutritional profile, the technological performance during post-harvesting processing plays a role in shaping the functional and dietary quality of the resulting product.

The adoption of intercropping may involve additional labour, technical expertise and even the use of specialized equipment. However, harvesting machines for these systems is still an understudied subject (Walter et al., 2024). In separation phase, due to their simultaneous cultivation and post-harvest processing, residues between the two crops can occur (Liu et al., 2025). This may lead to traces of one species remaining in the final yield of the other. While such cross-contamination is typically viewed as a drawback, especially when requiring product purity, it may also offer a nutritional advantage, as the presence of legume particles could enhance the wheat flour with additional value. This potential enrichment will be specifically evaluated in this study.

On the other hand, depending on the milling type, this process can lead to reduction in nutrient levels, in cases where the germ and barn are removed from the endosperm to produce a refined, starch-rich flour – classified as type W700 under Austrian code or T65 under Portuguese code. In contrast, when the entire grain is milled, the result is integral flour richer in fibres and with a greater ash content, designated as type W1600 (Austria) or T110 (Portugal). For this reason, chemical quality assessments are generally conducted after the milling, rather than on the crop, to better reflect the composition of the flour used in bread production (Mastromatteo et al., 2015).

Bread quality is fundamentally determined by the composition and structure of the cereal grains from which it is made. As one of the most widely consumed crops worldwide, wheat serves as a staple in both human and animal diets (Ghaley et al., 2005), as it contributes approximately to 55% of the carbohydrate's intake and 21% of total calories worldwide, making it the primary nutritional source for around 36% of the world's population (Khalid et al., 2023).

For the dough preparation, flour, water, salt and yeast are the indispensable ingredients being added, before fermentation. The hydration of the wheat flour combined with mechanical forces applied while kneading, causes disruption and reorganization of the gluten proteins, forming a visco-elastic protein network, as observed in Figure 3. This is stabilized by a combination of chemical interactions, including disulfide bonds (S-S) between glutenin chains, providing strength and elasticity. Gliadins are mostly monomeric and participate in weaker links, enhancing extensibility and viscosity. To further contribute to the cohesiveness and flexibility of the dough, non-covalent hydrogen bonds and hydrophobic interactions are formed within and between glutenin chains. The development of this structure can be assessed using a Farinograph or Mixograph (Goesaert et al., 2005).

A balanced ratio between the two (gliadins and glutenins) is crucial, as either excessive elasticity or resistance can negatively affect the final loaf volume (Ye et al., 2023). Current research supports the idea that differences in wheat quality are strongly associated with variations in glutenin structure and functionality (Khalid et al., 2023).

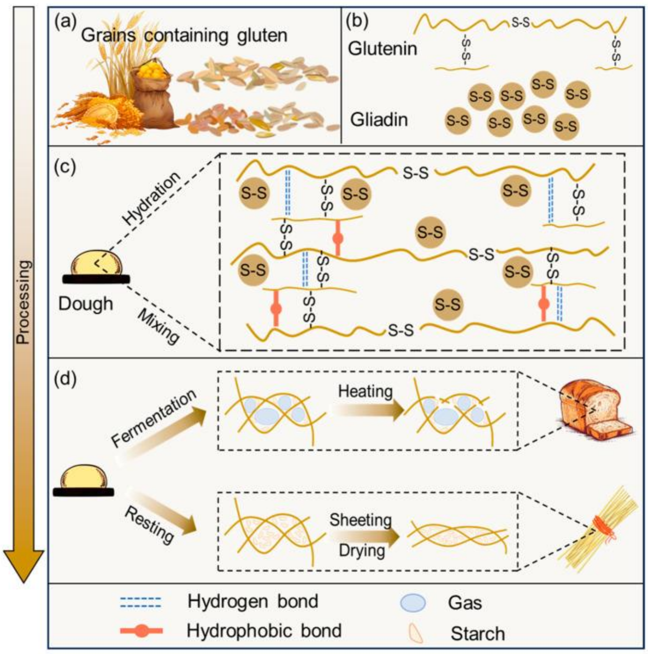
The gluten proteins in the flour-water matrix are below the isoelectric point, staying in an overall positive charge. This cause the proteins to repel and become more hydrated, creating shorter and stickier doughs. The salt (NaCl) plays a significant contribution to contradict this effect, by reducing the electrostatic repulsion between gluten proteins, promoting their aggregation through hydrophobic interactions. In addition, NaCl favours organoleptical features, like colour, texture and flavour (Avramenko et al., 2018).

The yeast (*Saccharomyces cerevisiae*) plays a fundamental role as a leavening agent by transforming substrate into carbon dioxide and ethanol, responsible for the dough expansion.

Yeast activity depends on temperature, with optimal fermentation occurring between 27 °C and 38 °C, peaking around 35 °C, remaining active within a broader range of 0 °C to 55 °C. As fermentation progresses, yeast metabolism not only inflates gas in the dough but also produces a variety of volatile and non-volatile compounds, such as, organic acids, aldehydes, esters, ketones and alcohols, that contribute significantly to the aroma and flavour of the final product as well as improve the gluten network extensibility (Avramenko et al., 2018).

During baking, due to water absorption and heat swelling, starch granules gelatinize, diffusing amylose, that rapidly retrogrades upon cooling. This procedure forms a firm network that contributes to the initial crumb structure (Figure 3).

In contrast, amylopectin retrogrades more slowly. Over time, this gradual crystallization process is the main driver of bread staling, leading to crumb firming and loss of softness. Thus, the balance and behavior of these two starch components are central to both the fresh quality and shelf life of bread (Nivelle et al., 2019).



Legend: (a) Grains containing gluten; (b) Glutenin and gliadin, the two main protein fractions; (c) Formation of the gluten network through hydration and mixing; (d) Functional changes in gluten structure during fermentation, heating, resting and drying.

Figure 3- Schematic representation of gluten development and structural interactions during processing (Ye et al., 2023)

Besides starch retrogradation, one of the key chemical reactions that occurs during baking is the Maillard reaction, a type of non-enzymatic browning reaction. It involves the interaction between reducing sugars and primarily free amino acids or peptides upon heating. It plays a fundamental role in developing a golden-brown crust in baked goods and contributes significantly to the generation of characteristic flavours and aromas (Avramenko et al., 2018).

1.6. Extrusion

Cooking by extrusion is a thermal processing widely used in the production of cereal-based foods, known for its abilities to modify the functional characteristics of flours. This technique is considered a HTST (high temperature, short time) method, where the flour is subjected to intense heat and mechanical forces, under low-moisture conditions. Within a matter of minutes, multiple unit operations, such as mixing, shearing, cooking and shaping, occur simultaneously. These lead to significant chemical transformations, including protein denaturation, starch gelatinization, enzyme activation or inactivation and Maillard reactions, providing the extrudate with distinct organoleptic aspects.

Beyond chemical changes, the rheological properties are also affected, depending largely on the specific extrusion parameters, such as, temperature, initial moisture, and screw speed. At higher processing intensities, amylopectin chains may break down, leading to further modifications in texture and overall functionality of the extruded product (Martínez et al., 2014; Nivelle et al., 2019).

1.7. Objectives of the Study

The main objective of this study was to evaluate the impact of an intercropping plantation of cereal-legume on the nutritional quality, breadmaking and processability of bread, seeking in giving a scientific foundation to farmers in assessing intercropping as a viable option, due to its sustainable approach related to the traditional monoculture regime.

To achieve this focal goal, this thesis anchored in the following specific objectives:

- To evaluate and compare the chemical composition and rheological behaviour of the five flours used in this study: W700, W1600 intercrop (IC), W1600 purecrop (PC), pea flour with hulls and pea flour without hulls, to understand how processing and cropping system affect flour functionality. The comparison between hulled and dehulled pea flours aimed to determine which form was more suitable for breadmaking applications, based on their impact on flour quality and functional performance.

- To assess the impact of pea flour incorporation (0% to 5%) into W700 wheat flour bread quality and processing performance. This range reflects the level of legume residues commonly found in intercropped wheat after harvest, when no sieving or sorting is applied. The use of W700 flour in this trial aimed to isolate the effect of pea flour content, independent of the intercropping factor.
- To compare the quality and processability of bread with W1600 PC and bread with W1600 IC, in order to determine whether intercropping influences the final product.
- To investigate the effect of intercropping on the performance of flour under extrusion processing assessing parameters such as expansion, texture and general processability of the extrudates.

2. Materials and Methods

2.1. Preparation of Intercropping

The intercropping system used in this study combined a modern winter wheat (*Triticum aestivum* L., variety Centurion) with chickpeas (*Cicer arietinum* L., variety Lambada), cultivated in Verfeil (31590), south-west France.

The field used had previously been cultivated with sole sunflower and ensured good homogeneity across the plot. In the wheat-chickpea intercrop system, plant density consisted of approximately 86% wheat and 14% chickpea, corresponding to 202 wheat and 34 chickpea plants, closely matching the 246 wheat plants in the pure wheat plot (monoculture). Importantly, no nitrogen fertilizer was applied to either system.

Finally, since pea flour was still required for bread formulation tests, and peas were not part of the current intercropping setup, the pea flour used for those tests was sourced from a different partner.

2.2. Chemical Analysis

2.2.1. Dry Matter

The dry matter content of the samples was determined, according to the method ICC 110/1 (International Association for Cereal Science and Technology (ICC), 2023). The method is based on gravimetric drying, where previously milled or ground samples (particle size < 1 mm, with at least 50% < 0.5 mm) were weighed (~5 g) into pre-dried aluminium dishes and dried at high temperature until constant weight was achieved. The drying was performed overnight at 103 °C in a Phoenix Instrument Incubator TIN-TF120 series. Between drying cycles, samples were cooled for at least 30 minutes in a desiccator with silica gel before weighing. The final dry matter percentage was calculated using the formula:

$$dry\,matter\,[\%] = \frac{Wx\ -\ T}{S} * 100$$

where Wx is the stable final weight (sample + dish), T is the tare weight of the dish, and S is the initial sample weight. All analyses were performed in triplicate.

2.2.2. Ash Content

The ash contente was determined following the method ICC 104-1(International Association for Cereal Science and Technology (ICC), 2023). The analysis was conducted in triplicate, with each sample (~5 g) weighed into pre-dried porcelain crucibles and incinerated in a rapid incinerator model SVD 95 P. Crucibles were first dried at 130 °C for at least 2 hours, cooled in a desiccator and their tare weight (T) recorded. Samples were then heated in a Carbolite AAF 11/7 ashing furnace, starting from room temperature and gradually increasing to 550 °C, where they were held for 3 hours until complete combustion. After ashing, crucibles were cooled again in a desiccator (60-90 min) and weighed to determine the ash residue (A). The ash percentage was calculated in relation to the initial sample weight (W) and adjusted to dry mass based on previously determined dry matter values (DM). The final results were expressed as:

$$ash\,[\%] = \frac{A\,[g] -\ T\,[g]}{W\,[g]}$$

$$ash\ in\ dry\ mass\ [\%] = \frac{ash\ [\%]}{DM\ [\%]} * 100$$

2.2.3. Soxhlet Extraction (Crude Fat content)

The crude fat content was determined by Soxhlet extraction, following the standard protocol AACC method 30-10 (AACC International, 2010). Approximately 2 g of finely milled sample (adjusted according to expected fat content) were weighed into cellulose extraction filters and placed in Soxhlet extraction units (behr Labor-Technik GmbH, Düsseldorf, Germany). The system was assembled with flat-bottom flasks connected to condensers containing ~150 mL of petroleum ether (boiling range 40-60 °C) and boiling stones. The extraction was carried out under reflux for a minimum of 4 hours (ideally ~6 h), with a flow rate of around 6 drops per second and at least 25 extraction cycles. After extraction, the majority of the solvent was recovered, and the remaining ether was evaporated by heating the flask until dry. The flasks were then dried at 103 °C for 1 hour, cooled in a desiccator and weighed. Crude fat content was calculated using the formula:

$$crude\ fat\ [\%] = \frac{m2\ -\ m1}{w} * 100$$

where m_1 is the tare weight of the flask, m_2 is the final weight after extraction, and w is the sample weight. All measurements were performed in triplicate, with proper safety measures under fume hoods due to the flammable nature of the solvent.

2.2.4. Kjeldahl Quantification (Crude Protein content)

The crude protein content of the samples was determined using the Kjeldahl method, in accordance with the ICC standard 105/2 (International Association for Cereal Science and Technology (ICC), 2023). The method is based on the quantification of total nitrogen, which is used as an indirect measure of protein. Approximately 2 g of homogenised sample were digested at a BUCHI KjelDigester K-449 in concentrated sulfuric acid with KjelTabs containing catalysts (K_2SO_4, $CuSO_4 \cdot 5H_2O_3$ TiO_2) to accelerate the oxidation of organic matter. The resulting ammonium ions were then neutralised with sodium hydroxide (NaOH 32%) and distilled via steam into a boric acid solution (4%, pH 4.65), forming ammonium borate complexes. These were subsequently titrated, using a BUCHI

MultiKjel K-365, with standardised hydrochloric acid. All analyses were performed in triplicate, with glycine standards and blanks used to verify method validity.

$$\% \text{ nitrogen (glycine)} = \frac{(\text{V [ml]} - \text{B [ml]}) \times \text{M(N)}\left[\frac{\text{g}}{\text{mol}}\right] \times \text{c}\left[\frac{\text{mol}}{\text{l}}\right] \times \text{z}}{\text{W [g]} \times 10}$$

$$\% \text{ protein (wet sample)} = \frac{(\text{V [ml]} - \text{B [ml]}) \times \text{M(N)}\left[\frac{\text{g}}{\text{mol}}\right] \times \text{c}\left[\frac{\text{mol}}{\text{l}}\right] \times \text{z x F}}{\text{W [g]} \times 10}$$

$$\% \text{ protein (dry sample)} = \frac{\% \text{ protein (wet sample)x 100}}{\% \text{ dry mass}}$$

Where V is the volume of titrant used for sample, B the volume of titrant used for blank sample, M (N) Atomic mass of nitrogen (14.0067 g/mol), c the concentration of acid standard solution, z valency of acid (1 for HCl, 2 for H_2SO_4), W the sample weight, F the factor for converting nitrogen into protein (6.25).

2.2.5. Starch Content

The total starch content of the samples was determined using the Neogen Rapid Total Starch (RTS) method, based on AOAC Method 996.11 (AOAC International, 2005) and AACC Method 76-13.01 (AACC International, 2010). In this enzymatic method, starch is hydrolysed into maltodextrins by thermostable α-amylase, followed by conversion into D-glucose using amyloglucosidase. The resulting glucose is then oxidised by glucose oxidase into D-gluconate and H_2O_2, which is quantified colorimetrically through the formation of a quinoneimine dye, measured at 510 nm. Each sample was prepared in triplicate, along with a sample blank to correct for free glucose content. Approximately 95-105 mg of sample were incubated in sodium acetate buffer (pH 5.0) and subjected to sequential enzymatic digestion at 50 °C in a Stuart SBH130D/3 block heater. After centrifugation (using and Eppendorf Centrifuge 5430 model) and dilution (standard dilution factor: 4), the supernatant was reacted with GOPOD reagent, and the absorbance was recorded using a Shimadzu UV-1800 VIS spectrophotometer. The final starch content was calculated incorporating glucose standards, dilution factors and correction for blank values, with results expressed as g/100 g sample (dry basis).

$$TS[g/100g] = \left[\frac{S - RB}{W}\right) - \left(\frac{SB - RB}{WB}\right)] \times \frac{100}{\left(\left(\frac{G1 + G2 + G3 + G4}{4}\right) - RB\right)} \times \left(\frac{E}{V}\right) \times DF \times \left(\frac{1}{1000}\right) \times \left(\frac{162}{180}\right) \times 100$$

Where S is the absorbance of the sample, SB the absorbance of sample blank, W the sample weight, WB the sample blank weight, RB the absorbance of reagent blank (zero, if values measured against it in photometer), G the absorbance of glucose standard, E the Extraction volume = ml Buffer + ml Enzymes (AA, AMG), usually 10.2 ml, V the volume of samples analyzed (0.1 ml), DF the Dilution Factor (4), 1/1000 is the conversion from µg to mg, 162/180 is the conversion factor from glucose to anhydroglucose, as found in starch and, finally, 100 the conversion to % = g/100g.

2.3. Rheological Analysis

2.3.1. Water Absorption Index (WAI) and Water Solubility Index (WSI)

The Water Absorption Index (WAI) and Water Solubility Index (WSI) were determined to evaluate the functional properties of the flours and extrudates, according to the method described by Anderson (1982). Approximately 1.5 g of ground sample was suspended in 30 mL of distilled water in a 50 mL Falcon tube, vortexed for 15 seconds and incubated in a GFL 1086 shaking water bath at 30 °C for 30 minutes, using vortex every 10 minutes. After incubation, the samples were centrifuged at 1739×g for 10 minutes at an Eppendorf Centrifuge 5804 R model. The supernatant was carefully poured into a pre-weighed aluminium dish and dried in an oven at 103 °C until constant weight. The weight of the pellet (gel) remaining in the tube was noted.

The WSI was calculated as the solubilized dry matter relative to the initial sample dry weight and the WAI was calculated as the mass of water absorbed by the non-solubilized fraction of the sample. All measurements were conducted in triplicate and results were expressed on a dry matter basis.

$$WAI\ (\%) = \frac{Weight\ of\ sediment}{Weight\ of\ dry\ sample} \text{ x } 100$$

$$WSI\ (\%) = \frac{Weight\ of\ dissolved\ solids\ in\ supernatant}{Weight\ of\ dry\ sample} \text{ x } 100$$

2.3.2. Rapid Visco Analysis (RVA)

The pasting properties of the flour samples were determined using a Rapid Visco Analyser (RVA), following the AACC International Method 76-21.01 (AACC International, 2010) and ICC Standard No. 162 (International Association for Cereal Science and Technology (ICC), 2023), as described RVA methods 01.05 e 05.02 by Perten Instruments. The RVA measures the viscosity changes of starch-containing samples under controlled heating, shearing and cooling, enabling the assessment of starch behaviour during processing. For each test, 3.50 g of flour (corrected depending on the moisture of each flour) were mixed with 25.0 mL of distilled water in an RVA 4500 model by Perten Instruments. The standard pasting profile was applied: initial temperature at 50 °C, ramped and held at 95 °C and, finally, cooled back to 50 °C, with stirring speeds of 960 rpm initially, then reduced to 160 rpm for the remainder of the test. The duration of the total test was 13 minutes.

The RVA software recorded the viscosity profile, such as the key parameters: pasting temperature (PT), peak viscosity (PV), breakdown (BD), final viscosity (FV) and setback (SB). These parameters provide valuable insights into the gelatinisation, stability and retrogradation behaviour of the starch in the samples. Each analysis was performed in triplicate and results were expressed in centipoise (cP).

2.3.3. Farinograph and Extensograph

The analysis of the dough properties was performed using a Farinograph (Brabender & Co. KG, Duisburg, Germany). Prior to testing, the moisture content of each flour sample was determined through many trials and entered into the system. The measurements followed the Brabender ICC/ISO standard method (International Association for Cereal Science and Technology (ICC), 2023), with the dough consistency target set at 500 Brabender Units (BU). Based on this setting, the following parameters were recorded: water absorption (WA), dough development time (DT), stability (S) and degree of softening (DS). All measurements were performed in triplicate for each flour blend. The bread formulations were subsequently adjusted in terms of water content, using the farinograph-derived water absorption values for each of the five flour types tested.

The Extensograph (Brabender & Co. KG, Duisburg, Germany) was employed to evaluate the elasticity and tensile strength of the doughs, offering insights into their viscoelastic properties. Dough samples were prepared based on the water absorption values previously determined by the farinograph analysis, following the same preparation protocol. However, in this case, 2% salt (relative to the flour weight) was incorporated into the formulation, which required a corresponding reduction of 2% in water content.

The mixing time for dough preparation was set to 5 minutes. After that, the dough was submitted to fermentation at 30 °C, and measurements were taken after 45, 90 and 135 minutes of resting. For each time point, two dough samples from the same batch were analysed in parallel and all tests were conducted in triplicate to ensure repeatability.

The Extensograph provided the following parameters: resistance to extension, measured in Brabender Units (BU); extensibility, recorded in mm; and the energy required until dough rupture, in cm^2.

2.4. Breadmaking

The bread doughs were prepared using a standardised formulation (Method ICC 130) (International Association for Cereal Science and Technology (ICC), 2023) adapted for a total dough weight of 1000 g. Six formulations were tested: one with 100% W700 wheat flour and the others from 1% to 5% substitution of hulled pea flour.

Table 1- Standardized wheat bread formulation.

Ingredients	0%** [g]	1% [g]	2% [g]	3% [g]	4% [g]	5% [g]
Wheat Flour	606.8	600.7	594.7	588.6	582.5	576.5
Pea Flour	0	6.1	12.1	18.2	24.3	30.3
Water*	358	358	358	358	358	358
Dry Yeast	18.2	18.2	18.2	18.2	18.2	18.2
Salt	10.9	10.9	10.9	10.9	10.9	10.9
Sugar	6.1	6.1	6.1	6.1	6.1	6.1

Legend: *Water content was adapted based on the outcome of the Farinograph (For W700 wheat flour, the value was 59% of water absorption)/ **This recipe was also used for the comparison of the breads with W1600 PC and W1600 IC.

This standardisation ensured consistent dough handling and allowed for reliable comparison of the impact of pea flour incorporation on bread quality and processing behaviour. The dry ingredients were first homogenised using a laboratory kneader at speed 2 for 30 seconds. Subsequently, water was added while kneading continued at the same speed. After that, the dough was then kneaded for 6 minutes at speed 4, followed by 1 minute of manual moulding.

The resulting dough was transferred to a fermentation chamber (MANZ Backtechnik GmbH, Creglingen, Germany) set at 30 °C and 85% relative humidity (RH) for a first fermentation of 30 minutes. After this rest period, the dough was divided into three equal 300 g portions, manually moulded, rolled into a screw shape and placed into oiled baking tins. A second fermentation was carried out under the same conditions (30 °C, 85% RH) for 50 minutes.

Baking was performed in a conventional deck oven (Model 60/rW, MANZ Backtechnik GmbH, Creglingen, Germany) at 220 °C for 25 minutes. After baking, the breads were allowed to cool at room temperature for 2 hours and then stored overnight at 20 °C and 50% RH in a climate chamber prior to further analyses. This process was repeated for the evaluation of W1600 wheat breads.

2.5. Physical Analysis

The physical properties of the breads were assessed through a combination of instrumental and gravimetric methods.

Firstly, the baking loss was calculated as the percentage of mass lost during baking, using the formula:

$$baking\ loss\ [\%] = \frac{m1 - m2}{m1} * 100$$

where m_1 is the mass of dough for each loaf of bread and m_2 is the final weight of the bread after cooling down.

The volume and specific volume [cm^3/g] was determined via Bread Volume Meter BVM 6600 for each bread loaf. This data was then used to calculate volume yield (%) dividing the volume by the weight of the amount of flour used for each bread.

Colour analysis was carried out on both crumb and crust using the IRIS colorimeter operating in CIELAB system (Figure 4), measuring L* (lightness), a* (red-green) and b* (yellow-blue) values. Each sample was measured in triplicate and averaged.

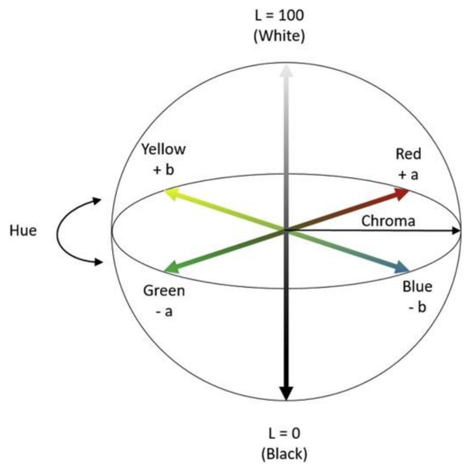


Figure 4- CIELAB colour space diagram (Ly et al., 2020).

In terms of texture, it was analysed, using a TA.XT Plus Connect Texture Analyser, conducted through a compression test on slices of 3 cm thickness, taken from the centre of each loaf. These measurements were performed immediately after slicing to reduce moisture loss effects. A 50 kg measuring cell and penetration plunger were used to apply compression with pre-determined settings (Table A1 - Appendice). The key parameters recorded included maximum force (F_{max}), minimum force after 120 seconds (F_{min} 120) and relative elasticity (Rel. El.), as indicators of crumb firmness and springiness.

Finally, regarding the crumb porosity, using ImageJ, an image analysis software, the images were processed with cross-sectional bread slices scanned at high resolution, to quantify pore size distribution, number and total porosity, providing a structural evaluation of the internal crumb.

2.6. Extrusion

Extrusion trials were carried out using a Brabender Twin-Screw Extruder TSE 20/40 (Brabender GmbH & Co. KG, Duisburg, Germany). The processing conditions were selected based on previous studies involving wheat flour extrusion and adapted according to equipment specifications. Temperatures were gradually increased across four zones

(30 °C to 130 °C) with screw speed set to 150 rpm. The moisture content of the feed was adjusted to 25%. Extrudates were collected for further analysis of dry matter, physical characteristics and hydration properties (WAI and WSI).

2.7. Statistical Analysis

The statistical analysis of the data was performed with the software STATGRAPHICS Centurion 19, Version 19.7.01 (Statgraphics Technologies, Inc., The Plains, VA, 20198, USA). One-way ANOVA test with a confidence interval of $\alpha = 0.05$ was conducted to determine the significance of differences. Means that differed significantly were denoted with different superscript letters in the tables and figures.

3. Results and Discussion

3.1. Chemical Analysis of Flours

All results of the chemical analysis performed to the flours, used during the study, are presented in **Table 2**. Although a wheat-chickpea intercropping system was implemented in the field due to the availability of this legume on the part of the collaborating farmer, pea flour was used for analytical purposes. This decision was based on the fact that pea is the legume species more commonly employed in intercropping systems, by this supplier, with wheat. In this specific context, the use of pea in the field was not feasible, selecting the more convenient legume, at the moment, as the synergetic partner.

Table 2- Results of the chemical analysis of conventional wheat flour (W700), purecropped wholegrain flour (W1600), intercropped wholegrain flour (W1600), pea flour with hulls and pea flour without hulls.

Sample	Dry Matter (%)	Ash [%;d.b.]	Fat [%;d.b.]	Protein [%;d.b.]	Starch [%;d.b.]
W700	88.04 ± 0.05^{a}	0.76 ± 0.02^{b}	1.73 ± 0.33^{a}	16.73 ± 0.07^{a}	71.20 ± 2.08^{a}
W1600 PC	87.83 ± 1.42^{a}	0.68 ± 0.01^{a}	1.60 ± 0.29^{a}	10.38 ± 0.03^{b}	72.87 ± 3.70^{a}
W1600 IC	88.71 ± 0.14^{a}	0.69 ± 0.01^{a}	1.57 ± 0.19^{a}	10.17 ± 0.05^{c}	84.07 ± 6.43^{a}
Pea Hulled	90.43 ± 0.11^{b}	2.95 ± 0.02^{c}	2.13 ± 0.09^{b}	22.89 ± 0.07^{d}	38.63 ± 3.07^{b}
Pea Non-Hulled	91.52 ± 0.04^{b}	2.97 ± 0.02^{d}	2.05 ± 0.08^{b}	28.42 ± 0.13^{e}	40.73 ± 0.85^{b}

All values are means ± SD (n = 3 for all analysis). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$).

From a general perspective, and as expected based on the nutritional composition of each crop, wheat flours exhibit significantly higher starch content compared to pea flours. The latter show greater concentrations of ash, fat and protein. These results are consistent with values reported by Padalino, et al (2014), regarding the chemical composition of pea flour, as well as those described for wheat flour by Steve (2012) and Chai, et al (2025).

A more detailed analysis reveals that W700 flour, being the refined white flour, should theoretically present lower ash and fat contents when compared to wholemeal flours, due to the absence of bran and germ (where the majority of minerals and lipids are located). Similarly, the starch content would be expected to be higher in W700, given that it is composed mainly of the endosperm, which is rich in starch and storage proteins. However, these compositional traits may be influenced by both environmental and agronomic factors, including differences in the origin of the samples, as the W700 and W1600 flours were sourced from different suppliers.

Protein content was the only parameter showing statistically significant differences between all samples. This result highlights the complexity of protein synthesis, which can be affected by several factors, including the specific legume species used in intercropping and the type and conditions of the agriculture utilized. While the underlying principle of cereal-legume intercropping, by enhancing nutrient availability through biological nitrogen fixation, remains constant, the degree of synergy achieved can vary depending on the species combination and environmental context. For instance, during sowing, mineral nitrogen levels in the soil were relatively similar between the systems, averaging 32.5 kg/ha overall: ranging from 24-32 kg/ha for the IC plot and 36-37 kg/ha for the pure wheat plot. This slight increase in initial nitrogen availability in the monoculture may explain the marginally higher protein content observed in PC wheat compared to IC.

A notable observation is the higher protein content found in non-hulled pea flour compared to hulled pea flour. This supports the notion that pea proteins are not strongly bound to the fibrous hulls, resulting in a concentration effect upon dehulling. Accordingly, the presence or absence of hulls in pea flours significantly affects protein and ash content, but not other chemical parameters.

In summary, the results presented in Table 2 indicate that the intercropped sample has no significant impact on the overall composition of the flours. Apart from variations in ash and protein content, the wholemeal flours show no meaningful differences when compared to conventional refined flour. However, substantial compositional differences are evident between wheat and pea flours, reflecting the distinct nutritional profiles of these two species.

3.2. Rheological Analysis of Flours

The measurements of the hydration properties (WAI and WSI) of the flours used throughout the study are summarized in **Table 3**.

Table 3- Results of the WAI and WSI analysis of conventional wheat flour (W700), purecropped wholegrain flour (W1600), intercropped wholegrain flour (W1600), pea flour with hulls and pea flour without hulls.

Sample	WAI [g/g;d.b.]		WSI [g/g;d.b.]	
	30ºC	90ºC	30ºC	90ºC
W700	1.31 ± 0.23^{a}	12.49 ± 0.49^{c}	6.99 ± 0.04^{a}	8.61 ± 0.70^{a}
W1600 PC	1.43 ± 0.04^{a}	13.41 ± 0.14^{d}	7.11 ± 0.07^{a}	10.73 ± 2.43^{a}
W1600 IC	1.43 ± 0.03^{a}	10.17 ± 0.55^{b}	6.97 ± 0.05^{a}	14.77 ± 3.29^{b}
Pea Hulled	2.41 ± 0.07^{b}	9.83 ± 0.30^{b}	27.10 ± 0.35^{b}	18.18 ± 0.86^{c}
Pea Non-Hulled	2.22 ± 0.04^{b}	8.37 ± 0.62^{a}	32.22 ± 0.50^{b}	29.22 ± 0.33^{d}

All values are means ± SD (n = 3 for all analysis). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$). WAI: Water Absorption Index; WSI: Water Solubility Index.

WAI measures the capacity of a material to absorb water and swell, reflecting its ability to reach a certain consistency, particularly relevant during breadmaking. It serves as an indicator of the starch gelatinization. On the other hand, WSI quantifies starch degradation by measuring the number of soluble polysaccharides released from starch granules when exposed to an excess of water (Choi et al., 2012). For both measurements, values tend to be higher at 90 ºC, as elevated temperatures promote greater water absorption and solubility. However, an exception to this trend is observed in pea flours in WSI analysis.

W700 and W1660 purecropped present significantly higher WAI values at 90ºC than W1600 intercropped and pea flours, demonstrating their greater capacity for water uptake and enhanced gelatinization at high temperatures (conditions typically encountered during breadmaking). This behaviour suggests a more stable starch structure capable of forming a well-developed gel, especially due to the increased starch contents present in wheat flours that favours gelatinization and enhances water absorption. In contrast, pea flours exhibit markedly lower WAI values (with the exception of the W1600 IC and pea hulled flour comparison), indicating a weaker gelatinization potential and, consequently, a less robust gel structure. This limitation in gelatinization observed in pea flours is further supported by the Rapid Visco Analyser (RVA), where the absence of characteristic peaks in Figure 5 confirms reduced starch functionality under heat.

Regarding WSI, pea flours display significantly higher values at both 30ºC and 90ºC compared to wheat flours, suggesting a higher degree of starch instability and lower thermal stability. This interpretation is corroborated by the breakdown and trough values from the RVA analysis, which reinforce the lower heat resistance of pea starch.

The limited gelatinization capacity observed in pea flours can be attributed to their higher contents of protein, ash and fat, as shown in Table 2. These components interfere with water absorption and hinder the proper swelling of starch granules, thereby preventing the formation of a cohesive gel network, assuming the less favourable performance of pea flours in gelatinization-related processes.

Choi, I. et al (2012) highlighted that flours with higher WAI and lower WSI, such as wheat flours, are better suited for applications requiring high viscosity.

The **Table 4** presents the pasting properties of the flours used throughout the study. **Figure 5** illustrates the viscosity behaviour of the flours according to the duration and temperature of the procedure.

Table 4- Results of the RVA analysis of conventional wheat flour (W700), purecropped wholegrain flour (W1600), intercropped wholegrain flour (W1600), pea flour with hulls and pea flour without hulls.

Sample	Peak viscosity [mPa.s]	Trough viscosity [mPa.s]	Breakdown viscosity [mPa.s]	Final Viscosity [mPa.s]	Setback viscosity [mPa.s]	Peak Time [min]	Pasting Temperature [ºC]
W700	2148 ± 112[d]	1297 ± 61[d]	851 ± 59[b]	2492 ± 116[c]	1195 ± 58[c]	6 ± 0.08[b]	81 ± 10[a]
W1600 PC	2039 ± 39[cd]	1157 ± 27[cd]	882 ± 16[b]	2331 ± 33[bc]	1174 ± 19[c]	6 ± 0.08[a]	85 ± 0.4[a]
W1600 IC	1939 ± 64[c]	1114 ± 32[c]	826 ± 42[b]	2243 ± 56[b]	1130 ± 25[c]	6 ± 0[ab]	86 ± 0.03[a]
Pea Hulled	680 ± 20[a]	670 ± 22[a]	11 ± 3[a]	1358 ± 29[a]	688 ± 8[b]	7 ± 0[c]	84 ± 0.4[a]
Pea Non-Hulled	984 ± 152[b]	944 ± 169[b]	40 ± 17[a]	1559 ± 220[a]	615 ± 54[a]	7 ± 0.2[c]	79 ± 1[a]

All values are means ± SD (n = 3 for all analysis). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$).

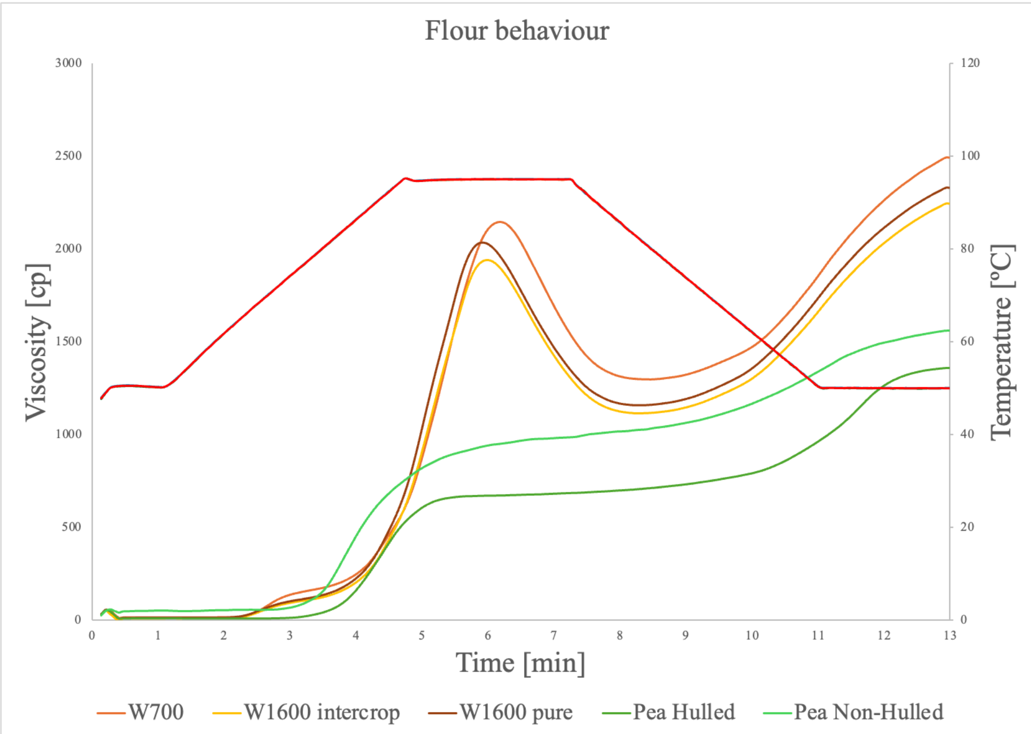


Figure 5- Pasting profiles of conventional wheat flour (W700), purecropped wholegrain flour (W1600), intercropped wholegrain flour (W1600), pea flour with hulls and pea flour without hulls.

W700 flour shows the highest peak viscosity, followed by the wholegrain wheat flours. This is an expected result for flours with high starch content (Table 2), as starch plays a key role in gelatinization, leading to a sharp increase in viscosity during heating. In contrast, pea flours exhibit a much less pronounced peak. This is not only due to their lower starch content, but also to their higher protein levels, which can interfere with starch swelling. As a result, their peak viscosity is both lower and reached at a significantly different time. The peak time parameter reflects how long it takes for a sample to reach its maximum viscosity. This graph follows a similar curve presented by Moreno-Araiza, O. et al (2023).

Breakdown viscosity, the difference between the peak and trough viscosities, indicates the stability of the starch paste under heat and mechanical agitation. A higher breakdown value suggests that the sample loses viscosity more easily and is thus less stable under processing conditions. Wheat flours exhibit much higher breakdown values than pea flours, which may suggest that pea flours are more stable under these conditions. This apparent stability could be advantageous in processes such as breadmaking, where dough is exposed to both heat and mixing.

However, when analysing the trough viscosity (the minimum viscosity recorded after the peak during sustained high temperature) it can be seen that pea flours have much lower values than wheat flours. At first glance, this seems to contradict the previous interpretation based on breakdown, but this can be explained by the fact that pea flours never reach high peak viscosities to begin with, as there is less gelatinized structure, there is also less viscosity to lose, resulting in both lower trough and lower breakdown values. Therefore, the low breakdown in pea flours does not necessarily indicate high stability, but rather a limited capacity for gelatinization.

Final viscosity and setback (calculated as final viscosity minus trough viscosity) are both significantly higher in wheat flours than in pea flours. This indicates that starch retrogradation (the re-association of starch molecules upon cooling) is more pronounced in wheat, leading to firmer gel structures. The presence of proteins and fibres in pea flours likely interferes with this process. For instance, the hulled pea flour, which contains more fibre, shows a lower final viscosity on average, supporting this interpretation.

Lastly, the pasting temperature (the temperature at which viscosity begins to rise sharply) does not differ significantly among the flour types. However, it is worth noting that the intercropped pea flour shows the higher value, on average. This may indicate the presence of certain compounds or residues from the intercrop system that delay the onset of gelatinization (Moreno-Araiza et al., 2023)

The **Table 5 and Table 6** presents the outcome of Farinograph and Extensograph rheological properties of the flours, respectively.

Table 5- Results of the Farinograph analysis of conventional wheat flour (W700), purecropped wholegrain flour (W1600) and intercropped wholegrain flour (W1600).

Sample	**Water Absorption [%]**	**Development Time [min]**	**Stability [min]**	**Degree of softening [FU]**	
				after 10 min	**after 12 min**
W700	59.10 ± 0.10^{c}	6.53 ± 0.16^{b}	12.63 ± 11.37^{b}	12.67 ± 1.53^{a}	43.00 ± 1.73^{a}
W1600 PC	53.0 ± 0.0^{b}	1.22 ± 0.10^{a}	1.48 ± 0.05^{a}	114.00 ± 6.08^{b}	125.67 ± 7.51^{b}
W1600 IC	52.5 ± 0.0^{a}	1.18 ± 0.065^{a}	1.27 ± 0.05^{a}	126.67 ± 11.37^{b}	136.00 ± 10.82^{b}

All values are means ± SD (n = 3 for all analysis). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$).

Table 6- Results of the Farinograph analysis of conventional wheat flour (W700), purecropped wholegrain flour (W1600) and intercropped wholegrain flour (W1600).

Sample	Extensibility [mm]			Resistance [BU]			Energy [cm²]		
	45 min	90 min	135 min	45 min	90 min	135 min	45 min	90 min	135 min
W700	237.67 ± 10.41[a]	222.67 ± 21.57[a]	207.67 ± 18.61[a]	235.33 ± 49.17[a]	289.00 ± 49.76[a]	302.00 ± 64.82[a]	132.67 ± 25.01[a]	147.00 ± 9.00[a]	138.67 ± 13.61[ab]
W1600 PC	147.00 ± 12.73[b]	147.00 ± 8.49[b]	190.5 ± 75.67[a]	365.00 ± 140.01[a]	327.50 ± 111.02[a]	332.50 ± 36.06[a]	90.00 ± 22.63[a]	82.00 ± 19.80[b]	124.00 ± 62.23[a]
W1600 IC	137.50 ± 7.78[b]	138.00 ± 2.83[b]	135.50 ± 2.12[a]	373.50 ± 57.28[a]	326.50 ± 10.61[a]	324.50 ± 12.02[a]	83.50 ± 7.78[a]	75.50 ± 0.71[b]	72.50 ± 6.36[b]

All values are means ± SD (n = 3 for all analysis). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$).

The physical properties of dough are critical in determining the final quality of baked products. One of the key tools used to assess flour quality is the farinograph, which monitors changes in dough consistency during mixing. Among the parameters obtained, water absorption (WA) is especially important, as it quantifies the amount of water required that can be added for an optimal consistency and structural stable dough (Branch, n.d.). For this reason, WA values were used to determine the water content in the bread formulation.

Results show that WA was highest in the conventional refined flour (W700), followed by purecropped then intercropped flours, with all these showing statistically significant differences. This trend implies that the more refined the flour, the greater its capability to absorb water and develop a stable dough structure.

The Development Time (DT) represents the time elapsed from the initial addition of water until the dough reaches its maximum consistency (peak torque) (Lei et al., 2008). While Lei et al. (2008) did not observe a direct correlation between WA and DT in their experiments, they acknowledged the relationship between an increase in WA often corresponding to an increase in DT. This pattern can be observed in Table 5, where water absorption and development time values rise almost proportionally across the samples.

Dough stability, another farinograph parameter, reflects the flour's resistance to mechanical stress during mixing. W700 flour reveals the highest average stability, although with greater variability, especially when compared to the more consistent results from wholegrain flours. This shows that refined flours may tolerate mixing better, but their performances can be more affected by batch variability (Lei et al., 2008).

An additional parameter of technological importance is the degree of softening, which indicates the dough's tolerance to mechanical stress. Lower values are associated with better dough quality (Branch, n.d.). The results demonstrate that W700 flour has the lowest softening values, indicating superior dough strength and tolerance during processing.

The Extensograph results further support the differences observed between refined and wholegrain flours. W1600 flours produced doughs have lower extensibility (significantly lower at 45 and 90 min), higher resistance (although not statistically different from W700) and lower energy until rupture (less energy required to break the dough structure), correlated between all three samples. This highlighted the weaker and inextensible doughs derived from W1600 flour, indicator of poorer gluten matrix. This can be attributed to the presence of bran in W1600 flours, which physically disrupts gluten development and interferes with proper dough formation (Fu et al., 2017). This was also noticeable during breadmaking, where doughs made from wholegrain flours were more hardly to manipulate, presenting greater resistance to moulding and shaping, confirming the mechanical limitations imposed by the higher bran content.

3.3. Physical analysis of Breads

Table 7 demonstrates the physical properties of the breads produced throughout the study. In **Figure 6,** it is possible, at naked eye, to observe some of those features in the crust and crumb of the breads produced.

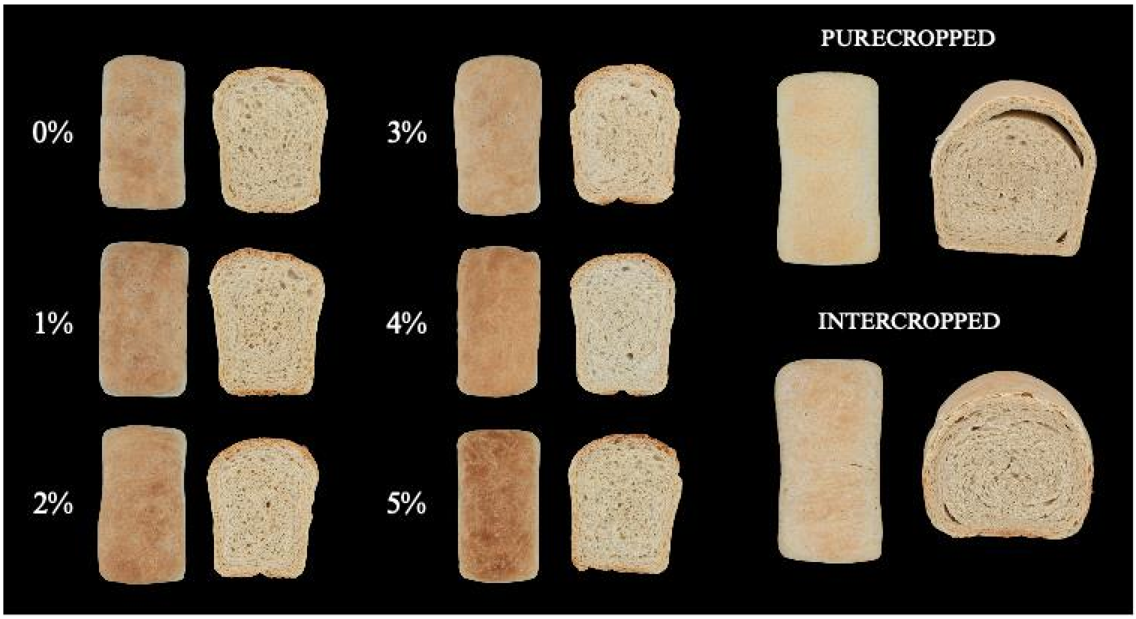


Figure 6- Crust and Crumb of the breads produced: W700 + 0-5% breads are from the same batch, as well as PC and IC breads - Images taken by VeriVide's DigiEye Colour Measurement System.

By the observation of Figure 6 it can be noted the progressive darkening of the bread crust with increasing levels of pea flour incorporation. In contrast, breads made with wholegrain flours exhibit noticeably lighter crusts compared to those produced with refined W700 flour plus pea flour, a trend that is also supported by the colorimetric data presented in Table 7.

In terms of crumb structure, breads produced with wholegrain flours display a denser crumb with reduced air cell dispersion, which results in a lower loaf height. This suggests that the inclusion of bran and germ components affects gas retention during fermentation and baking. Between pure and intercrop breads there's no noticeable differences, but to further understand these, a detailed analysis of the numerical values presented in Table 7 follows.

Table 7- Results of the physical analysis of breads with percentage of pea flour (W700 + 0-5%) and breads W1600 IC and PC.

Sample	Baking loss [%]	Specific volume [cm3/g]	Volume yield [%]	Fmax [N]	F120 [N]	Relative elasticity [%]	Color crust			Color crumb			Pore Analysis			
							L*	a*	b*	L*	a*	b*	Pore count	Average pore size	Total Area [%]	Uniformity
W700 + 5 % pea	15.13 ± 0.009^{bc}	3.30 ± 0.22^{ab}	464.16 ± 27.38^{a}	17.76 ± 2.65^{c}	9.03 ± 1.34^{c}	50.85 ± 0.80^{b}	59.73 ± 2.69^{c}	9.87 ± 1.68^{ab}	48.04 ± 1.86^{a}	78.70 ± 0.62^{ab}	1.89 ± 0.98^{bc}	32.19 ± 2.01^{bc}	337.33 ± 58.50^{a}	0.27 ± 0.059^{ab}	21.67 ± 3.25^{a}	0.75 ± 0.19^{a}
W700 + 4 % pea	14.48 ± 0.88^{ab}	3.40 ± 0.21^{ab}	481.89 ± 26.01^{a}	18.20 ± 2.13^{a}	9.37 ± 0.99^{a}	51.58 ± 2.16^{ab}	65.57 ± 5.43^{bc}	9.48 ± 2.51^{bc}	49.87 ± 1.59^{ab}	78.82 ± 1.13^{ab}	1.84 ± 1.22^{c}	31.92 ± 1.53^{b}	332.67 ± 47.22^{a}	0.26 ± 0.05^{bc}	21.17 ± 2.37^{a}	0.78 ± 0.23^{ab}
W700 + 3 % pea	15.54 ± 0.67^{c}	3.49 ± 0.27^{b}	488.28 ± 34.76^{a}	16.85 ± 2.59^{ab}	8.91 ± 1.29^{a}	53.01 ± 1.64^{a}	68.04 ± 4.90^{ab}	8.92 ± 1.67^{c}	47.17 ± 4.16^{b}	80.12 ± 0.82^{a}	2.51 ± 1.33^{c}	29.87 ± 2.11^{b}	372.78 ± 64.00^{a}	0.24 ± 0.05^{c}	21.41 ± 3.45^{a}	0.71 ± 0.23^{b}
W700 + 2 % pea	14.31 ± 0.01^{a}	3.34 ± 0.19^{ab}	474.53 ± 26.62^{a}	17.02 ± 1.67^{a}	8.65 ± 0.86^{ab}	50.86 ± 1.11^{c}	62.50 ± 2.14^{c}	11.28 ± 1.60^{a}	49.77 ± 1.32^{a}	78.33 ± 1.15^{c}	2.67 ± 1.42^{c}	30.99 ± 1.11^{a}	318.81 ± 52.13^{b}	0.29 ± 0.07^{a}	22.43 ± 3.13^{a}	0.90 ± 0.31^{a}
W700 + 1 % pea	14.43 ± 0.75^{ab}	3.44 ± 0.22^{ab}	488.02 ± 28.81^{a}	16.71 ± 1.68^{bc}	8.54 ± 0.84^{bc}	51.10 ± 1.36^{ab}	65.22 ± 1.55^{bc}	10.45 ± 1.17^{ab}	49.30 ± 2.35^{b}	78.61 ± 0.82^{b}	2.64 ± 1.26^{a}	30.91 ± 0.70^{cd}	332.70 ± 56.79^{a}	0.27 ± 0.07^{abc}	21.64 ± 3.79^{a}	0.79 ± 0.19^{a}
W700 + 0 % pea	14.43 ± 0.98^{ab}	3.26 ± 0.33^{a}	461.89 ± 42.54^{a}	18.37 ± 2.37^{abc}	9.49 ± 1.20^{abc}	51.69 ± 1.34^{a}	67.52 ± 2.93^{a}	9.40 ± 0.90^{abc}	46.99 ± 1.99^{ab}	78.76 ± 0.64^{ab}	2.53 ± 1.25^{ab}	31.20 ± 0.61^{d}	340.89 ± 37.99^{a}	0.25 ± 0.05^{bc}	21.03 ± 2.70^{a}	0.74 ± 0.20^{a}
W1600 - purecropped	13.16 ± 0.90^{a}	2.22 ± 0.13^{a}	297.76 ± 15.54^{a}	45.05 ± 6.51^{a}	21.82 ± 3.12^{a}	48.44 ± 0.58^{a}	77.77 ± 3.45^{a}	5.13 ± 1.17^{a}	37.19 ± 4.78^{a}	75.60 ± 0.84^{a}	2.83 ± 0.56^{a}	29.45 ± 1.93^{a}	497.07 ± 71.74^{a}	0.15 ± 0.02^{a}	18.85 ± 2.92^{a}	0.49 ± 0.09^{a}
W1600 - intercropped	12.82 ± 0.012^{a}	2.20 ± 0.12^{a}	293.85 ± 13.89^{a}	44.85 ± 6.87^{a}	21.53 ± 3.44^{a}	47.96 ± 0.77^{a}	77.80 ± 1.08^{a}	4.62 ± 0.46^{a}	35.57 ± 2.88^{a}	75.18 ± 0.76^{a}	2.89 ± 0.54^{a}	29.69 ± 1.76^{a}	495.67 ± 98.21^{a}	0.15 ± 0.031^{a}	18.03 ± 2.08^{a}	0.50 ± 0.21^{a}

All values are means ± SD (n = 9 for breads with W700 + 0-5% pea flour, except n=27 for crumb colour and texture and pore properties; n=5 for breads W1600 IC and W1600 PC, except n=15 for crumb colour and texture and pore properties). The statistical analysis was performed separately between breads with W700 + 0-5% pea flour and breads W1600 IC and W1600 PC, sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$). Fmax: Crumb firmness; F120: Crumb firmness after 120 seconds

According to Table 7, the incorporation of wheat flour from intercropping systems did not negatively affect the overall bread quality, as none of the evaluated parameters showed statistically significant differences when compared to those from PC wheat. This finding is particularly relevant, as it suggests that adopting more sustainable cultivation practices, such as intercropping, can be implemented without compromising the quality of the final product, offering a promising alternative for both the environment and the food industry.

On the other hand, the baking loss of the breads with incorporated pea flour remained consistent across most samples, with values within a narrow range. The only exception was the 3% pea flour bread, which showed a statistically significant difference.

For the specific volume, most samples were statistically correlated, except for the control and the 3% bread, which deviated from the others. However, when assessing volume yield, the differences across the samples became less evident, and no statistically significant variation was observed with increasing pea flour percentages.

In terms of texture, both F_{max} (maximum force) and F_{120} (residual force after 120 seconds) remained closely aligned with the control sample across all formulations, indicating consistency in crumb firmness and structural resistance. However, the relative elasticity parameter revealed more variation: breads containing 2% and 5% pea flour differed significantly from the control, revealing changes in dough elasticity with increasing legume content.

The colour parameters, particularly for crust and crumb, showed more variability. For the crust, only the L* value (lightness) was statistically different in all samples compared to the control, with the exception of the 3% bread. This indicates a general darkening of the crust as more pea flour is added. In the crumb, L* was significantly different only in the 2% formulation, while the a* (red-green) and b* (yellow-blue) values showed two clear correlation groups: the 0% and 1% breads, and the 2% to 5% formulations.

Pore analysis showed that pore count remained mostly consistent among samples, except for the 2% bread, which differed significantly. This same sample also presented the most pronounced difference in average pore size, indicating a structural deviation in crumb formation. Total pore area was not significantly affected by the addition of pea flour, suggesting that although the size and distribution of pores may vary, the overall gas retention was similar. Regarding uniformity, which reflects the consistency of pore size, the 3% bread was the only sample statistically different from the control, although it showed some degree of similarity with the 4% formulation.

The results indicate that breads with 0% and 1% pea flour are the most similar to the control in terms of the evaluated quality parameters, as expected. In contrast, formulations containing 2% and 3% pea flour exhibited the most significant deviations, suggesting a more pronounced impact of legume addition on the physical characteristics of the bread. Recipes with 4% and 5% also presented some differences, particularly in relation to crust and crumb colour, although the overall structural properties were less affected.

These findings confirm that the inclusion of pea flour does lead to measurable physical alterations in the final product. Therefore, the next critical step in assessing the viability of incorporating intercropped pea flour is to conduct a sensory analysis. In case the sensory evaluation reveal negative consumer feedback, it may be necessary to implement or maintain post-harvest strategies, such as sieving or sorting, to reduce or eliminate residual pea flour particles from the wheat flour matrix in order to maintain product acceptability.

3.4. Extrudates analysis

Table 8 shows all the analysis executed to the extrudates (outcome of the extrusion process) including dry matter, WAI and WSI analysis and physical properties.

Table 8- Results of the analysis of the extrudates from W1600 IC flour and from W1600 PC flour.

Sample	Dry Matter		Expansion Index [cm]	Bulk Density [g/cm3]	WAI [g/g;d.b.]		WSI [g/g;d.b.]		Color			Pore Analysis		Texture		
	before storage	after storage			30°C	90°C	30°C	90°C	L*	a*	b*	Pore count	Average pore size	Peak Count	Linear Distance [g*mm]	Hardness [N]
W1600 - purecropped	93.93 ± 0.16[a]	91.71 ± 0.08[a]	3.01 ± 0.23[a]	0.40 ± 0.0019[a]	7.85 ± 0.054[b]	21.19 ± 0.82[a]	25.35 ± 0.18[b]	51.45 ± 2.15[a]	81.27 ± 0.99[a]	4.27 ± 0.31[a]	28.10 ± 0.88[a]	78.33 ± 19.02[a]	0.35 ± 0.13[a]	61.90 ± 13.14[a]	19,1786 ± 19,467[a]	409.46 ± 59.25[a]
W1600 - intercropped	92.83 ± 2.20[a]	91.83 ± 0.35[a]	3.03 ± 0.23[a]	0.39 ± 0.0019[a]	8.16 ± 0.094[a]	18.19 ± 2.59[a]	27.49 ± 0.68[a]	49.15 ± 5.04[a]	80.74 ± 0.74[a]	4.02 ± 0.31[a]	27.58 ± 1.32[a]	81.44 ± 16.46[a]	0.25 ± 0.08[a]	63.00 ± 6.85[a]	19,9467 ± 23,864[a]	380.92 ± 39.63[a]

All values are means ± SD (n = 3 for dry matter, WAI and WSI; n=9 for all physical properties, except n=5 for bulk density). Sample means with different lowercase letters within the same parameter are significantly different ($p < 0.05$). WAI: Water Absorption Index; WSI: Water Solubility Index.

Table 8 shows that intercropping did not significantly affect any of the evaluated parameters, with the exception of hydration properties at 30 °C. At this temperature, both WAI and WSI were higher in intercropped wheat flour, suggesting greater starch gelatinization and degradation at lower temperatures compared to the PC wheat flour extrudate. At 90 °C, the trend was reversed, with PC wheat extrudates showing slightly higher values than IC extrudates, although without statistical significance. This difference can be explained by the compositional characteristics reported in Table 2: IC wheat contains more protein and less starch, while PC wheat contains less protein and more starch. A higher protein content can limit starch gelatinization, whereas a higher starch content can promote starch degradation (Zhang et al., 2014)

These findings are particularly relevant as they demonstrate, once again, that the use of intercropping, a more sustainable cultivation practice, can be implemented without adversely affecting the functional quality of the final product.

Dry matter content was measured before and after storage of the extrudates in a climate-controlled chamber. As expected, an increase in moisture content over time resulted in a corresponding decrease in dry matter.

The expansion index revealed that the extrudates approximately triplicate in size relative to their original diameter, indicating substantial air incorporation. This parameter is inversely related to bulk density, as confirmed in Table 8: higher expansion is associated with lower density. The slightly higher fat content observed in PC wheat flour (Table 2) may have contributed to its marginally lower expansion index, since increased lubrication in the extruder can reduce mechanical shear and expansion (Robin et al., 2015).

All colour parameters (lightness (L*), redness (a*) and yellowness (b*)) were higher in PC wheat extrudates. Pore analysis, as shown in Figure X, revealed greater values in IC samples.

In terms of texture, hardness is considered one of the main drivers of consumer preference in extruded cereal products. When compared to the results reported by Robin et al. (2015), who analysed a variety of cereal-based extrudates including wheat, the hardness values obtained in this study are markedly higher. This indicates that the extrudates produced in this study have a less hard texture to bite, which is generally regarded as a desirable quality trait.

4. Conclusion

This thesis investigated the impact of intercropping on the chemical, functional and technological properties of the processing performance and bread quality, aiming to assess whether this sustainable cultivation practice could be integrated without compromising the food chain.

From the chemical characterization, pea flours were confirmed to contain higher protein, fat and ash levels, whereas wheat flours presented greater starch content. The comparison between purecropped and intercropped wheat flours revealed no statistically significant differences in the main compositional parameters, with protein content being the only attribute occasionally affected. These findings suggest that intercropping does not alter the intrinsic chemical properties of wheat flour in a way that would compromise its processing.

The technological analysis of doughs demonstrated that the W700 wheat flour absorbed more water (59.1%) and yielded stronger and more stable doughs. In contrast, wholegrain doughs (PC and IC) were less extensible and more resistant, because of the presence of the bran disrupting gluten development. Importantly, IC and PC wholegrain wheat behaved similarly (e.g. WA 53.0% vs 52.5%; comparable DT, stability, softening), indicating no rheological penalty due to intercropping. A similar pattern was observed in the RVA analysis, where IC and PC flours showed highly correlated profiles, both displaying higher gelatinization capacity and greater gel stability than pea flours, the latter being caused by their higher protein content.

Breadmaking trials revealed that breads made with intercropped wheat flour displayed no significant differences in quality parameters to those from purecropped wheat, supporting the conclusion that intercropping did not influence bread quality. In parallel trials, with W700 + 0-5% pea flour (to simulate possible legume residues), the results were very dispersed, but most attributes remained close to the control. To strengthen this part, an adequate sensory analysis can be performed to further evaluate its impact on consumer acceptance.

Extrusion trials further supported no significant differences between using IC and PC wheat flours in the process. Although hydration properties (WAI and WSI) showed slight variations at different temperatures, intercropped wheat extrudates behaved similarly to purecropped ones in terms of expansion, bulk density, colour, pore structure and texture. Importantly, the softer texture of the extrudates obtained in this study can be considered favourable for consumer acceptance when compared with values reported in the literature.

Overall, the results consistently demonstrate that wheat produced under intercropping systems can be successfully processed into bakery products and extrudates without significant loss of quality. This highlight intercropping as a viable and sustainable agricultural strategy, capable of reducing environmental impact while maintaining technological performance and product acceptability.

Future research should incorporate sensory analysis and long-term storage trials to fully assess consumer perception and shelf-life stability. Additionally, this study was constrained to a single harvest from one region and one season due to material limitations. Thus, while the findings provide valuable indicators, broader monitoring across diverse cultivation conditions and over extended periods is needed to obtain deeper insights. Nevertheless, the present work demonstrates that intercropping can sustain grain yield while preserving bread quality and processability. Within the food chain, this approach is not only technologically feasible but also environmentally beneficial, representing a meaningful step toward more sustainable cereal production systems.

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Appendix

Table A 1- Settings input for the texture analyser.

Settings:	3 Hold until time		
Testmode	Kompression		
Pre-Test speed	1,00 mm/s		
Test speed	0,5 mm/s		
Post-test speed	10 mm/s		
Target mode	Distance		
Distance	15 mm	bei 30 mm Scheiben)	
Hold time	120 s		
Trigger type	Auto Force		
Trigger force	0,049 N		
Advanced options	off		