EFFECTS OF CHICKPEA MILLING AND PRETREATMENT ON THE PHYSICAL AND MICROSTRUCTURAL CHARACTERISTICS OF ENRICHED BREAD AND PASTA

By

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THESIS ABSTRACT

Chickpeas, with their rich nutritional profile, present a promising ingredient for food enrichment. However, their incorporation into staple foods such as pasta and bread requires understanding the effects of milling and pretreatment of chickpeas on their microstructural behaviour in chickpea-wheat blend formulation. This research investigates the impact of milling and pretreatment methods on the microstructure and physicochemical properties of chickpea-enriched wheat staple products using X-ray microcomputed tomography (X-ray μCT).

Chickpeas were milled using a multi-stage roller mill and a single-stage (Ferkar) mill, yielding five flour types: four flours were obtained from the roller mill (viz., high-protein flour, low-protein flour, reconstituted whole flour, straight-grade flour), and one flour from Ferkar mill (Ferkar-milled flour). These flours were blended with durum wheat semolina in equal amounts for pasta production. The effects of three pretreatment methods - germination, micronization, and roasting - were also studied before milling the chickpeas into straight-grade flour and blending it (in a 20:80 ratio) with wheat flour for bread production.

Physicochemical analysis revealed significant differences ($p < 0.05$) among pasta samples, with high-protein flour pasta exhibiting lower optimum cooking time, reduced cooking loss, and higher protein and ash contents. Microstructural analysis showed that the high-protein blend resulted in low porosity with closed pores and increased structural thickness, contributing to enhanced firmness and texture. Similarly, the physicochemical properties of chickpea-enriched bread were influenced by roasting and micronizion pretreatment methods, with significant ($p < 0.05$) effects on loaf volume, colour, and protein content. The microstructural evaluation demonstrated that bread made from germinated chickpeas exhibited superior pore structure, leading to improved overall quality.

In conclusion, this study provides valuable insights into optimizing chickpea milling and pretreatment methods for integration into staple foods such as pasta and bread. Given the rising demand for alternative protein sources, incorporating chickpea flour with minimal microstructural impact presents opportunities for innovation and sustainability within the food industry.

Keywords: Chickpea, milling, germination, micronization, roasting, chickpea-enriched pasta, chickpea-enriched bread, X-ray microcomputed tomography

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CHAPTER 1

INTRODUCTION

1.1 Scope of the research

The increasing global population, which is expected to reach nine billion by 2050, and the prevalence of diet-related chronic diseases have increased the urgent need for healthier, palatable, and more sustainable food products among consumers (Boukid, 2021; Oliveira et al., 2023; United Nations, 2019). Cereal-based staples such as bread and pasta, primarily made from wheat flour, constitute a significant portion of global diets. However, they are limited in health-promoting bioactive compounds, including vitamins, polyphenols, and dietary fibre, as well as essential amino acids like lysine, leading to nutritional imbalances (Begum et al., 2023; McDermott & Wyatt, 2017). Pulses, such as chickpeas, present a promising solution to address these nutritional deficiencies, given their high protein content, dietary fibre, and lysine-rich composition (Byanju & Lamsal, 2023; Singh, 2017). These nutritional advantages over wheat have driven increasing interest among researchers, ingredient manufacturers, and food companies in incorporating pulse flours as a partial substitute for wheat in staple foods to enhance nutritional quality and functional properties to meet market demand.

Chickpea (*Cicer arietinum* L.), a member of the *Fabaceae* family, is one of the most widely cultivated and consumed pulses globally (Rachwa-Rosiak et al., 2015). It is a rich source of protein (17–22%), dietary fibre (18–22%), carbohydrates (60–70%), lipids (7%), minerals, vitamins, and bioactive compounds (Begum et al., 2023; Kaur & Prasad, 2021; Wallace et al., 2016). Additionally, research indicates that chickpeas could help regulate glucose and insulin levels and reduce the risk of cardiovascular disease, obesity, and cancer (Priyadarshini et al., 2020; Wallace et al., 2016). These health benefits have driven increasing interest in chickpeas as a functional

ingredient for enhancing the nutritional profile of wheat-based foods, particularly in developed countries such as Canada.

According to Agriculture and Agri-Food Canada, chickpea production in Canada increased by 80% from 159,000 tonnes in 2023-24 to 287,000 tonnes for the 2024–25 crop year, marking the highest level in over two decades (AAFC, 2024). Despite this growth, chickpea consumption remains low, with an annual per capita intake of only 12 kg (Szczebyło et al., 2019). This underutilization is attributed to challenges such as the presence of anti-nutritional factors (ANFs) and limited knowledge of how chickpeas impact the quality, especially the microstructural-mixability with wheat flour in food products (Kaur & Prasad, 2021; Thakur et al., 2019).

Milling techniques and pretreatment methods, such as germination, micronization, and roasting, can significantly reduce ANFs and enhance the functional properties of chickpea flour. Milling, a physical processing technique, is primarily used for grain size reduction to produce flour while removing coarse and undesirable particles. The characteristics of the resulting flour, including particle size, shape, and distribution, are influenced by the milling technique employed and the inherent properties of the pulse seed (Sivakumar et al., 2022; Thakur et al., 2019). While the milling of pulse flours is crucial for their functional properties, research highlights the importance of evaluating their characteristics beyond whole flour to understand their behaviour in cereal blends, particularly with wheat, for optimized product formulation (Scanlon et al., 2018; Sivakumar et al., 2022, 2023). Roasting and micronization are thermal pretreatment methods that utilize high temperatures and radiation to alter grains' physical and chemical properties, effectively reducing off-flavours and heat-labile ANFs such as phytic acid, which is predominant in pulses.

In contrast, germination is a biochemical pretreatment method that involves exposing grains to a moist environment, triggering the development of sprouts. This process enhances the nutritional

attributes and mineral bioavailability while reducing ANFs such as oligosaccharides, tannins, and phytic acid in pulses (Kaur & Prasad, 2021; Vaz Patto et al., 2015). While these pretreatment methods exist, identifying the most suitable and innovative technique remains a challenge, as pretreatment alters the nutritional composition, physical properties, and microstructure of pulse flour, which in turn affects its compatibility and mixability with wheat in staple food formulations (Kaur & Prasad, 2021; Sivakumar et al., 2024; Thakur et al., 2019). To effectively evaluate these structural changes, X-ray microcomputed tomography (X-ray μCT), a high-resolution, non-destructive method that utilizes radiation beyond the visible spectrum, characterizes the internal structure of food in three dimensions (Olakanmi et al., 2023; Schoeman et al., 2016; Sivakumar et al., 2023). This technique enables samples to be examined in their natural state and at atmospheric temperature with minimal preparation (Schoeman et al., 2016). Analyzing microstructural features such as total porosity, open and closed pores, cell connectivity, and structural thickness within food formulations provides vital image texture analysis, an essential aspect of quality evaluation in the food industry (Schoeman et al., 2016; Zheng et al., 2006).

Therefore, to enhance the utilization of pulses like chickpeas, a comprehensive understanding of the appropriate milling technique and pretreatment method is required, focusing particularly on their effects on the microstructure of chickpea-wheat blend formulations. Food microstructure plays a crucial role in determining the physicochemical and sensory properties of food products, making it integral to product quality assessment and the development of novel formulations (Blonk, 2002). To address this gap, the present study was conducted to evaluate the physical and microstructural characteristics of chickpea-enriched wheat pasta and bread using the X-ray μCT technique.

1.2 Thesis objectives

1) To evaluate the effect of multi-stage roller and single-stage Ferkar milling of chickpea on the quality and microstructure of chickpea-enriched wheat semolina pasta.
2) To analyze the impact of pretreatment methods (germination, micronization or roasting) of chickpeas on the physical properties and microstructure of chickpea-enriched wheat bread.

Fig 1.1 shows the experimental design for the research objectives grouped in two phases (Phase 1 and 2)

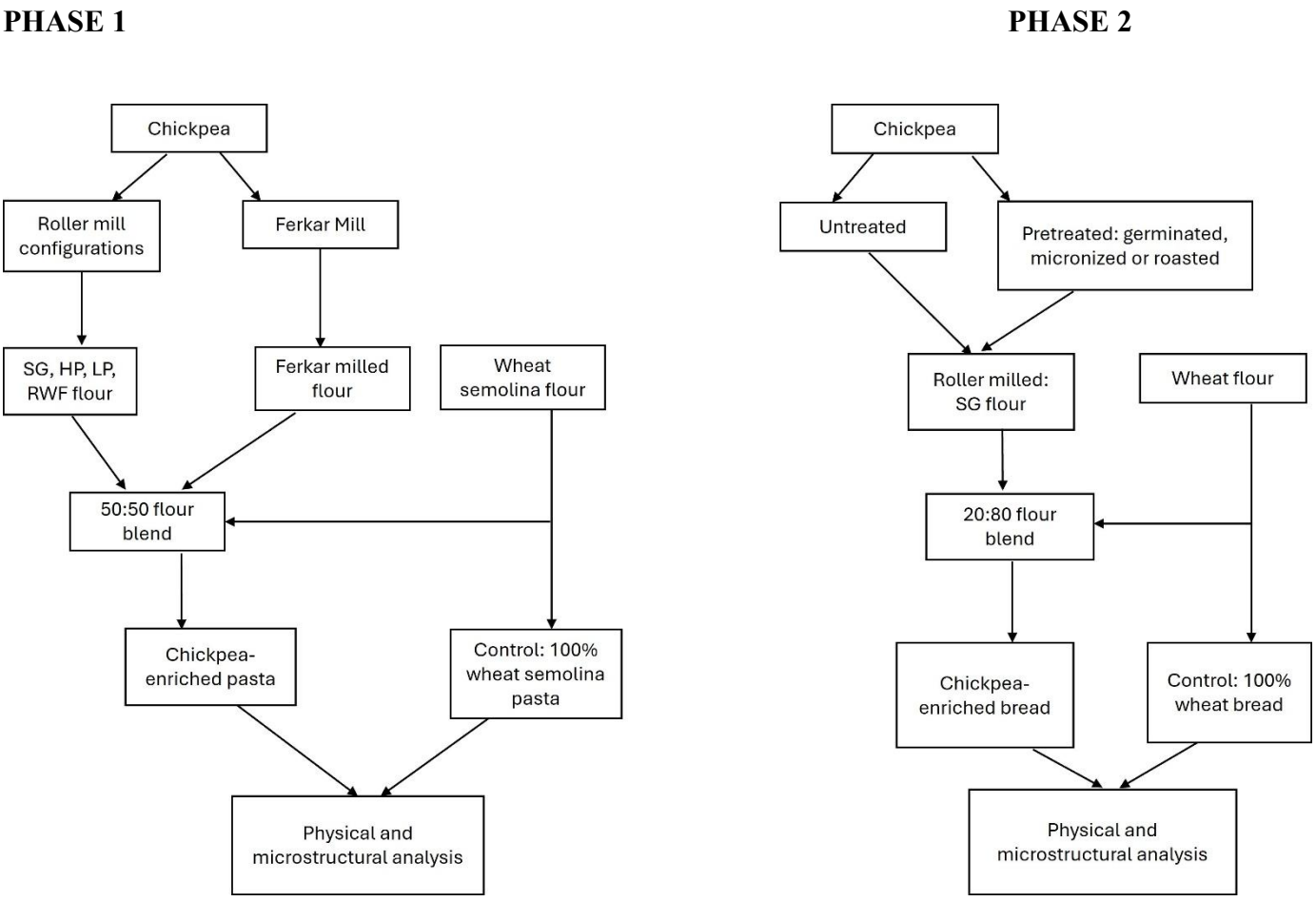

Fig 1.1 Flowchart of the experimental design. SG- Straight grade, HP- High Protein, LP- Low Protein, and RWF- Reconstituted Whole Flour.

1.3 Structure of the thesis

This thesis is organized into five chapters as follows:

Chapter 1 provides the scope of the research and lists the objectives of this research.

Chapter 2 reviews published literature on chickpeas, the different milling techniques, pretreatment methods, and the use of X-ray μCT in evaluating the quality of manufactured foods.

Chapter 3 presents the microstructural evaluation of chickpea-enriched wheat semolina pasta using X-ray micro-computed tomography in a manuscript format.

O.U. Ukoji, M.M.A. Chaudhry, L. Boyd, E. Sopiwnyk and J. Paliwal, (2024). "Quality and microstructural analysis of chickpea-enriched wheat semolina pasta using X-ray micro-computed tomography". *Journal of Cereal Science*. *119*: 104009. https://doi.org/10.1016/j.jcs.2024.104009

Chapter 4 discusses the effect of pretreatment methods of chickpeas on microstructure and physical properties of enriched bread.

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Chapter 5 presents the overall conclusion, limitations of the conducted work and future recommendations. Also, it presents the authors' contributions to chapters 3 and 4 of the thesis.

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CHAPTER 2

LITERATURE REVIEW

2.1 Pulses

Pulses, also known as grain legumes, belong to the *Fabaceae or Leguminosae* family and play a crucial role in environmental sustainability by enhancing food security and promoting human health. These grain legumes serve as low-carbon protein sources that require minimal water and fertilizer while contributing to improved soil fertility and productivity through crop rotation practices (Priyadarshini et al., 2020). Recognizing their significance, the Food and Agriculture Organization of the United Nations declared the year 2016 as the International Year of Pulses to raise awareness of their nutritional benefits and role in sustainable food systems. Global pulse production continues to rise, reaching approximately 96 million metric tonnes (MMT), with over 72 MMT comprising major pulses such as chickpeas, dry beans, and lentils. Asia dominates global pulse production, accounting for over 80%, followed by Africa and the Americas (FAOSTAT, 2022; Szczebyło et al., 2019). Among individual countries, India is the largest producer, followed by Canada and China (Bhat et al., 2022). However, Canada achieves the highest pulse crop yield per hectare, likely due to the widespread adoption of crop rotation practices (MacWilliam et al., 2014; Patil et al., 2024). Only 19.0 metric tonnes (Mt) of pulses are traded globally, with Canada leading at 5.0 Mt, followed by Australia at 2.0 Mt (Bhat et al., 2022). Beyond their agronomic benefits, pulses are nutritionally rich, providing essential micronutrients, proteins, carbohydrates, and dietary fibre, making them a vital component of a balanced diet.

2.2 Global chickpea trend

Chickpeas (*Cicer arietinum* L.), often called garbanzo beans, are the only cultivated species in the genus *Cicer*. This annual plant grows as a small bush with branches that spread outward from the base. There are two primary varieties: Kabuli and Desi. The Kabuli variety is noted for its large, light-coloured seeds with smooth surfaces, while the Desi variety features smaller, angular to round seeds with dark brown coats and rough textures (Kaur & Prasad, 2021).

Chickpeas are widely recognized as an economically significant and highly nutritious crop, accounting for approximately 15% of the global pulse market and ranking as the second most produced pulse worldwide, following dry beans (Rawal & Navarro, 2019). The Desi variety is predominantly cultivated in Africa and Asia, contributing to 80% of total global chickpea production, while the Kabuli variety is primarily grown in temperate regions such as Europe, North America, and West Asia, representing 20% of the global output. According to the Food and Agriculture Organization of the United Nations, global chickpea production exceeded 15 million metric tonnes in 2022, with Canada achieving the highest yield (FAOSTAT, 2022; Patil et al., 2024).

In Canada, chickpea cultivation is concentrated in the southern regions of Alberta and Saskatchewan, where soil conditions are well-suited for its growth. Saskatchewan accounts for 78% of the country's chickpea production, while Alberta contributes 22% (Lavergne & Oleson, 2018). Canada ranks amongst the largest chickpea exporters, with over 75% of its production exported to markets including the United States, Turkey, and the European Union. However, chickpea exports from Canada have been declining, decreasing from 198,000 tonnes in 2022–2023 to 183,000 tonnes in 2023–2024, reflecting a 7.58% reduction, with a further forecasted decline of 4.37% to 175,000 tonnes in 2024–2025, according to Agriculture and Agri-Food Canada (AAFC,

2024). Additionally, the average price of Canadian chickpeas is projected to drop by 19% to $815 per tonne due to increased global supply.

2.3 Composition of chickpea

Chickpeas are primarily consumed for their high nutritional composition and protein content. Additionally, they are a rich source of carbohydrates, minerals, vitamins, and bioactive compounds. Due to their availability and nutritional value, chickpeas play a significant role in the food systems of developing countries and are an integral ingredient in various traditional culinary dishes (Begum et al., 2023). Table 2.1 summarizes the nutritional composition of Canadian chickpeas based on the Canadian Grain Commission report (Wang & Daun, 2004).

Table 2.1 Composition and nutritive value of Canadian chickpeas. Modified from Wang and Daun, (2004).

Composition (g/100 g dry matter)	Kabuli type		Desi type	
	Mean	Range	Mean	Range
Protein (N * 6.25)	24.4	17.9 – 30.8	23.0	20.3 – 27.5
Starch	41.1	38.2 – 43.9	36.4	33.1 – 40.4
Amylose (% of total starch)	26.2	24.4 – 29.2	23.8	20.5 – 25.9
Acid detergent fibre	3.7	3.0 – 5.7	13.1	12.7 – 13.5
Neutral detergent fibre	5.0	4.2 – 7.7	12.8	10.1 – 13.6
Phytic acid	0.98	0.78 – 1.25	0.92	0.63 – 1.24

a) Protein

Pulses, such as chickpeas, are valuable sources of protein and are widely recognized as sustainable and cost-effective alternatives to meat. In chickpeas, water-soluble albumins and salt-soluble globulins are the predominant protein fractions, with globulins significantly contributing to protein bioavailability (Yust et al., 2003). Albumins primarily consist of enzymatic proteins, enzyme inhibitors, and lectins, whereas globulins are composed of vicilin (7S), convicilin (15S), and legumin (11S). Chickpea seeds are deficient in sulfur-containing amino acids, such as methionine and cysteine, but are rich in lysine. In contrast, cereals have the opposite composition, being abundant in sulfur-containing amino acids while lysine remains the limiting amino acid (Rachwa-Rosiak et al., 2015). The protein content (in dry mass) in chickpeas varies based on the dehulling process (before dehulling – 25 to 29%, after dehulling – 17 to 22%). Additionally, significant differences in protein concentration have been reported between Kabuli and Desi chickpea varieties across various studies (Begum et al., 2023; Jukanti et al., 2012). According to Gaur et al. (2016), the protein concentration of Kabuli chickpeas (20.5%) was lower than that of Desi chickpeas (29.2%). Conversely, Kou et al. (2013) reported protein concentrations of 23.4% in Kabuli and 22.2% in Desi chickpeas.

Pretreatment methods have been shown to influence the protein concentration and digestibility of chickpeas. Germination has been reported to enhance protein concentration by 9.8%, whereas fermentation leads to a 32% reduction (Yaver, 2022). Similarly, Baik & Han, (2012) found that roasting, cooking, and fermentation increased protein content by 1.5%, 5.0%, and 5.1%, respectively. The in-vitro protein digestibility of chickpeas ranges from 79.4 to 87.47%, with Kabuli chickpeas exhibiting higher digestibility than Desi varieties (Kaur & Prasad, 2021; Patil et al., 2024; Sánchez-Vioque et al., 1999). Digestibility is assessed to evaluate protein susceptibility

to proteolysis and serves as a key indicator of protein bioavailability (Singh, 2017). Comparatively, chickpea protein digestibility exceeds that of other pulses such as soy (70 to 72%) and green peas (72.65 to 75%), further underscoring its significance as a high-quality, plant-based protein source (Patil et al., 2024).

b) Carbohydrates

Carbohydrate is the major component of pulses, accounting for 60 to 70% of dry weight. The total starch content in chickpeas is 60.3%, which is higher than that of other legumes, which have a greater amylose content (32.3%) (Begum et al., 2023). Chickpeas contain oligosaccharides, including verbascose, stachyose, ciceritol, and raffinose, in the ranges of 5.54 to 8.82%, 0.25 to 0.73%, 1.54 to 3.18%, 2.04 to 5.26%, and 0.42 to 0.86%, respectively (Begum et al., 2023; Tosh & Yada, 2010). According to Sánchez-Mata et al. (1998), the concentrations of monosaccharides in chickpeas were ribose (0.11 g per 100 g), galactose (0.7 g per 100 g), glucose (0.05 g per 100 g), and fructose (0.25 g per 100 g), while the abundant free disaccharides in chickpeas, such as maltose and sucrose, were 0.6% and 1 to 2%, respectively. Chickpeas consist of both available and unavailable carbohydrates. The available carbohydrates include monosaccharides and disaccharides, which can be digested by enzymatic action in the small intestine, whereas unavailable carbohydrates are oligosaccharides, resistant starch, pectin, hemicellulose, and cellulose, which cannot be digested in the small intestine (Kaur & Prasad, 2021). Starch is the most abundant carbohydrate in chickpeas, ranging from 36.4 to 41.1% of the dry matter (Wang & Daun, 2004). Chickpea starch has a high amylose content, which increases its tendency for retrogradation, making it more resistant to enzymatic breakdown and consequently lowering its glycemic index (Kaur & Prasad, 2021; Wang & Daun, 2004).

c) Dietary fibre

Dietary fibre refers to the component of plant-based foods that resists complete digestion in the human digestive tract while playing a crucial role in reducing the risk of chronic diseases such as diabetes and cardiovascular disorders (Eastwood & Kritchevsky, 2005). In chickpeas, dietary fibre is classified into soluble fibre, which undergoes slow digestion in the colon, and insoluble fibre, which remains metabolically inert, with concentrations of 1.42% and 27.84%, respectively (Kaur & Prasad, 2021). The ratio of soluble to insoluble fibre in pulse flour is significantly influenced by the applied pretreatment method. The study by Tosh & Yada, (2010), reported that the total dietary fibre content in chickpeas ranges from 18 to 22%, with soluble fibre comprising 4 to 8% and insoluble fibre accounting for 10 to 18%. Similarly, Aguilera et al. (2009) found that the total dietary fibre content of chickpeas was between 18 and 22 g per 100 g, higher than that of other pulses. The Kabuli chickpea variety contains less acid detergent fibre and neutral detergent fibre than the Desi type, likely due to its thinner hull and seed coat, which make up only 4.3–4.4% of the total seed weight, compared to 11.5% in the Desi variety (Begum et al., 2023; Wang & Daun, 2004).

d) Anti-nutritional factors

Antinutritional factors (ANFs) are naturally occurring compounds produced during the metabolism of plants, which can reduce nutrient intake, absorption, and utilization (Shah et al., 2021). Plants primarily synthesize these compounds as a defence mechanism against predators or as a survival trait. However, excessive consumption of ANFs can have adverse physiological effects in humans (Shah et al., 2021). In pulses such as chickpeas, common ANFs include phytates or phytic acid, which contribute to undesirable sensory attributes such as off-flavours and a beany taste. Phytic acid, a negatively charged compound, binds to essential minerals such as iron, zinc,

calcium, and magnesium, reducing their intestinal absorption and potentially leading to deficiencies (Shah et al., 2021; Wang & Daun, 2004). Processing methods such as milling, as well as pretreatment methods including germination, micronization, and roasting, have been shown to effectively reduce the concentrations of ANFs in pulses (Kaur & Prasad, 2021; Shah et al., 2021; Singh, 2017).

2.4 Milling methods

Milling is the process of reducing large materials into smaller particles. The milling of pulses dates back to ancient times when early tools such as quern stones were used to produce dhal or flour (Bar-Yosef, 1998; Wood & Malcolmson, 2020). Pulse milling serves three primary functions: reducing particle size, separating components, and making mechanochemical modifications (Scanlon et al., 2018; Sivakumar et al., 2022, 2023; Thakur et al., 2019). The resulting flour varies in particle size, shape, and distribution depending on the milling method and properties of the pulse seed (Sivakumar et al., 2022, 2023; Wood & Malcolmson, 2020). Common milling methods include stone milling, knife milling, roller milling, pin milling, and hammer milling. Given the commercial relevance and distinct operating mechanisms of milling systems, this study specifically evaluates roller mills and Ferkar mills.

A roller mill is an attrition mill that produces four to ten flour fractions through shear and compression forces. It consists of two roll types: break and reduction rolls. Break rolls feature a fluted surface that fractures the kernel, allowing larger bran particles to be separated while the finer endosperm is sifted away. In contrast, reduction rolls have a smooth, frosted surface and operate at lower differentials, influencing water absorption capacity and pasting properties by damaging starch granules (Choo et al., 2024; Sivakumar et al., 2022, 2024).

The Ferkar mill, a single-stage knife mill, processes whole seeds into a single flour fraction, yielding uniformly granulated, high-quality flour (Sivakumar et al., 2022). Choo et al. (2024) reported significant differences in the particle size distribution of pulse flours produced by the Ferkar and roller mills, observing that Ferkar-milled flours exhibited smaller particle sizes at the 10^{th} and 50^{th} percentiles than roller-milled flours. Similarly, Bourré et al. (2019) identified substantial differences in the bread-baking properties (viscosity, proofing, mixing time, resilience, colour, texture, softness, and crumb strength) and nutritional attributes (starch content, starch damage, protein, water absorption capacity, and oil absorption capacity) of pulse flours from commercial mills and the Ferkar mill, attributing these variations to differences in particle size distribution. They concluded that the choice of whole or dehulled pulses significantly impacts particle size and, consequently, flour functionality.

2.5 Pretreatment methods

Pulse pretreatment is one of the oldest and most important food processing, an integral part of the food production chain (Tiwari et al., 2020). Pretreatment conditions can significantly change the chemistry, physical, functional and nutritional values of pulses (Meda et al., 2018). It is, therefore, necessary to understand the changes induced by the pretreatment methods on pulses and pulse-based products. Among conventional pretreatment methods, germination, micronization, and roasting are regarded as minimally processed methods for producing modified ingredients with enhanced properties (Patterson et al., 2017).

2.5.1 Germination

Germination involves tempering pulses to a target moisture level required for sprouting, followed by incubation under cool, humid conditions. Soaking is an essential pretreatment, enabling water

absorption necessary for seed activation. Seed germination occurs in three phases: (i) imbibition, characterized by rapid water uptake; (ii) biochemical activation, marked by reduced water absorption and enzyme activity; and (iii) radicle emergence, where the root penetrates the seed coat (Kaur & Prasad, 2021; Wolny et al., 2018).

According to the American Association of Cereal Chemists (AACC), germinated grains qualify as whole grains if they retain the bran, germ, and endosperm, as long as the sprout length does not exceed the kernel length and the nutrient composition remains intact. Germination has been shown to reduce antinutritional factors while enhancing the physical, functional, and sensory properties of pulses and pulse-based products (Marengo et al., 2017; Wood & Malcolmson, 2020). Frohlich et al. (2019) reported that the pre-germination of yellow peas increased starch damage and water absorption capacity, reduced peak and final viscosities, and improved bread crumb softness, aroma, and flavour compared to untreated yellow pea flour. Similarly, Milán-Noris et al. (2018) found that germinated chickpea seeds exhibited higher protein content than non-germinated seeds. Marengo et al. (2017) reported that sprouted chickpea-wheat flour blends had superior dough mixing properties and protein content compared to non-sprouted blends, demonstrating the potential of germination to enhance flour functionality.

2.5.2 Micronization

Micronization is a rapid, high-temperature process that utilizes electromagnetic radiation in the infrared (IR) region to achieve internal heating and increased water vapour pressure in materials (Mwangwela et al., 2007; Wood & Malcolmson, 2020). IR radiation is artificially generated using electrical or gas-fired generators (Bellido et al., 2003; Pan & Griffiths, 2011). Electrical generators are commonly employed in laboratory-scale studies due to their user-friendly operation and ease of control, whereas gas-fired generators are better suited for large-scale applications owing to their

lower operational costs (Bellido et al., 2003). The primary distinction between these two systems lies in their operational parameters: electrical generators produce IR energy for micronization at temperatures up to 2200°C, with a peak wavelength of approximately 1.15×10^{-9} m. In contrast, gas-fired generators operate from 400°C to 700°C, emitting radiation with wavelengths between 1.8 and 3.4×10^{-9} m (Bellido et al., 2003; Cenkowski & Sosulski, 1998). The micronization process is cost-effective due to the superior efficiency of radiation heating compared to conventional heating technologies (Bellido et al., 2006).

Several studies have shown that achieving an optimal balance of moisture control and micronization temperature improves the functionality, colour, and flavour of pulse flour (Bellido et al., 2003; 2006; Wood & Malcolmson, 2020). Frohlich et al. (2019) discovered that micronizing pea flour at a moisture level of 18–20% and a lower temperature (105–110°C) enhanced functionality and baking performance; however, higher temperatures (135–140°C) resulted in bread of inferior quality. Similarly, Mwangwela et al. (2007) stated that micronized cowpea flour had higher water absorption and the lowest gelatinization concentration compared to untreated flour, indicating improved hydration properties. These findings emphasize the significance of micronization in customizing the functional characteristics of pulse flours for specific food applications.

2.5.3 Roasting

During roasting, heat is transferred to pulses either through direct contact with hot surfaces or via hot air in furnaces or ovens. Simultaneously, moisture migrates from the grain to the surrounding air and is removed by circulation within the heating chamber (Meda et al., 2018). Depending on the heat transfer mode, the outcome of the roasting process will vary. Roasting requires approximately 450–650 kJ/kg of grain, primarily for moisture removal, crust formation, browning,

and caramelization reactions (Meda et al., 2018). Soaking is also a prerequisite for seeds before roasting, as it allows them to absorb moisture, enhancing crispness and texture after roasting (Kaur & Prasad, 2021). Roasted chickpeas, known as *Leblebi*, are widely consumed as snacks in India, Afghanistan, Iran, and Turkey (Kaur & Prasad, 2021). The duration and intensity of the roasting process significantly affect the physical, textural, and nutritional composition of the grain and grain-based products (Meda et al., 2018).

Baik & Han, (2012) reported that bread made from roasted pulses exhibited a more appealing aroma and greater loaf volume than those made from cooked or untreated pulses. Young et al. (2020) observed that roasting peas at 120°C produced flours with improved functionality and enhanced bread-baking properties. Similarly, Fahmi et al. (2019) used a combination of a Revtech heating system and steam to process yellow peas for pan bread formulations. Their findings indicated that bread made with 10% steam and thermal roasting at 140°C had superior textural, colour, and nutritional attributes compared to untreated yellow pea pan bread.

2.6 Non-destructive characterization of food

Non-destructive techniques have become essential for assessing food quality without compromising product integrity (Olakanmi et al., 2023). These advancements are particularly valuable in the food industry, where evaluating internal quality attributes without sample destruction is a priority (Kotwaliwale et al., 2014). High-resolution imaging methods, particularly those utilizing wavelengths beyond the visible spectrum, enable the exploration of microstructural properties and sub-molecular functions (Olakanmi et al., 2023; Sivakumar et al., 2023). One such technique with growing applications in food quality assessment is high-resolution X-ray microcomputed tomography (X-ray μCT).

2.6.1 X-ray µCT technique

X-rays have frequencies ranging from 30×10^{15} Hertz to 30×10^{18} Hertz, with corresponding energies between 145 electron volts and 124 kilo electron volts and wavelengths from 1×10^{-12} m to 1×10^{-9} m, placing them between ultraviolet and gamma rays in the electromagnetic spectrum (Li et al., 2019; Olakanmi et al., 2023). Due to their high frequency and short wavelength, X-rays offer superior imaging resolution, allowing for the visualization of structures that are opaque to visible light (Barigou & Douaire, 2013; Sivakumar et al., 2023).

X-ray µCT is a non-destructive, non-invasive technique for characterizing food microstructure in three dimensions. This method enables scanning an entire sample in its natural state without requiring preparation, maintaining conditions such as temperature and pressure (Schoeman et al., 2016). It integrates X-ray microscopy with tomographic algorithms to analyze variations in X-ray attenuation caused by density differences within the sample (Mousavi et al., 2007; Olakanmi et al., 2023). A significant advancement in X-ray µCT imaging is the use of synchrotron radiation, which enhances image quality through high-flux X-ray beams, rapid detection systems, and advanced reconstruction techniques, enabling near real-time 3D imaging (Calo et al., 2020; Pathare & Rahman, 2022). However, the limited availability of synchrotron facilities and the requirement for advance scheduling restrict widespread use in research (Olakanmi et al., 2023; Sivakumar et al., 2023).

Alternatively, benchtop X-ray µCT systems provide practical solutions by utilizing X-ray penetration to capture multiple 2D radiographs. These are computationally reconstructed into cross-sectional images, offering detailed internal structural analysis (Kareh & Whitham, 2021). X-rays in this system are generated by accelerating electrons from cathode-ray tubes within a high-voltage electric field, producing a cone-shaped X-ray beam capable of resolutions as low as 5

microns. The technique provides both qualitative and quantitative microstructural information through three primary stages: image acquisition, reconstruction, and visualization, as shown in Fig 2.1.

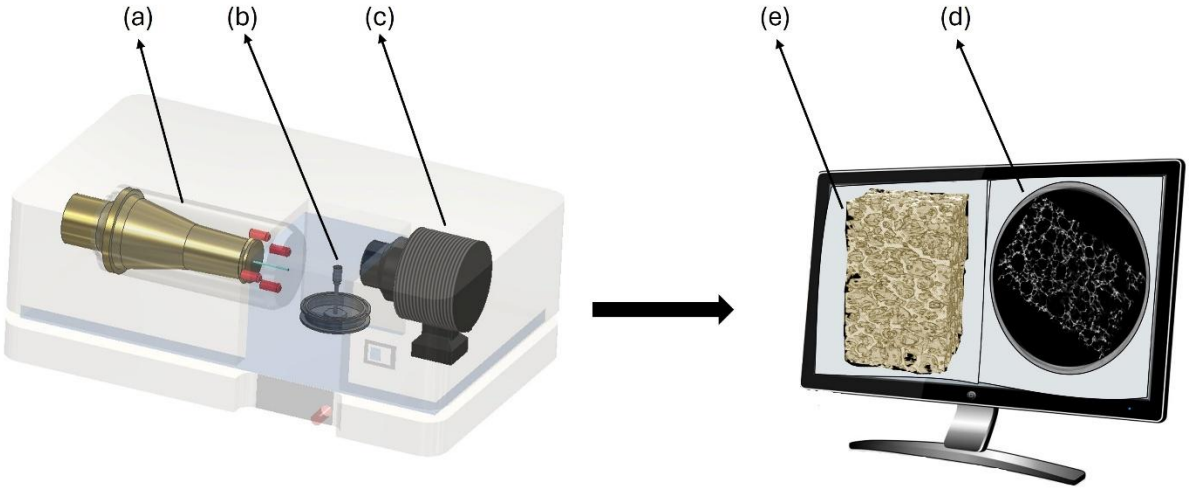

Fig 2.1 Schematic layout of the X-ray µCT system. (a) X-ray source, (b) sample holder, (c) detector (CCD camera), (d) image reconstruction, and (e) visualization.

a) Image acquisition

A benchtop X-ray µCT scanner comprises an X-ray source, a sample stage, and an X-ray detector. The X-ray source, typically a tube, generates polychromatic beams that are transmitted, absorbed, or scattered as they pass through the sample. During scanning, the X-ray source and detector remain stationary while the sample rotates incrementally (180° or 360°), capturing radiographs from multiple angles. The detector converts transmitted X-rays into visible light, which is then digitized by a CCD camera. Scanning time depends on sample size, resolution, and exposure time (Kareh & Whitham, 2021). The tube's voltage, current, and exposure time are precisely controlled to ensure optimal imaging conditions (Kotwaliwale et al., 2014). The resulting shadow image,

which reflects X-ray attenuation along the beam path, is then computationally reconstructed for detailed analysis.

b) Reconstruction

Reconstruction integrates multiple projections taken from various angles to create a three-dimensional image (Barigou & Douaire, 2013; Olakanmi et al., 2024). The projection data acquired is back-projected along recorded attenuation profiles, which reconstructs the internal features of the sample. Additional algorithms, such as filtering and noise reduction, enhance image clarity by minimizing distortions introduced by the back-projection process (Calo et al., 2020). Specialized algorithms also correct artifacts caused by the X-ray beam, including beam hardening and ring artifacts.

c) Visualization

Reconstructed 3D images, composed of voxels, are visualized as tomographs through the conversion of 2D radiographs and applying post-processing steps such as background subtraction, windowing, and segmentation/thresholding in specialized software (Olakanmi et al., 2023; 2024). Image visualization begins with smoothing filters and segmentation, where voxel groups represent specific sample regions of interest (ROI) (Kotwaliwale et al., 2014). Converting grayscale images into binary representations enhances the differentiation between solid and porous components, improving segmentation accuracy. ROI selection further refines targeted analysis, increasing data reliability and relevance (Calo et al., 2020; Chidanand et al., 2017). X-ray μCT allows detailed analysis of pore size distribution, volume fractions, and density variations, crucial for understanding sample properties (Chidanand et al., 2017).

Qualitative methods describe image characteristics during the analysis phase, while quantitative techniques assess structural properties and density distribution (Kotwaliwale et al., 2014). Ensuring the selection of statistically representative ROI enables thorough microstructural evaluation, including identifying voids and inclusions. Quantitative analysis involves virtually slicing the 3D volume and utilizing continuous voxel grids to examine density variations. Integrating multiple 2D slices into a 3D reconstruction allows for volumetric assessment, providing significant advantages over conventional microscopy methods (Calo et al., 2020).

2.6.2 Applications of X-ray μCT in food

X-ray μCT is particularly well-suited for characterizing the internal morphology of dry foods such as cereals, bread, pasta, crackers, and biscuits, all of which exhibit a cellular structure (Barigou & Douaire, 2013; Olakanmi et al., 2023; Van Dalen et al., 2011). This technique enables precise quantification of microstructural features using 3D image analysis, allowing comparison with computationally generated models (Barigou & Douaire, 2013). Several studies have demonstrated the efficacy of X-ray μCT in food microstructure analysis. Wang et al. (2011) investigated different types of bread and found that porosity is largely dependent on the degree of open interconnected cells. Cafarelli et al. (2014) combined X-ray μCT with a chi-squared algorithm to classify bread morphology, demonstrating that integrating statistical analysis with microstructural parameters such as object surface and volume enhances quality assessment and classification. Similarly, Whitworth & Alava, (2005) compared X-ray μCT and magnetic resonance imaging (MRI) in studying the structural evolution of bread, muffins, and cakes during proofing and baking. Their findings indicated that X-ray μCT provided superior microstructural detail, whereas MRI was more effective for analyzing water mobility throughout processing.

Recent studies have further expanded the application of X-ray μCT in snack and baked food characterization. Olakanmi et al. (2024) examined the effects of particle size and substitution ratios of fava bean flour blended with wheat in bread formulations. The authors reported that the substitution ratio influenced porosity and structural thickness, not particle size. Boudina et al. (2023) used X-ray μCT to analyze the crispness of cornflakes, establishing a relationship between mechanical behaviour under compression and perceived crispiness. Similarly, Lisiecka et al. (2023) investigated the structural characteristics of ready-to-eat snacks produced with fresh carrot pulp subjected to different expansion methods, including hot-oil frying, hot-air frying, and microwave treatment. Their results showed that expansion methods significantly increased pore size distribution while reducing cell wall thickness.

2.6.3 Relationship between microstructure, physical, and functional properties of food

The microstructure of a product determines its mechanical, physical, and functional properties, particularly texture (Barigou & Douaire, 2013; Barrett, 2002). According to Barrett, (2002), the deformation behaviour of food is highly dependent on its physical structure, while functional properties often influence sensory perception. Barigou & Douaire, (2013) highlighted the importance of characterizing food microstructure through non-destructive and non-invasive methods to better understand the complex relationships between microstructural, physical, and functional properties. Haedelt et al. (2007) linked the spatial distribution of cells in aerated chocolate to its sensory and functional properties, particularly texture. Similarly, Robin et al. (2011) investigated the effect of wheat bran concentration on the functional properties of extruded foam, observing increased mechanical stress in extrudates with a finer microstructure. Wang et al. (2011) further reported that the distribution and size of pores significantly affect a food product's mechanical properties, thereby influencing its texture and sensory attributes. Establishing the

relationship between microstructure, physical, and functional properties is essential as it enables predictive capabilities and facilitates the development of targeted food products with specific textural and sensory characteristics.

2.7 Research gap

Identifying an optimal milling and pretreatment method for chickpea-enriched wheat-based products while minimizing microstructural impact is essential. To our knowledge, no study has utilized non-destructive techniques like X-ray μCT to analyze and compare the microstructure of these products. Therefore, this study aims to determine the most suitable milling type, configuration, and pretreatment method for chickpeas with the least microstructural alteration.

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CHAPTER 3

Quality and microstructural analysis of Chickpea-Enriched Wheat Semolina Pasta Using X-ray Micro-Computed Tomography

(This chapter has been published in a peer-reviewed scientific journal)

O.U. Ukoji, M.M.A. Chaudhry, L. Boyd, E. Sopiwnyk and J. Paliwal, (2024). "Quality and microstructural analysis of chickpea-enriched wheat semolina pasta using X-ray micro-computed tomography". *Journal of Cereal Science*. *119*: 104009. https://doi.org/10.1016/j.jcs.2024.104009

3.1. Highlights

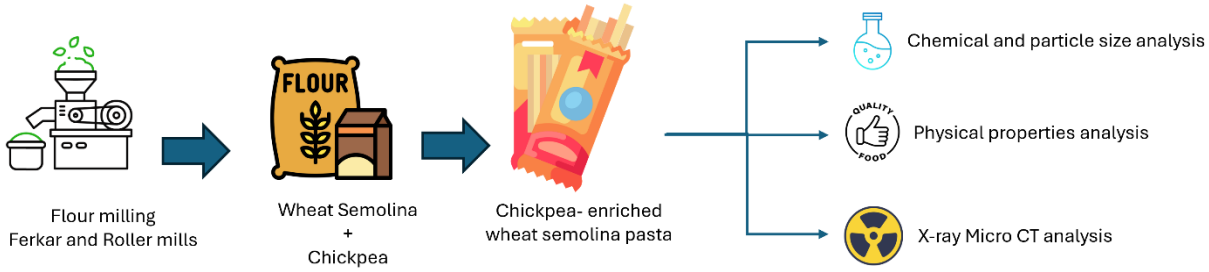
- Mill type and configuration influenced the microstructure of wheat-chickpea pasta
- Reduction roll of roller mill minimally impacts wheat-chickpea pasta microstructure
- The texture analysis correlates with the microstructural analysis of pasta

3.2. Abstract

The microstructure of manufactured foods largely governs palatability qualities such as crunchiness, crispness, mouthfeel and texture. While enriching cereal flour with leguminous flour increases its nutritional value, it should not compromise the palatability of the end products. This study investigated the flour blend properties as well as the physical and microstructural characteristics of durum wheat semolina pasta enriched with 50% chickpea flour obtained from single-stage (Ferkar) and multi-stage (roller) mills. Results of the flour blend analysis indicated the influence of milling on the protein, ash and particle size distribution of pasta. High protein chickpea blend showed minimal impact on the microstructure of the pasta. Also, the textural analysis of the pasta samples showed that the hardness and firmness of pasta made with roller-milled flours, specifically reduction and straight-grade flours, were not statistically significantly different ($p < 0.05$). However, correlating the texture and total porosity indicated that flour from

roller mill reduction rolls resulted in pasta with a firmer texture along with a more compact internal 0and external microstructure. The findings of this study provide useful insight into the quality attributes of chickpea-enriched wheat semolina pasta as influenced by the mill type and stream blend of the chickpea flour.

3.3. Graphical abstract



Keywords: microstructure, milling, chickpea-enriched wheat pasta, micro-computed tomography

3.4. Introduction

Cereal-based pasta products (spaghetti or macaroni) are highly recognized staple foods that can easily be enriched nutritionally by adding pulse flour ingredients (Goñi and Valentín-Gamazo, 2003). The nutritional quality of the pasta produced depends on the type of grain, milling technique, added ingredients, and nature of extrusion (warm or cold). Traditionally, durum wheat semolina has been the primary raw material for pasta production; however, concerns regarding high gluten content and essential amino acid deficiency have prompted the inclusion of other cereal and pulse flours to enrich wheat-based pasta nutritionally (Kumar et al., 2021).

Pulse crops are widely recognized for their ecological and nutritional benefits. They contribute to soil fertility through nitrogen fixation while providing protein-rich nutritional foods that are an

ideal alternative to animal protein sources (De Pasquale et al., 2021; Malhotra et al., 2023). Canada is a leading producer and exporter of pulse crops worldwide. According to 2020 farm receipts, Canada's production of key pulses such as lentils, chickpeas, peas, and beans reached a record high of 8.8 million tonnes, generating a revenue of $1.5 billion (Malhotra et al., 2023). Among these pulses grown in Canada, chickpeas, owing to their neutral taste and colour, have become a popular ingredient blended with wheat flour to manufacture extruded products. Globally, chickpea (*Cicer arietinum L.*), belonging to the family Fabaceae, is ranked as the third most important pulse crop, following common beans and peas, with primary cultivars identified as Kabuli and Desi (De Pasquale et al., 2021). Chickpea is particularly noted for its high protein and fibre content with a low environmental footprint, making it the most desirable ingredient for enriching conventional wheat flour in manufacturing foods, including extruded and baked products (Malhotra et al., 2023; Saget et al., 2020).

Previous studies on chickpea-enriched pasta include microstructural analysis using scanning electron microscopy (SEM) (Bouasla et al., 2017), investigating structural characteristics (Garcia-Valle et al., 2021a) and quality attributes (El-Sohaimy et al., 2020; Schettino et al., 2019; Singh et al., 2024) at different substitution ratios. However, none of the studies have investigated the important aspects of milling and flour stream blending on the quality characteristics and microstructure of chickpea-enriched pasta.

According to Scanlon et al. (2018), it is essential to mill pulse crops to adequate particle sizes to optimize the benefits of enrichment. These particle sizes are achieved from the various stream blends and mill configurations, which significantly influence the separation of the flour components after milling. Industrially popular multi-stage roller milling is an encouraged approach in studying the influence of pulse milling because its degree of compression and shear added

during milling can be manipulated independently (Scanlon et al., 1988). Single-stage milling, on the other hand, offers a comparative approach given its singular mill configuration. However, issues such as poor miscibility of ingredients due to large particle size can occur during blending, influencing the quality of finished products achieved with differently milled flours. To verify that an enrichment target is met in a product, the ingredient value from co-stream(s) and mill types needs to be established (Scanlon et al., 2018).

Research has demonstrated that the choice of pulse type and milling method significantly influences the suitability of pulses for specific food products, impacting the quality and texture of the final product (Sivakumar et al., 2022; Wood and Malcolmson, 2011). In pasta production, the milling process affects the product quality, texture, and overall acceptability (Thakur et al., 2019). An SEM study on rice pasta enriched with chickpea flour milled to a particle size of 0.5 mm revealed uniform particle distribution and a smoother pasta surface (Bouasla et al., 2017). However, 2D imaging techniques such as SEM are often insufficient for comprehensive microstructural evaluations of food products (Ramachandran et al., 2021).

To overcome these procedural limitations and bridge the existing knowledge voids, this study employed high-resolution X-ray micro-computed tomography (X-ray μ-CT), which enables detailed qualitative and quantitative 3D analysis. This technology provides intricate details on the distribution, shape, and size of pores, as well as morphometric parameters such as porosity, wall thickness, and void cell size distribution (Ramachandran et al., 2021; Schoeman et al., 2016). Previous studies applied small-angle X-ray scattering (SAXS) and X-ray diffraction (XRD) generated using synchrotron X-ray technology to study the microstructural characteristics of pasta formulations (Aravind et al., 2013; Muneer et al., 2018). The SAXS and XRD techniques provided information on the crystallinity and molecular arrangement of starch and protein, as well as their

interactions in the pasta studied. However, for high-resolution spatially-resolved 3D visualization, X-ray μ-CT is preferred (Schoeman et al., 2016).

A few studies have been conducted to investigate the effect of mill type and mill configuration on the microstructure of chickpea flour (Sivakumar et al., 2022). However, there is a lack of research on the overall quality attributes and microstructural influence of the mill type and mill configuration, specifically on extruded products enriched with chickpea flour (such as pasta). Additionally, even though ingredients such as wheat (Garcia-Valle et al., 2021a), barley (Singh et al., 2024), rice (Bouasla et al., 2017), and tiger nut (Llavata et al., 2020) have been enriched with chickpea to produce pasta, there has been no comparative analysis of the milling techniques used and their impacts on the final product quality. The goal of this research work is to address this knowledge gap for chickpea-enriched pasta by considering different milling types and mill configurations. Therefore, this study aims to investigate the impact of incorporating chickpea flour milled using two different methods (i.e. Ferkar and roller milling) into durum wheat semolina to create protein-rich pasta. The pasta quality was determined in terms of the physical properties of the flour blends and pasta microstructure.

3.5. Materials and Methods

3.5.1. Raw materials and milling

Kabuli chickpea (CDC Orion), acquired from Reisner Farm Ltd. in Limerick, Saskatchewan, was selected for this study. Chickpeas were scoured prior to roller milling to remove the hulls using a scourer (Bühler MHXN 80/90; Bühler Group, Uzwil, Switzerland). The samples were milled using a pilot-scale roller mill (Bühler MLU 202 pilot mill; Bühler Group, Uzwil, Switzerland) with the mill configuration setup comprising three break rolls (B1/B2, B3, B4), five reduction rolls (1M,

2M, 3M, 4M, 5M), one low-grade roll (LG), one tailing roll (T) and one 2nd quality roll (2Q) optimized for chickpea milling. The resulting hull and shorts were collected as by-products. Straight-grade chickpea (CP_SG) flour was prepared by combining all flour streams (B1/B2, B3, B4, 1M, 2M, 3M, 4M, 5M, 2Q, LG, T). Reconstituted whole (CP_RW) flour was formulated by combining 92.1% CP_SG blend with 7.9% by-products, which were ground using a Jacobson hammer mill (Model 120-B; Jacobson Machine Works, Inc., Iowa, USA) with a 1.19 mm screen. The low protein (CP_LP) flour blend was prepared from flours obtained from five roll passes (B1/B2, B3, B4, 1M, and 2Q), while the high protein (CP_HP) flour blend was created from flours obtained from six roll passes (2M, 3M, 4M, 5M, LG, and T) of the roller mill, based on composition, yield and industrial suitability. Additionally, chickpeas were milled with a Jacobson hammer mill (Model 120-B; Jacobson Machine Works, Inc., Iowa, USA) fitted with a 3.18 mm screen for pre-breaking, followed by a single-stage Ferkar multipurpose knife mill (Ferkar 5 Model; KFM, Velenje, Slovenia) to produce the Ferkar milled (CP_FM) flour.

3.5.2. Chemical analysis and particle size distribution

Protein content was determined by combustion nitrogen analysis (CNA) method using the LECO-FP282 (LECO Corp., Michigan, USA) (William et al., 1998). A protein conversion factor of N × 5.7 was used for durum semolina and N × 5.98 for 50/50 semolina-chickpea flour blends. Drift corrections were done using ethylenediaminetetraacetic acid (EDTA) and ash content was determined using the AACC, 2000 Method 08-01.01. Flour samples were weighed into dishes previously dried at 600°C for a minimum of 1 h. The samples were then incinerated for 16 h at 600°C and ash content was measured. The particle size distribution of the flour blend in dry dispersion conditions was determined by laser diffraction using a Malvern Mastersizer 2000 with Scirocco 2000 accessory (Malvern Instruments Inc., Massachusetts, USA). Results were presented

as the size of the particles (μm) at different percentiles of the distribution curve. At d (0.1), 10% of the flour particles by volume is at or below the indicated size (μm), at d (0.5), 50% of the flour particles by volume is at or below the indicated size (μm), and at d (0.9), 90% of flour particles by volume is at or below the indicated size (μm) or lower. Span and uniformity describe the width of the distribution of particles and the absolute deviation from the median, respectively. The experiments were conducted in duplicates to account for variance in the data.

3.5.3. Pasta formulation and processing

Spaghetti-type pasta was prepared in 3 kg batches using a Namad Extruder (60 VR, Namad, Rome, Italy) following the manufacturer's instructions. Chickpea flour was blended with durum wheat semolina at a 50% inclusion level, which was determined based on preliminary trials using straight-grade flours at 30%, 40%, and 50% inclusion levels. The water bath temperature during dough making was set to 20.5°C, with a mixing time of 12 min. During the extrusion process, the water absorption (%) of the dough was determined by the target dough pressure (74-85 bar) which is an indicator of the wetness or dryness of the sample as presented in Table 3.1. After extrusion, the spaghetti was dried in a batch dryer (Bühler C-line; Bühler group, Uzwil, Switzerland) with the drying conditions presented in Fig. 3.1. Each sample was processed twice on separate days, resulting in two processing replicates. Spaghetti made using 100% durum wheat semolina was included as a control.

Table 3.1. Water absorption (%) used in pasta processing.

Sample	Water absorption (%)
Control	32
50% CP_FM	27
50% CP_SG	26
50% CP_RW	26
50% CP_LP	26
50% CP_HP	25

Control – durum wheat semolina; 50% CP_FM – Ferkar mill flour blend; 50% CP_SG – straight grade flour blend; 50% CP_RW – reconstituted whole flour blend; 50% CP_LP – low protein flour blend; 50% CP_HP – high protein flour blend.

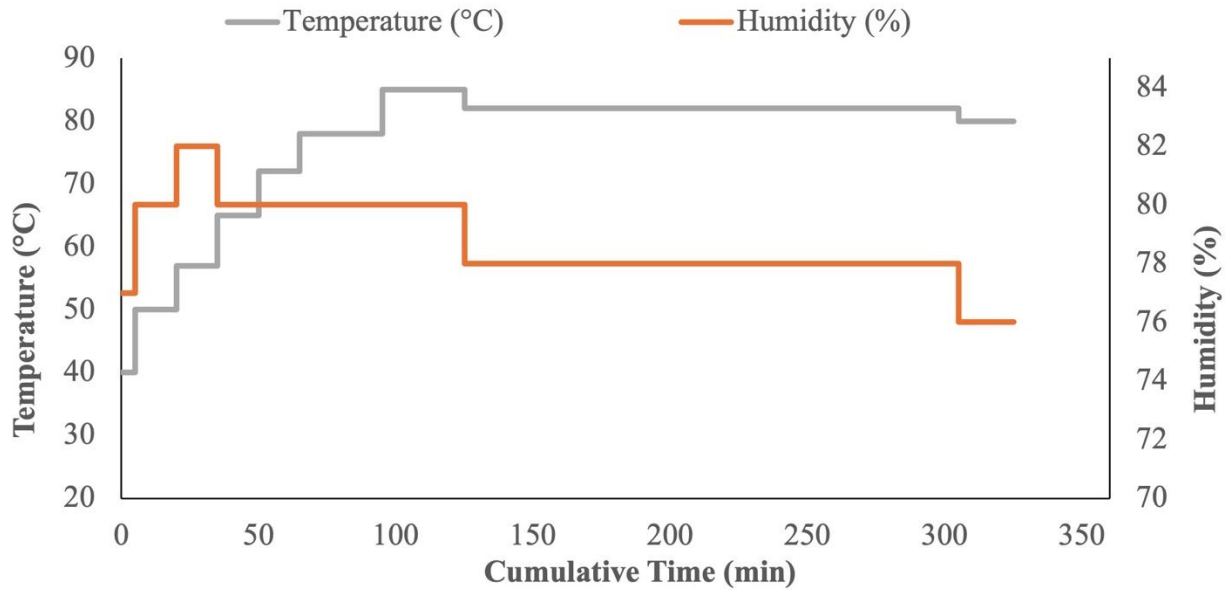


Fig. 3.1. Spaghetti drying profile.

3.5.4. Cooking quality

The cooking quality was determined according to the AACC-approved methods 66-51.01 (2000). Dried spaghetti (30 g, 1 cm length) was cooked in 300 mL of boiling water to its optimum cooking time (OCT) which is the time when the center core of the spaghetti just disappears when squeezed between two Plexiglas plates. After the OCT was reached, the spaghetti was drained, and the cooking water was retained. The cooking water was evaporated for 16 h at 130°C and the residue was weighed and expressed as a percentage of the initial spaghetti weight which described the cooking loss (CL). The experiment was determined in duplicate for each pasta type.

3.5.5. Colour analysis

The colour of flour blends and pasta samples were analyzed using the Minolta CR-410 colorimeter (Konica Minolta Inc., Osaka, Japan) with D65 illuminant and at a 2° standard observer angle according to the manufacturer's instructions. A slurry of flour and water was made according to the AACC Method 14-30.01 with respect to the volume of water, mixing time and waiting time. A flour weight equivalent to 15 g on a dry moisture basis was used for the pulse blends while the durum semolina (15 g) was corrected to 14% wet moisture basis. Also, dry uncooked spaghetti strands were mounted using double-sided tapes on a standard white cardboard (7.5 cm x 7.5 cm) and measurements were recorded following the CIE $L^*a^*b^*$ scale. The following parameters were measured: L^* (0 = black to 100 = white); a^* ($-a^*$ = green to $+a^*$ = redness); b^* ($-b^*$ = blue to $+b^*$ = yellow). The overall change ΔE in colour from the control sample was calculated using eq 1.

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2} \quad (1)$$

3.5.6. X-ray micro-computed tomography

The 3D microstructural analysis of spaghetti samples was conducted using an X-ray μ-CT system (SkyScan 1275; Bruker microCT, Kontich, Belgium), operating in the range of 20-100 kV. Single spaghetti strands, accurately cut from the 50% chickpea flour spaghetti and the control samples, were mounted on a cylindrical sample holder using low-density wax. Image acquisition was conducted with no filter, a pixel size of 10 μm, a source voltage of 40 kV, and a current of 180 μA. The sample was then scanned with a rotation angle of 0.2° over 180° to obtain high-resolution 2D images (699 × 1382 pixels). Each sample was scanned in quadruplicate, with each scan taking 15 min.

3.5.6.1. Image reconstruction and analysis

The 2D images obtained at each rotational angle were reconstructed into 3D hypercubes utilizing NRecon software (version 1.7.0.4; Bruker microCT, Kontich, Belgium), enabling a profound analysis of the internal and external geometries of the pasta samples. Corrections were made for ring artifacts (set at 13%), misalignment, and beam hardening (set at 23%) in the 2D images. During reconstruction, the grayscale frequency scatter plot for each spaghetti sample was manually adjusted to set the minimum and maximum ranges. The 3D analysis and reconstruction of voxel images were conducted using the CT-Analyzer software package (version 1.16.9.0; Bruker micro-CT, Kontich, Belgium). This process involved the application of a custom-developed algorithm to the selected regions of interest (ROI). The threshold for gray level values was manually set to lower and upper gray thresholds of 200 and 255, respectively, to differentiate the microstructures and generate binary images while comparing slices from each pasta sample. Morphological operations, such as de-speckle, were employed to maintain pore structures while removing

artifacts. Bitmaps were utilized to convert images to monochrome and preserve the object structure. A bitwise separation was employed to customize the pores in the images. The 3D structural properties, including open porosity, closed porosity, number of closed pores, and total porosity, were quantitatively assessed. Also, to facilitate a rigorous comparison of the effects of different milling methods and flour blends on the microstructure of pasta, a qualitative analysis of the internal structure was performed. Visualization of the 3D images was conducted using CT Vox software (version 3.3.0.0; Bruker microCT, Kontich, Belgium), comparing each sample against the control.

3.5.7. Texture analysis of cooked and uncooked pasta

Dry spaghetti (12 strands, 5 cm length) was cooked in boiling water (300 mL) for 9 min. After cooking, the spaghetti was drained and placed in a fine sieve. A texture analyzer (TA.HD *plus*; Texture Technologies Corp., New York, USA) equipped with a firmness blade (TA-47) and 5 kg load cell was used to measure firmness (g), which is defined as the peak force observed for each texture curve. The blade compressed the spaghetti strands at a test speed of 1 mm/s for a 2.9 mm distance with, duplicate measurements recorded. On the other hand, the hardness of dry uncooked pasta samples was measured using a snap test procedure modified from (Jyothi et al., 2009). A three-point bending rig (code: HDP/3PB) with an adjustable length of 80 mm was set up onto a texture analyzer (TA.XT plus; Texture Technologies Corp., New York, USA). The dried pasta was accurately cut to a length of 100 mm and placed onto the support bars kept 40 mm apart from their end. The probe's distance from the sample was set to 3 mm with a trigger force of 0.1 N. The probe was lowered at a pre-test speed of 10 mm/s, test speed of 2.0 mm/s and post-test speed of 10 mm/s. The force peak in the force-deformation curve was recorded as the hardness, with 10 replicates obtained for each sample.

3.5.8. Statistical analysis

Data was analyzed using SPSS software (version 27.0, IBM SPSS Statistics Inc., Illinois, USA) to perform a one-way analysis of variance (ANOVA) at a 95% confidence interval ($p < 0.05$), and Duncan's multiple range test was used to compare the mean. All results were presented as means along with standard error.

3.6. Result and Discussion

3.6.1. Chemical analysis, particle size distribution and colour of semolina-chickpea flour blends

The result of the protein and ash content of the durum wheat semolina and chickpea flour blend is presented in Table 3.2. It can be observed that the different mill and mill configurations influenced the protein and ash content of the flour blends. This observation is in agreement with Choo et al. (2024) who studied the impact of milling methods on pulse flour quality. The 50% CP_LP blend had the lowest protein (~16%) and ash (1.65) content among the flour blends while the highest protein and ash content was observed in the 50% CP_HP blend (~18% and 1.86, respectively). However, the protein and ash content of the chickpea flour blends were significantly higher ($p < 0.05$) than the control sample indicating a positive nutritional enrichment of the semolina-chickpea flour blends. The particle size distribution of the semolina-chickpea flour blends is presented in Table 3.2 with the size of particles shown to be in the range of 14 - 450 μm. A previous study on chickpea flour obtained from the Ferkar mill and different streams of the roller mill observed a bimodal particle distribution in the range of 5 – 150 μm (Sivakumar et al., 2022). This significant increase in the particle size distribution of the semolina-chickpea flour blends can be attributed to the monomodal particle distribution (160 – 1000 μm) of durum wheat semolina flour (Petitot et al., 2010). The 50% CP_FM showed a lower particle distribution in the 10% volume of flour which

was statistically significantly different ($p < 0.05$) from the other chickpea flour blend. This difference could be attributed to the narrow particle size of the chickpea flour obtained using this mill type (Sivakumar et al., 2022). Similarly, 50% CP_RW showed a statistically significant increase ($p < 0.05$) in the 90% volume of flour when compared with other roller-milled flour blends, which could be due to the added hammer-milled by-products (i.e. hull and shorts).

Further analysis of the flour blends showed a higher median deviation and width distribution of particles in the 50% CP_HP which was statistically significantly different ($p < 0.05$) from other chickpea flour blends and control. However, the varying chemical composition and particle size of semolina and chickpea flour can impact the hydration properties and physical properties of dough and pasta (Petitot et al., 2010; Scanlon et al., 1988). The colour analysis of the semolina-chickpea flour blend showed a statistically significant difference ($p < 0.05$) when compared to the control as presented in Table 3.2. The least brightness (L^*) was observed in the 50% CP_FM and 50% CP_RW flour blend while a more reddish (a^*) and yellowish (b^*) colour was observed for 50% CP_HP flour blend. However, this change in colour of semolina-chickpea flour blend is not attributed to the mill type or configurations but rather to the presence of pigments in chickpeas (Bouasla et al., 2017).

Table 3.2. Chemical properties, particle size distribution and colour of chickpea flour blends

Sample	Protein content (%db)	Ash content (%db)	Particle size distribution (μm)					Colour of flour blends			
			d (0.1)	d (0.5)	d (0.9)	Uniformity	Span	L^*	a^*	b^*	ΔE
Control	13.80±0.00^{a}	0.75±0.01^{a}	176.00±0.00^{e}	302.00±0.00^{e}	495.50±0.50^{d}	0.33±0.00^{a}	1.06±0.00^{a}	83.34±0.01^{e}	-1.84±0.03^{a}	26.66±0.01^{a}	-
50% CP_SG	17.00±0.00^{e}	1.78±0.02^{c}	20.00±0.00^{c}	175.50±0.50^{a}	438.50±0.50^{ab}	0.78±0.00^{e}	2.39±0.01^{e}	80.58±0.04^{c}	0.25±0.00^{c}	32.06±0.02^{d}	6.41
50% CP_FM	16.55±0.05^{c}	1.83±0.01^{d}	14.00±0.00^{a}	200.50±0.50^{d}	446.00±0.00^{b}	0.74±0.00^{c}	2.16±0.01^{b}	79.18±0.10^{a}	0.38±0.05^{d}	30.72±0.05^{c}	6.25
50% CP_RW	16.80±0.00^{d}	1.86±0.01^{de}	20.00±0.00^{c}	193.50±1.50^{c}	453.50±2.50^{c}	0.73±0.01^{b}	2.24±0.01^{c}	79.34±0.10^{a}	0.55±0.04^{e}	30.07±0.06^{b}	5.77
50% CP_LP	16.00±0.00^{b}	1.65±0.01^{b}	19.00±0.00^{b}	188.00±0.00^{b}	443.00±3.00^{ab}	0.74±0.01^{c}	2.25±0.01^{c}	81.01±0.06^{d}	-0.04±0.06^{b}	31.06±0.29^{c}	5.29
50% CP_HP	17.85±0.05^{f}	1.86±0.00^{e}	21.00±0.00^{d}	176.50±0.50^{a}	436.50±3.50^{a}	0.76±0.00^{d}	2.36±0.01^{d}	80.30±0.08^{b}	0.49±0.02^{de}	32.64±0.13^{e}	7.10

The data presented are the mean ± standard error of duplicate experiment, values within the same column and having different superscript are significantly different ($p < 0.05$). Control – durum wheat semolina; 50% CP_FM – Ferkar mill flour blend; 50% CP_SG – straight grade flour blend; 50% CP_RW – reconstituted whole flour blend; 50% CP_LP – low protein flour blend; 50% CP_HP – high protein flour blend.

3.6.2. Physical properties of pasta

The percentage OCT and CL of pulse-enriched pastas have been established to be lower and higher, respectively, from previous studies (Bouasla et al., 2016; Garcia-Valle et al., 2021a). A similar trend was observed in this study, as presented in Table 3.3. The analysis illustrates 50% CP_RW to have the lowest OCT, which was significantly different ($p < 0.05$) than the other pasta blends and control. Similarly, the OCT of 50% CP_SG, 50% CP_FM and 50% CP_LP were statistically significantly different ($p < 0.05$) from that of 50% CP_HP and control. However, the OCT of 50% CP_HP pasta was not statistically significantly different ($p < 0.05$) from that of the control. The reduced OCT could be due to the increased chemical composition from chickpea enrichment of pasta (El-Sohaimy et al., 2020). A higher CL was observed for all chickpea-enriched pasta (~7%) as compared to the control (~5%). This increased CL across the chickpea-enriched pasta is attributed to the weak starch network, increased fibre content and particle size of the flour blend (Bouasla et al., 2016; Petitot et al., 2010). However, the OCT and CL of the chickpea-enriched pasta samples observed in this study are within the acceptable threshold for industrial pasta production (Garcia-Valle et al., 2021a). The colour of a product is crucial to the consumer perception of that product which is an important consideration during pasta production. The result of the colour analysis of the pasta samples presented in Table 3.3 showed variability in the colour of semolina-chickpea pasta, which was also observed in previous studies involving chickpea pasta (Schettino et al., 2019; Singh et al., 2024). A decrease in L^* and b^* with an increase in a^* value in all semolina-pasta samples was observed, which was statistically significantly different ($p < 0.05$) when compared to the control. The highest overall ΔE change of the pastas from the control was observed in the 50% CP_FM, although this increased change could be attributed to the choice of substitution ratio used in this study.

Table 3.3. Physical properties of semolina-chickpea pasta

Pasta	OCT (min)	CL (%)	Firmness of cooked pasta (g)	Hardness of uncooked pasta (N)	Colour of dry uncooked pasta			
					L^*	a^*	b^*	ΔE
Control	11.25 ± 0.25^{c}	4.70 ± 0.20^{a}	760.50 ± 14.50^{d}	3.00 ± 0.10^{c}	74.85 ± 0.25^{c}	2.56 ± 0.30^{a}	59.05 ± 0.15^{d}	-
50% CP_SG	10.50 ± 0.00^{b}	6.60 ± 0.20^{bc}	689.50 ± 18.50^{bc}	2.18 ± 0.06^{b}	61.55 ± 2.45^{ab}	16.96 ± 2.00^{bc}	46.80 ± 3.00^{c}	23.12
50% CP_FM	10.25 ± 0.25^{b}	7.00 ± 1.00^{c}	649.50 ± 10.50^{b}	2.37 ± 0.07^{b}	61.70 ± 1.50^{ab}	15.19 ± 0.98^{bc}	35.90 ± 1.20^{a}	29.47
50% CP_RW	9.50 ± 0.00^{a}	6.40 ± 1.00^{b}	661.50 ± 17.50^{b}	1.30 ± 0.17^{a}	58.65 ± 1.75^{a}	17.15 ± 1.75^{bc}	41.30 ± 0.80^{b}	28.11
50% CP_LP	10.00 ± 0.00^{b}	6.60 ± 0.00^{bc}	579.00 ± 11.00^{a}	1.31 ± 0.25^{a}	64.65 ± 1.75^{b}	13.85 ± 2.10^{b}	43.85 ± 0.55^{bc}	21.51
50% CP_HP	11.00 ± 0.00^{c}	6.75 ± 0.15^{bc}	732.00 ± 3.00^{cd}	2.18 ± 0.08^{b}	58.40 ± 0.70^{a}	19.60 ± 0.65^{c}	44.90 ± 1.10^{bc}	27.59

Mean ± standard error of values with the same superscript in a column are not significantly different ($p < 0.05$). Control – durum wheat semolina; 50% CP_FM – Ferkar mill flour blend; 50% CP_SG – straight grade flour blend; 50% CP_RW – reconstituted whole flour blend; 50% CP_LP – low protein flour blend; 50% CP_HP – high protein flour blend.

3.6.3. Image analysis and 3D model

A visualization model of the 2D gray level and binarized images of specific slices of the pasta samples is presented in Fig. 3.2a. The control and 50% CP_HP pasta exhibited the lowest porosity after binarization compared to the other pasta samples, with the highest porosity observed in the 50% CP_RW pasta. Similarly, the 3D model of the wheat semolina and chickpea-enriched pasta, revealing the solid structure, closed pores, and open pores through slicing, is shown in Fig. 3.2b. The high number of pores coloured in red in the 50% CP_RW pasta were distributed from the top to the bottom layer, unlike in the other samples. Additionally, a random distribution of pores is evident in the 50% CP_FM and 50% CP_LP samples, whereas a few widely spread pores were observed in the 50% CP_SG and 50% CP_HP samples.

3.6.4. Pasta microstructure

A comparative analysis of the microstructural characteristics of the different pasta samples revealed variations in semolina-chickpea pasta depending on the milling technique and mill configuration (Fig. 3.2a and 3.2b). The 3D analysis primarily focused on a quantitative understanding of the porosity parameters, including the number of closed pores, closed porosity, open porosity, and total porosity, as well as the connectivity of pores and the structure/cell wall thickness. These findings are detailed in Table 3.4.

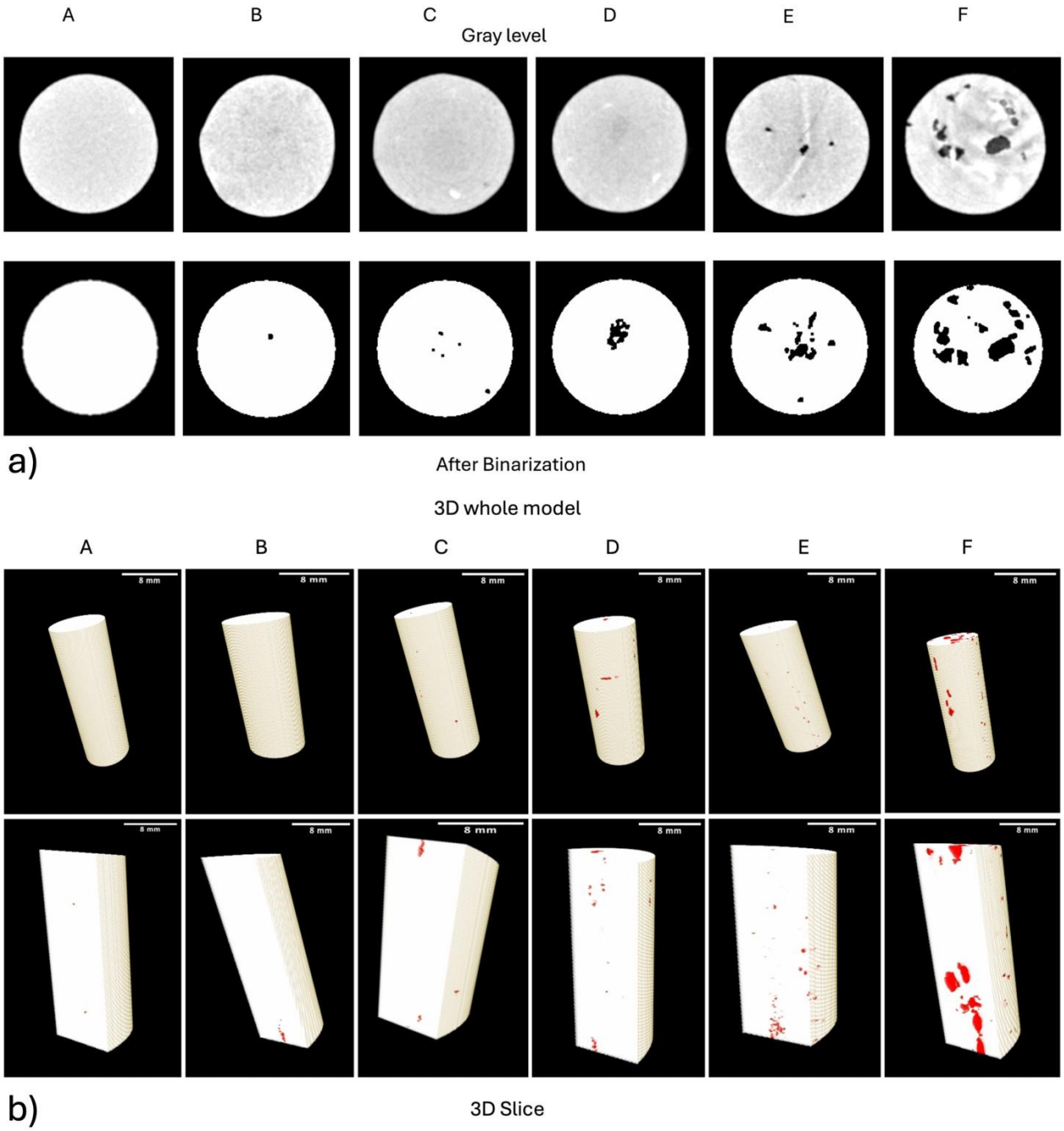


Fig. 3.2. a) 2D images of the cross-section of pasta at the gray level (top row) and after binarization (bottom row); b) 3D model of pasta samples showing pores (red) and solid structure (white) in whole (top row) and sliced (bottom row) sections

A) Control – durum wheat semolina; B) 50% CP_HP – high protein flour blend; C) 50% CP_SG – straight grade flour blend; D) 50% CP_FM – Ferkar mill flour blend; E) 50% CP_LP – low protein flour blend; F) 50% CP_RW – reconstituted whole flour blend.

Table 3.4. Microstructure parameters of pasta samples.

Pasta	Number of closed pores	Closed porosity (%)	Open porosity (%)	Total porosity (%)	Structural thickness (mm)	Connectivity (μm)
Control	1.75 ± 0.63^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.37 ± 0.01^{d}	0.00 ± 0.00^{a}
50% CP_SG	43.00 ± 7.78^{ab}	0.03 ± 0.01^{a}	0.01 ± 0.00^{a}	0.04 ± 0.00^{a}	0.97 ± 0.05^{c}	-1.50 ± 0.65^{a}
50% CP_FM	68.25 ± 5.38^{ab}	0.12 ± 0.03^{a}	0.11 ± 0.02^{a}	0.22 ± 0.05^{a}	0.76 ± 0.03^{b}	5.00 ± 1.68^{a}
50% CP_RW	74.75 ± 8.85^{bc}	3.01 ± 0.42^{b}	3.41 ± 1.00^{b}	6.33 ± 0.69^{b}	0.53 ± 0.03^{a}	36.50 ± 5.69^{b}
50% CP_LP	141.75 ± 35.42^{c}	0.35 ± 0.23^{a}	0.26 ± 0.21^{a}	0.61 ± 0.44^{a}	0.73 ± 0.09^{b}	9.50 ± 7.58^{a}
50% CP_HP	11.25 ± 2.21^{ab}	0.01 ± 0.00^{a}	0.00 ± 0.00^{a}	0.02 ± 0.00^{a}	1.09 ± 0.07^{c}	0.00 ± 0.00^{a}

The data is the mean of the quadruple experiment ± standard error. Values within the same column followed by the same letter are not significantly different. Control – durum wheat semolina; 50% CP_SG – straight grade flour blend; 50% CP_FM – Ferkar mill flour blend; 50% CP_RW –reconstituted whole flour blend; 50% CP_LP – low protein flour blend; 50% CP_HP – high protein flour blend.

3.6.4.1. Number of closed pores

A higher number of closed pores was observed in pasta made from the 50% CP_LP flour blend, which was significantly different ($p < 0.05$) from the other analyzed pasta samples, as shown in Table 3.4. This high number of closed pores could be attributed to the break rolls of the roller mill used to achieve the flour blend as the result of particle size distribution of flour blends presented in Table 3.2 showed break roll blend (50% CP_LP) had statistically larger particle in the 50 and 90 percentile range than the reduction roll blend (50% CP_HP). Sivakumar et al. (2022) identified chickpea flour from the break rolls of the roller mill (B1+ B2 + B3) had a larger particle size compared to flour from the reduction rolls. According to Wójtowicz and Mościcki (2014), the number of closed pores in pasta varies according to the particle size of the flour blend and the processing condition. Similarly, pasta made from the 50% CP_RW flour blend had a high number of closed pores, likely due to the particle size of the hammer-milled by-products (i.e. hulls and shorts) used in the flour blend which led to the observed higher particle distribution (Table 3.2). However, the differences between the 50% CP_RW, 50% FM, and 50% CP_SG were not statistically significant ($p < 0.05$) (Table 3.4). Additionally, the least number of closed pores was observed in the 50% CP_HP sample, which could be attributed to the reduction rolls of the roller mill used to achieve the flour blend. However, in this study, the number of closed pores observed in the 50% CP_HP, 50% CP_SG, and 50% CP_FM pasta were not statistically significantly different ($p < 0.05$) from that observed in the control pasta sample (Table 3.4).

3.6.4.2. Open and closed porosity

The results presented in Table 3.4 showed that the percentage of open and closed porosity was similar across all pasta samples. However, a significantly higher percentage of open and closed

porosity was observed in the 50% CP_RW pasta, which was statistically different ($p < 0.05$) from the control and other semolina-chickpea pasta samples. This increase could be attributed to the particle size of the hammer-milled chickpea by-product added to the flour blend, which influences the microstructure of the products, as reported in previous studies (Scanlon et al., 2018; Sivakumar et al., 2022). Similarly, increased percentages of open and closed porosity were observed in the 50% CP_LP and 50% CP_FM samples, although this increase was not statistically significant ($p < 0.05$) compared to the control sample. The increase in percentage open and closed porosity observed in the 50% CP_LP and 50% CP_FM could be attributed to the increased particle distribution in the 50 and 90% volume of flour (Table 3.2) resulting from the compressive and shear forces imparted by the break roll of the roller mill and the cutting forces of the Ferkar mill used to obtain the chickpea flour (Sivakumar et al., 2022).

The lowest percentage of open and closed porosity was observed in the 50% CP_HP and 50% CP_SG pasta, with no statistically significant difference ($p < 0.05$) compared to the control, 50% CP_FM, and 50% CP_LP pasta samples. This compact internal structure of the pasta could be attributed to the proper interconnection of starch, protein, and fibre molecules in the dough of the enriched pasta (Sudha and Leelavathi, 2012). Previous 2D microstructural studies of pulse-enriched pasta using SEM revealed the presence of homogenous compact surface and internal structures, which aligns with our observation of low percentage open and closed porosity (Bouasla et al., 2017; Muneer et al., 2018; Wójtowicz and Mościcki, 2014). Bouasla et al. (2017) studied rice pasta enriched with chickpea at substitution levels of 10, 20 and 30% and concluded a homogenous structure of starch and protein matrix in the substituted pasta. Similarly, wheat semolina pasta enriched with white bean and yellow pea showed a compact structure at 40% substitution levels, although a non-homogeneous compact structure was observed for lentil flour

at 40% substitution, which the authors attributed to the pulse type and level of substitution (Wójtowicz and Mościcki, 2014). A study by Singh et al. (2024) on barley substituted with chickpea showed a non-homogeneous internal structure of the pasta, which the authors attributed to a weak matrix of starch, fibre, and protein network. However, the contradiction observed in their study and the present one could result from the difference in the cereal grain flour enriched with chickpea flour to make pasta.

3.6.4.3. Total porosity and structural thickness

Results presented in Table 3.4 depict total porosity as a summation of the percentage of open and closed porosities in the individually analyzed pasta samples. The semolina-chickpea pasta made from 50% CP_HP had the lowest total porosity value of 0.02%, while the highest total porosity value was observed in 50% CP_RW pasta (6.33%). According to Joardder et al. (2016), it is suggested that a non-porous material has a porosity below 0.25%, whereas a porous material has a porosity greater than 0.40%. Therefore, in this study, it can be assumed that the pasta made from 50% CP_HP, 50% CP_SG, and 50% CP_FM, with percentage total porosity values of 0.02%, 0.04%, and 0.22%, respectively, are non-porous. Moreover, these values were not statistically significantly different ($p < 0.05$) from the percentage total porosity of the control sample (Table 3.4). The zero percentage total porosity observed for the control sample is consistent with previous microstructural studies of wheat semolina pasta using SEM, which revealed compact starch granules with no holes or cracks (Kamble et al., 2021). However, the pasta made from 50% CP_LP and 50% CP_RW had total porosity values of 0.61% and 6.33%, respectively, which exceed the 0.40% threshold for non-porous products and can thus be regarded as porous. The total porosity of the 50% CP_RW pasta was significantly ($p < 0.05$) higher compared to the other pasta samples. This increased total porosity observed in 50% CP_RW could be a reason for the reduced OCT

observed in Table 3.3. In a previous study, Petitot et al. (2010) reported a porosity of 6% in wheat semolina pasta enriched with 35% roller-milled split pea and faba bean, which is greater than most of the percentage total porosity observed in this study. The lower percentage of total porosity observed in our study could be attributed to the pulse type, milling technique, and mill configuration. Further analysis showed that as the percentage of total porosity increases, structural thickness decreases simultaneously. This relationship was also reported in a previous study on bread microstructure (Gondek et al., 2013). The decrease in structural thickness showed a significant difference ($p < 0.05$) between the control (1.37 mm) and the chickpea-enriched pasta. The lowest structural thickness was observed in the 50% CP_RW pasta (0.53 mm), which is attributed to the high percentage of total porosity observed in this pasta (6.33%). Studies have concluded that large cell wall thickness hinders the release of nutrients from food products, while a lower cell wall thickness results in a rapid collapse of the cell wall, leading to nutrient loss during processing (Joardder et al., 2016). In contrast, the 50% CP_RW had the least percentage cooking loss, although it was not statistically significantly different ($p < 0.05$) from the other roller mill pasta types (Table 3.3). This could be attributed to the minimal disruption of the protein gluten network by the added by-products leading to reduced leaching of solid material into the cooking water (Garcia-Valle et al., 2021a).

3.6.4.4. Connectivity of pores

As can be observed from the results in Table 3.4, the connectivity values for pasta made from 50% CP_SG, 50% CP_HP, 50% CP_FM, and 50% CP_LP were not statistically significantly different ($p < 0.05$) from the control sample. However, the negative pore connectivity value observed in the 50% CP_SG suggests the lowest connectivity of the pores in the pasta. The highest pore

connectivity value observed in 50% CP_RW can be linked to the high porosity in the pasta sample; as such, the high connectivity of the pores can be seen in the 3D model presented in Fig. 3.2b.

3.6.5. Texture analysis

The texture of cooked and uncooked pasta is presented in Table 3.3. It can be observed that the blending of durum semolina with chickpea reduced the pasta firmness and hardness, which agrees with results reported in previous studies on precooked barley pasta enriched with chickpea flour (Singh et al., 2024), wheat pasta enriched with legume flour (Wójtowicz and Mościcki, 2014), and rice pasta enriched with chickpea flour (Bouasla et al., 2017). The reduction in the gluten content of enriched pasta compared to wheat semolina pasta creates a weak and destabilized protein network, which can decrease the hardness and firmness of pasta (Singh et al., 2024). However, in this study, the mill types and mill configuration influenced the firmness of cooked and uncooked semolina-chickpea pasta as observed in Table 3.3. The result of the analysis showed a statistically significant difference ($p < 0.05$) in the hardness of uncooked pasta, control and chickpea-enriched pasta. However, upon cooking the pasta, the firmness of the control (760 g) was not statistically significantly different ($p < 0.05$) from the 50% CP_HP. This can be attributed to the low microstructural influence of the mill configuration observed in this pasta sample (Table 3.4). Similarly, the 50% CP_LP and 50% CP_RW showed the least hardness for uncooked pasta, though, upon cooking, a low firmness value was observed for 50% CP_LP (579 g), which was significantly different ($p < 0.05$) from the other chickpea pasta samples and the control. This finding could be due to the increased number of closed pores (Table 3.4) in the 50% CP_LP which led to more internal swelling in the pasta thus influencing the structure. In addition, it can be inferred that the lower the microstructural influence in a pasta, the less influenced its texture attributes would be.

However, the pretreatment of chickpeas and the milling technique need to be considered, as they influence the correlation between the microstructure and texture of the pasta.

3.7. Conclusion

This study showed that chickpea flour produced using different mill types and configurations influences the chemical composition, physical characteristics and microstructure of cereal-based pasta enriched with chickpea flour. X-ray μ-CT provided a visual representation of the pore and solid structure of the pasta samples relative to the influence of the mill type and configurations. The results showed the microstructure of the 50% CP_HP pasta was less affected by the inclusion of chickpea flour compared to the other chickpea samples. Therefore, the authors recommend 50% CP_HP as the most suitable chickpea flour blend to incorporate into a cereal-based product such as pasta to achieve the purpose of enrichment. This attribute was also observed in the texture analysis as the firmness and hardness of the 50% CP_HP pasta correspond to the least porosity observed in the microstructure. Conclusively, the study provides valuable information for the milling and baking industry on the choice of milling technique and configuration required to achieve a good quality chickpea flour that maintains the microstructure of enriched products even at high substitution levels.

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CHAPTER 4

Effect of pretreatment methods of chickpea on microstructure and physical properties of enriched bread

This chapter is under review in the journal "*Food Structure*" entitled 'Effect of pretreatment methods of chickpea on microstructure and physical properties of enriched bread'.

4.1. Highlights

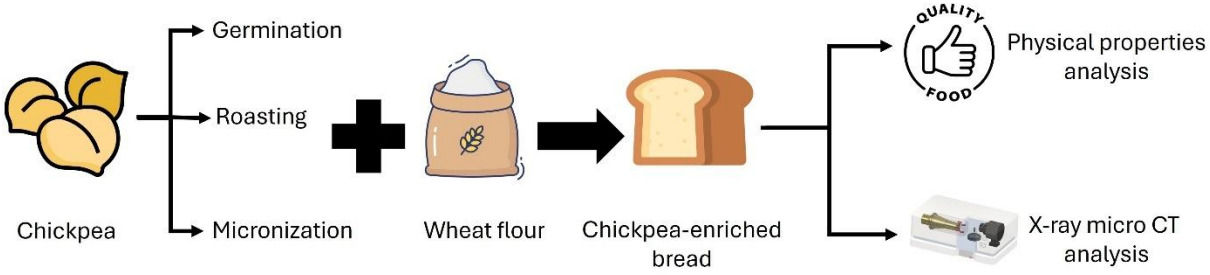
- Chickpea pretreatments impact the quality of chickpea-enriched bread.
- Germination enhances key microstructural traits in chickpea-enriched bread.
- Microstructure correlates with physical attributes in chickpea-enriched bread.

4.2. Abstract

This study evaluates the effect of chickpea pretreatment methods on the physical and microstructural properties of chickpea-enriched bread, aiming to identify the most effective treatment for optimizing bread quality. Five bread formulations were analyzed: a control (100% wheat flour), a sample with 20% untreated chickpea flour, and three samples containing 20% chickpea flour derived from germinated, roasted, or micronized chickpeas. The physical properties of the breads were measured using standard methods, and the microstructure was analyzed using X-ray microcomputed tomography. The inclusion of pretreated chickpea flours increased bread weight and reduced specific volume compared to the control; however, these differences were not statistically significant relative to untreated samples. Microstructural analysis of breads made with flour from roasted and germinated chickpeas depicted increased open pores, greater uniformity in porosity distribution, thinner crumb walls, and a well-defined, interconnected 3D structure. An expert panel's scoring evaluation confirmed that bread made with flour from germinated chickpeas retained quality scores comparable to untreated ones. In conclusion, germination is a promising pretreatment that can be used on chickpeas to enhance bread quality, ensuring physical integrity

while enhancing microstructural properties. This study offers valuable insights to advance the development of nutritionally enriched bread by incorporating pulse flour.

4.3. Graphical abstract



Keywords: X-ray microcomputed tomography, chickpeas, germination, roasting, micronization, chickpea-enriched bread, bread microstructure

4.4. Introduction

Worldwide, bread is part of a staple diet that can be enriched with nutritionally-dense constituents to provide enhanced health benefits (Collar Concha, 2015; W.H.O, 2019). Opportunities exist to create value-added bread products by including nutritionally dense ingredients such as pulse flour to improve the overall nutritional profile of bread from wheat flour (Boukid et al., 2018; Papanikolaou et al., 2020). In 2023, retail sales of packaged foods in Canada totalled CAD$77.6 billion, with baked goods ranking among the top categories (CAD$11.2 billion), driven largely by consumer demand for natural, functional, and plant-based high-protein products (Government of Canada, 2024). The rising consumer demand for nutritious foods supports supplementing wheat flour with plant-based proteins in bread making (Nhouchi et al., 2018). This is because the addition of lysine-rich pulse proteins helps enhance the amino acid profiles of wheat (Byanju and Lamsal, 2023; Olakanmi et al., 2022).

Among pulses, chickpeas (*Cicer arietinum* L.) are nutritionally rich, containing high levels of protein (22.4%), carbohydrates (57.8%), dietary fibre (10.8%), and fats (6.7%), along with essential B-vitamins and minerals (USDA, 2019). Despite its nutritional benefits, chickpea consumption remains low in Western countries, including Canada (Baik & Han, 2012; Priyadarshini et al., 2020). This underutilization of pulses, including chickpeas, is primarily attributed to anti-nutritional factors (ANFs), which reduce nutrient bioavailability and affect sensory properties (Byanju & Lamsal, 2023). Various pretreatment methods, including germination (Atudorei et al., 2021), micronization (Frohlich et al., 2019), and roasting (Kotsiou et al., 2021), have been explored to reduce ANFs before integrating pulses into bakery products. These pretreatments fall in the category of minimal processing for producing modified ingredients with enhanced properties. Micronization and roasting utilize infrared radiation and dry heat, respectively, to degrade heat-labile ANFs, such as phytic acid, while also improving sensory characteristics. Conversely, germination activates enzymatic processes that break down macronutrients, improving nutrient release and reducing ANFs like oligosaccharides, tannins, and phytic acid (Kaur & Prasad, 2021). Selecting an appropriate pretreatment is crucial for optimizing the functional properties of chickpea flour when blended with wheat flour in a bread application; however, the impact on its microstructural compatibility with wheat flour in bread formulation remains underexplored (Priyadarshini et al., 2020; Sivakumar et al., 2023; Thakur et al., 2019; Wood & Malcolmson, 2020).

Microstructural features, including porosity, cell size and distribution, cell wall thickness, and volume, can influence the textural, physical and sensory characteristics of bread (Ali et al., 2021; Rathnayake et al., 2018). Numerous studies have established a strong link between bread functionality and its cellular microstructure (Besbes et al., 2013; Cafarelli et al., 2014; Scanlon

and Zghal, 2001). Thus, different techniques such as scanning electron microscope (SEM), X-ray micro-computed tomography (μCT), light microscopy, and magnetic resonance imaging (MRI) have been used to characterize the microstructure of bread (Ishida et al., 2001; Kahraman et al., 2022; Olakanmi et al., 2024). Among these techniques, μCT provides a non-invasive and non-destructive approach for the quantitative characterization of the internal structure of bread, eliminating any drawbacks (such as sample preparation) common in microscopy and MRI techniques (Lassoued et al., 2007; Rathnayake et al., 2018; Olakanmi et al., 2023). While μCT has been applied to study the internal structure of enriched baked and extruded products (Van Dyck et al., 2014; Li et al., 2022; Olakanmi et al., 2024), no existing study has investigated the effect of germination, micronization, or roasting of chickpeas on the microstructural features of bread made with pretreated chickpea flour using μCT.

The present study examines the physical and microstructural characteristics of five bread samples, including a control (100% wheat flour), 20% untreated chickpea flour, and three 20% pretreated chickpea (germinated, micronized or roasted) flours. Further analysis establishes relationships between the physical properties of chickpea-enriched bread and μCT-derived microstructural parameters. The outcome of this study will provide the ingredient manufacturers and the food industry with the information needed to enhance the utilization of pulses as nutritionally-dense flour.

4.5. Materials and Methods

4.5.1. Raw materials and pretreatment methods

Kabuli chickpeas (CDC Orion; 2020 crop year) were sourced from McDougall Acres Ltd. (Moose Jaw, SK). Flour milled from No. 1 Canada Western Red Spring (CWRS) wheat was used for blending and as the control.

4.5.1.1. Germination

Chickpeas were germinated following the method described by Setia et al. (2019), in which 5 kg of chickpeas were rinsed in tap water and soaked in 0.07% sodium hypochlorite for 30 min. Upon bleaching, the seeds were rinsed and placed into a large container to soak for 24 h. After soaking, the water was drained, and the seeds were spread on screens covered with paper towels. The screens were placed on a rack and stored in the dark at room temperature for 48 h. After 24 h, the seeds were sprayed with water to maintain moisture. Once germinated, the seeds were placed on the screens and dried at 75°C for 15 min. After 15 min, the temperature was reduced to 55°C to complete the drying of germinated seeds (8-12 h) until moisture content dropped below 10%. The dried seeds were then stored in plastic bags at room temperature until milling.

4.5.1.2. Roasting

Whole chickpeas (5 kg) were tempered to a moisture content of 30%. Water was added gradually in 30 to 60 min intervals, ensuring thorough mixing for uniform absorption. The seeds were tempered overnight (16–18 h) in a sealed container, then roasted for 30 min at 160°C using a Picard reel oven (Model RE-8-24, Drummondville, QC, Canada). The roasted chickpeas were stored in plastic bags at room temperature until milling.

4.5.1.3. Micronization

Micronization of chickpeas was carried out at InfraReady Products (Saskatoon, SK, Canada) using a standardized procedure. Initially, chickpeas (5 kg) were tempered to a moisture content of 20%, following the AACC Official Method 26-95.01 (AACC International, 2010) for tempering. The tempered chickpeas were then subjected to infrared heating at a temperature of 140°C using a micronizer (Model A 156379-B0, FMC Syntron® Bulk Handling Equipment, Homer City, PA, USA). The micronizer setup included a heat-generating unit with two sets of three ceramic tiles

(Model Type R 1603-2 pat, Rinnai Corporation, Nagoya, Aichi Prefecture, Japan), a Syntron feeder (Model F010, Riley Automatic Ltd., Sinfin Ln, Derby, England) to regulate the flow rate and volume of chickpeas, and a Syntron magnetic feeder (Model BF2 A, FMC Corporation, Homer City, PA, USA) that transported the chickpeas through the heating zone. The burners were positioned 19 cm above a 152 cm long conveyor to ensure uniform heating of the chickpeas. Throughout the process, the temperature at the surface of the chickpeas was monitored using a handheld infrared thermometer (Model R2300-NIST, Oakton, Vernon Hills, IL, USA). After micronization, the treated chickpeas were stored in plastic bags at room temperature until milling.

4.5.2. Chickpea milling

Straight grade (SG) flour was obtained as described in Chapter 3, section 3.5.1, for all chickpea samples.

4.5.3. Protein analysis of pretreated chickpea flour

As illustrated in Chapter 3, section 3.5.2, a conversion factor of N × 6.25 was used to calculate the crude protein value, where N represents the nitrogen content.

4.5.4. Dough preparation and bread baking

The bread samples for the study were baked in the pilot bakery at Cereals Canada, Winnipeg, utilizing a no-time dough baking process. The flour blends used were 100% wheat flour (control) and 20% chickpea flour with 80% wheat flour for the chickpea-enriched breads designated as UT, GR, MN, and RR. The control and chickpea bread samples were formulated (expressed in baker's percentage) using 4% fresh yeast, 1.3% salt, 4% sugar, 4% vegetable shortening, 2% milk powder, and 2% dough conditioner (Puratos, Mississauga, ON, Canada). Gluten at 2% was added only to the chickpea-based bread, with the amount determined from preliminary trials. All ingredients were placed in a spiral mixer (Model SR120, Erka 120 Quart, Robert Köhler GmbH & Co,

Winnenden, Germany) with chickpea flour dough pre-mixed on slow speed for 4 min while the wheat control was pre-mixed for 2 min and then kneaded until the doughs were fully developed. Farinograph absorption was used for initial water addition, with optimum absorption assessed at the mixer to ensure a soft dough. The dough was left to rest on a bench for 10 mins, then scaled into 640 g pieces, rounded by hand, and rested again for 10 mins. The dough pieces were moulded into loaves using a B & B moulder (Oliver Packaging & Equipment Co., Walker, MI, USA), placed into baking pans (length 25.5 cm × width 12.7 cm × depth 8.5 cm), and proofed in a controlled chamber (85% relative humidity; 37°C) to a target height of 120 mm (measurement taken at the centre of the pan using a proof height gauge). Baking was done using a Picard reel oven at 200°C for 25 min, and each sample was prepared in duplicate. All samples were prepared using a standardized baking procedure, ensuring that the type of chickpea flour remained the only experimental variable.

4.5.5. Bread quality test

The specific volume of the bread loaves was measured after cooling according to AACC method 10-14.01 (AACC International, 2010) using the TexVol bread volume meter (Model BVM—L370, Perten Instruments, Shelton, CT, USA) equipped with VolCalc software (Perten VolCalc BVM L370, Shelton, CT, USA). A commercial bread slicer (Model 732-N, Oliver Machinery, Grand Rapids, MI, USA) was used to slice the bread loaves, and selected slices were used to measure C-Cell properties and crumb colour.

Two experienced bakers performed bread scoring on a loaf of bread that had been cut in half cross-wise. A consensus judgment was reached prior to the bread scoring, with the breads made with the flours from pretreated chickpeas scored relative to the UT bread, which received the highest score for each parameter. The breads were evaluated for external characteristics (maximum score of 40)

and comprised of the following parameters: (i) symmetry - a high score indicating greater symmetry, (ii) crust character - a high score indicating an even and smooth crust, (iii) crust colour - a high score showing a homogeneous light brown colour, and (iv) break and shred - a high score indicating a smooth and large break and shred. The breads were also scored for internal characteristics (maximum score of 60) comprised of the following parameters: (i) crumb colour - a high score indicating a brighter crumb, (ii) crumb structure - a high score indicating a fine crumb, (iii) cell wall thickness - a high score indicating thin cell walls, (iv) cell size - a high score indicating cells that are neither overly large nor small, (v) cell shape - a high score indicating cells that are elongated as opposed to round, and (vi) cell distribution - a high score indicating an even distribution of cells. The scoring system allowed for a comparative evaluation of bread containing pretreated chickpea blends against those with untreated blends, highlighting the influence of the flour blends on various visual and textural characteristics of the bread.

The C-Cell (Model CC.400, Colour system, Calibre Control International Ltd., Warrington, UK) imaging was used to assess the breadcrumb characteristics using three slices from the centre of the loaf. The following parameters were measured according to AACC International Method 10-18.01: number of cells/slice area (higher values indicate finer cell structure), cell contrast (higher values reflect more uniform cells), cell wall thickness (lower values indicate thinner walls), and cell diameter (higher values represent coarser, more open structures).

Bread crumb colour was measured using the Minolta Chroma Metre CR-410 (Model CR-410, Konica Minolta Ltd, Mississauga, ON, Canada) with a D65 illuminant at a 2° standard observer angle. The CIE L^*, a^*, b^* colour measurements were taken in triplicate in the centre of two stacked slices of bread from the centre of the loaf, and the colour change difference (ΔE) was determined

similar to Chapter 3, section 3.5.5. The sliced breads were placed in double-layered polyethylene bags and kept frozen until further analysis.

4.5.6. Bread microstructure

4.5.6.1. Image acquisition

The structure of the breadcrumb was observed as described by Olakanmi et al. (2024) and Wang et al. (2011), with some modifications. Frozen samples were removed from the freezer, and a representative section from three regions (top, center, and bottom) was cut to dimensions of 65 mm × 20 mm. These sections were imaged as described in Chapter 3, section 3.5.6, with the source current, voltage, and scan set at 50 kV, 140 µA and 30 µm, respectively, to ensure good image quality (Reiter et al., 2014). Six slice replicates of each bread type were scanned, resulting in a total of 90 scans, generating 1,056 2D images.

4.5.6.2. Image processing

A rectangular region of interest (ROI) with dimensions of 307 µm in width and 9210 µm in height was extracted from the 2D images, avoiding the borders to eliminate any edge effects from the cutting process. This process was similar to what is described in Chapter 3, section 3.5.6.1. Since thresholding is a less significant source of error in grayscale image processing (Wang et al., 2011), it was performed using the Otsu algorithm (Otsu, 1979), with lower and upper gray threshold values set at 37 and 255, respectively. The 3D microstructural parameters used to characterize the internal structure of breadcrumb include percentage porosity (open/closed/total), volume of closed pores (V.cl), number of closed pores (N.cl), crumb thickness (C.Th), object surface/volume ratio (Obj.S/Obj.V), object surface (Obj.S), object volume (Obj.V), and Euler number (Eu.N). Percentage porosity (open/closed/total) is the number of voxels designated as pores divided by the volume of interest (VOI, which was 1.18×10^{12} for all the analyzed samples). The volume and

number of closed pores are the percentage and amount of closed porosity within the total VOI. The crumb thickness of the bread refers to the average thickness of the cell spaces, determined from the average cell walls within the VOI. The object surface/volume ratio is the surface area of the sample structure present in the total VOI. The object volume and object surface from an individual binarized 3D object are the total volume or surface within the VOI. The Euler number of bread shows the connectedness of the 3D structure. A lower value indicates a better-connected structure, while a higher value indicates a poorly connected internal structure (Gondek et al., 2013).

4.5.7. Statistical analysis

Statistical analysis was conducted using OriginPro software (version 2024b, OriginLab Corporation, Northampton, MA, USA). A one-way analysis of variance (ANOVA) was performed to determine significance, with differences among samples assessed using Tukey's post-hoc test at a significance level of $p \leq 0.05$. RStudio version 2023.121+402 was used to perform the degree of variation plot using the lattice package.

Principal component analysis (PCA) was performed using the PLS Toolbox software (version 8.7.1) compatible with MATLAB (version 9.6, R2019a, MathWorks Inc., Natick, MA, USA) to explore the multivariate relationships among all measurements. As the physical, colour and C-Cell measurements were done on different samples to those used for the microstructural analysis, a dataset was created in which the breadcrumb's microstructural features at each region (top, centre, bottom) were used to obtain a single data point. This was done for three replicates of the bread types. Data were normalized before dimensional reduction into a set of statistically uncorrelated orthogonal variables called principal components (PCs).

4.6. Results and Discussion

4.6.1. Protein content of pretreated chickpea flour

The results of the protein content in the chickpea flours are presented in Fig 4.1. It can be observed that, upon germination, the protein content of the chickpeas increased significantly ($p < 0.05$) compared to the other chickpea flours. Similar results were observed in previous studies for germinated black chickpea flour and pigmented desi chickpea (Domínguez-Arispuro et al., 2018; Yaver, 2022). Atudorei and Codina (2020) attributed the increase in the protein content observed after chickpea germination to the loss of dry matter, such as carbohydrates, during seed respiration in the germination process. The MN chickpea flour also showed increased protein content, which was statistically significantly different ($p < 0.05$) from the RR and UT flour samples. However, a previous study on 20% tempered peas micronized at 140°C observed no significant difference in the protein content of untreated and micronized peas (Frohlich et al., 2019). The increase observed in this study for MN samples could be attributed to the pulse type studied. There was no statistical difference ($p < 0.05$) in the protein content of RR compared to UT chickpea flour, as shown in Fig 4.1. Therefore, it can be concluded that thermal treatment does not significantly increase the protein content in chickpeas.

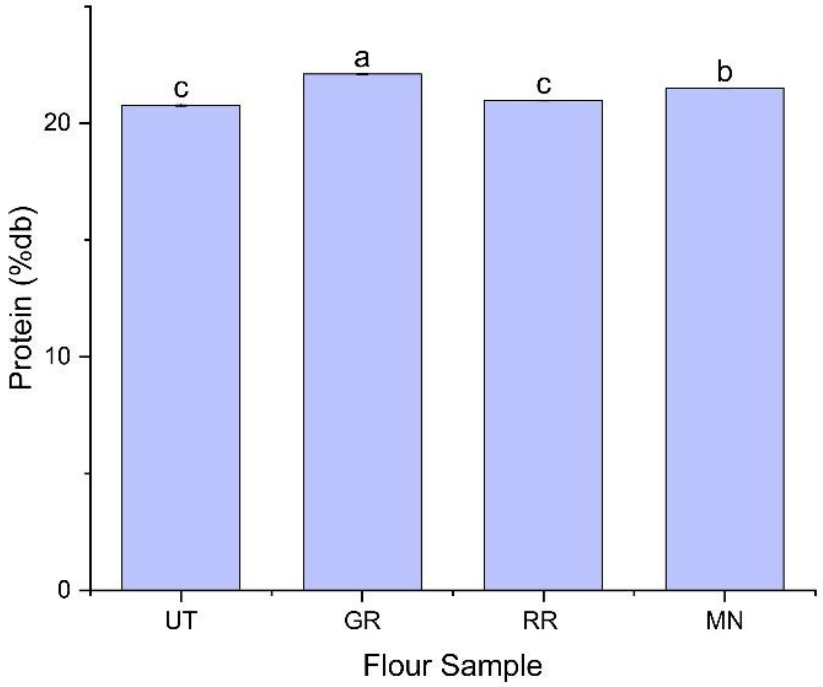


Fig 4.1. Protein content of chickpea flours. (UT- untreated, GR- germinated, RR- roasted, MN- micronized)

4.6.2. Bread physical characteristics and colour attributes

The physical characteristics of bread made with UT and pretreated chickpea flour are presented in Table 4.1. It can be observed that the weight of the chickpea-enriched bread samples were statistically significantly different ($p < 0.05$) from the control bread. An increase in the weight of chickpea-enriched bread may be attributed to increased fibre content, as fibre has a greater water-holding capacity and low baking loss rate (Mohammed et al., 2012; Yaver, 2022). A comparison between the pretreated and UT chickpea breads showed no statistical difference ($p < 0.05$), revealing that the pretreatment of chickpeas does not impact the loaf weight of bread.

The pretreatment methods influenced the specific volume of the chickpea-enriched bread, with a greater impact observed in the RR and MN bread samples, as presented in Table 4.1. The GR sample exhibited a specific volume comparable to the control but significantly lower ($p < 0.05$)

than the UT sample. The largest specific volume was observed in the UT sample, which was statistically different ($p < 0.05$) than the pretreated chickpea samples except for the control sample. The decrease in specific volume observed in the pretreated chickpea-enriched breads could be due to the pretreatment of chickpeas before bread making. Atudorei et al. (2022) reported a similar reduction in the specific volume of wheat enriched with 20% germinated chickpea in bread making. The authors attributed this decrease to the presence of enzymes, such as proteases activated during seed sprouting, that could disrupt the protein network in the dough, thus inhibiting the dough's ability to enclose air. Young et al. (2020) also observed a reduction in the specific volume of bread enriched with roasted yellow pea flour. However, given the lowest specific volume observed in the MN bread, it can be concluded that the micronization method significantly impacts the bread volume.

Colour influences consumers' perceptions of most baked products, including bread. The bread made with MN chickpea flour had lower L^* (brightness) and higher a^* (redness) and b^* (yellowness) values than the RR, GR and UT bread types, as shown in Table 4.1. Since the baking process was consistent across all samples, the variation in the colour parameters may be attributed to the pretreatment method of the chickpeas before its flour was combined with wheat flour. The darker crumb colour in MN bread could be because infrared heat increased the availability of reducing sugars in the chickpea, thus forming caramelization and the Maillard reaction during the baking process (Xing et al., 2021). A previous study on micronized yellow peas used in breadmaking observed darker crumb for all the micronized samples with increasing infrared temperature (Frohlich et al., 2019).

The L^* value of the RR bread sample was also significantly lower ($p < 0.05$) than the GR and UT bread. The RR sample a^* value was similar to that of the UT and higher than the GR sample. A

comparison of the $b*$ values showed no difference between the RR and MN samples, but they were lower in the GR and UT breads indicating less yellowness. The $L*$ value of the control bread was similar to the GR and UT breads. However, it was statistically significantly higher ($p < 0.05$) than the MN and RR breads. The control bread appeared to have less redness and yellowness than the chickpea-enriched bread samples. However, consumers are not negatively affected by red or yellow shades in bread, as these resemble familiar ingredients like red wheat or corn (Kotsiou et al., 2021). Despite the noticeable shift in yellow colour in pan bread enriched with thermally treated yellow split peas, consumer acceptability remained unaffected (Fahmi et al., 2019). When comparing the total colour difference (ΔE) between the chickpea-enriched bread samples and the control, the GR bread showed the least colour variation from the control bread (Table 4.1).

Table 4.1. Processing properties, physical characteristics and crumb colour of bread samples

Bread type	Gluten (%)	Processing properties			Physical characteristics		Crumb colour			
		Baking absorption (%)	Mixing time (min)	Proof time (min)	Bread weight (g)	Specific volume (cm^3/g)	L^*	a^*	b^*	ΔE
Control	-	70	5.7	65	548.4 ± 2.13^{b}	7.23 ± 0.20^{ab}	81.1 ± 0.1^{a}	-0.62 ± 0.02^{d}	14.53 ± 0.11^{d}	-
UT	2	59	7.5	72	562.1 ± 0.41^{a}	7.40 ± 0.06^{a}	81.9 ± 0.1^{a}	-0.26 ± 0.02^{c}	17.39 ± 0.08^{b}	2.99
GR	2	62	7.5	72	557.5 ± 3.01^{a}	6.87 ± 0.07^{b}	81.6 ± 0.1^{a}	0.08 ± 0.02^{b}	16.16 ± 0.09^{c}	1.84
RR	2	64	7.7	72	555.0 ± 1.77^{ab}	6.10 ± 0.00^{c}	79.0 ± 0.3^{b}	-0.21 ± 0.05^{c}	21.26 ± 0.23^{a}	7.05
MN	2	73	7.9	72	558.7 ± 0.91^{a}	5.30 ± 0.12^{d}	77.1 ± 0.3^{c}	0.44 ± 0.07^{a}	20.89 ± 0.02^{a}	7.59

Control (100% wheat), UT (20% untreated), GR (20% germinated), MN (20% micronized), and RR (20% roasted) chickpea bread. The data presented are mean ± standard error of the mean, values within the same column and have the same superscript are not significantly different ($p < 0.05$).

Table 4.2. Bread scoring characteristics of chickpea-enriched bread samples

Bread type	External characteristics (40)				Internal characteristics (60)					
	Symmetry (/10)	Crust character (/10)	Crust colour (/10)	Break & Shred (/10)	Crumb colour (/20)	Crumb structure (/20)	Cell wall thickness (/5)	Cell size (/5)	Cell shape (/5)	Cell distribution (/5)
UT	10	10	10	10	20	20	5	5	5	5
GR	10	10	11	9	22	20	5	5	5	5
RR	10	10	12	6	12	18	5	5	4	5
MN	6	7	10	4	11	13	3	3	3	5

UT (20% untreated), GR (20% germinated), MN (20% micronized), and RR (20% roasted) chickpea bread.

4.6.3. Bread scoring

The pretreatment of chickpeas prior to milling influenced the external and internal quality indicators of the bread samples, as presented in Table 4.2. In terms of external quality, the GR and RR breads scored higher than the UT bread, particularly in crust colour. In contrast, the MN bread had a significantly lower score for symmetry and crust quality. Regarding the internal characteristics, the GR bread scored higher in crumb colour when compared to the untreated bread. However, all the pretreated chickpea-enriched bread samples had a lower break and shred quality score. Overall, the GR bread outscored the other pretreated bread samples and had improved crust and crumb colour scores than the UT bread.

4.6.4. C-Cell analysis

The results from the C-Cell characteristics of the control and chickpea-enriched breads are presented in Table 4.3. The breads made with pretreated chickpea flours exhibited high cell contrast, except for the MN bread, which had lower cell contrast. When compared to the control bread, the chickpea-enriched breads had no significant difference ($p < 0.05$) in cell contrast except for the GR bread, which had a value significantly higher ($p < 0.05$). The crumb cell contrast strongly influences the mechanical properties of bread, with higher contrast associated with finer crumb structure (Rathnayake et al., 2018). All the chickpea-enriched breads had a higher number of cells/slice area than the control, which indicates a finer bread structure. This increase in the number of cells per unit area suggests a more aerated and porous bread structure. However, the values were not statistically significantly different ($p < 0.05$) compared to the control bread. The bread made with GR had the lowest cell wall thickness (0.47 mm) and diameter (2.10 mm), which was similar to the RR and UT samples but statistically significantly different ($p < 0.05$) from the MN and control samples. The MN bread had C-Cell parameter values similar to those of the control

bread, indicating that the bread was within the acceptable range. These results align with those obtained in a previous study by Frohlich et al. (2019) for micronized yellow pea flour used in bread making. The authors observed consistent C-Cell values between the control bread (100% wheat) and bread enriched with yellow peas micronized at 140°C. The fine and uniform cell structure within the cell wall observed in the GR and RR bread samples indicates a more elastic and tender bread texture, with these properties impacting the bread's overall mouthfeel (Hager and Arendt, 2013; Wang et al., 2017).

Table 4.3. C-Cell characteristics of bread samples

Bread type	Gluten (%)	Cell contrast	No. of cells/Slice area (cells/mm^2)	Cell wall thickness (mm)	Cell diameter (mm)
Control	-	0.74 ± 0.01^{a}	0.47 ± 0.01^{a}	0.493 ± 0.003^{ab}	2.45 ± 0.06^{a}
UT	2	0.76 ± 0.00^{a}	0.51 ± 0.01^{a}	0.473 ± 0.003^{bc}	2.15 ± 0.01^{bc}
GR	2	0.76 ± 0.00^{a}	0.52 ± 0.00^{a}	0.470 ± 0.000^{c}	2.10 ± 0.01^{c}
RR	2	0.75 ± 0.01^{ab}	0.49 ± 0.02^{a}	0.483 ± 0.007^{abc}	2.26 ± 0.07^{abc}
MN	2	0.73 ± 0.00^{c}	0.48 ± 0.01^{a}	0.500 ± 0.006^{a}	2.36 ± 0.04^{ab}

Control (100% wheat), UT (20% untreated), GR (20% germinated), MN (20% micronized), and RR (20% roasted) chickpea bread. The data presented are mean ± standard error of the mean, values in the same column and have the same superscript are not statistically different.

4.6.5. Bread microstructure

The total porosity of crumbs, reflecting open and closed cells, varies across the regions of a bread slice (Besbes et al., 2013; Van Dyck et al., 2014; Wang et al., 2011). To understand the degree of variation at each crumb region, a box and whisker plot was employed to establish the variation at the 25th and 75th percentile ranges (Fig 4.2). The GR and RR bread samples showed less variation in the total porosity at the top, centre and bottom crumb regions. This shows a consistent porosity within a bread loaf made with GR or RR chickpeas. In contrast, MN and UT bread showed a high variation in total porosity at the bottom crumb region, indicating inconsistent porosity in the bread loaf. A similar degree of variance was observed for the bottom crumb region compared to the

control bread. Therefore, it can be concluded that chickpea-enriched breads made with GR or RR chickpea flour can maintain the porosity of the loaf.

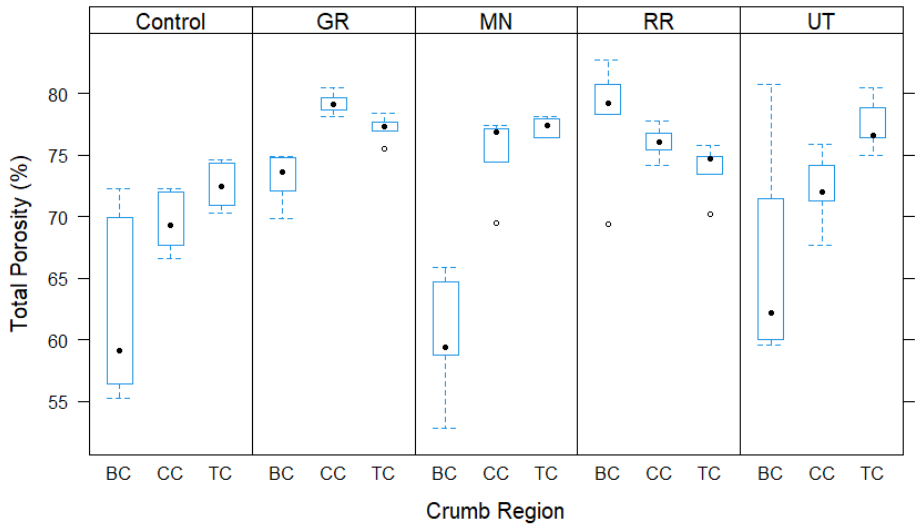


Fig 4.2. Degree of variation in total porosity at different crumb regions for 100% wheat bread (control), and bread enriched with 20% each of chickpea untreated (UT), germinated (GR), micronized (MN), and roasted (RR) flour. BC- bottom crumb, CC- centre crumb, TC- top crumb.

To compare the microstructure of the bread samples, the central VOI was analyzed for 3D morphological parameters (Fig 4.3), owing to consumer interest in the central region of bread. The amounts of open and closed porosity in a foamed food structure such as bread crumb are of interest, with the latter greatly influencing its mechanical properties (Sozer et al., 2011; Wang et al., 2011). Previous studies have shown that the development of the porous crumb structure in bread depends on the dough ingredients, processing conditions and gas bubble formation (Bajd and Serša, 2011; Scanlon and Zghal, 2001). Adding chickpea flour to wheat bread improved the open porosity, as seen in Fig 3a. A higher increase was observed in GR bread, which was significantly different ($p < 0.05$) than the other chickpea-enriched bread samples except for the RR. This increased open porosity in GR bread could be attributed to the presence of enzymes such as amylase that increased

starch breakdown and subsequently, enhanced the release of gases by yeast in the bread (Atudorei et al., 2022; Perri et al., 2021).

On the other hand, thermal treatment of pulses enhances the dough's handling ability and CO_2 retention, resulting in more open voids during baking (Hoque et al., 2022; Kadharmestan et al., 1998). This may explain the observed open porosity in the RR bread samples. Kahraman et al. (2022) noted a higher crumb porosity in bread made from a blend of roasted chickpea and rice flour. High open porosity is associated with an increased specific volume (Table 4.1), a characteristic seen in previous studies (Wang et al., 2011). The percentage of open porosity in MN bread was not statistically different ($p < 0.05$) from the UT bread sample. In terms of closed porosity, GR bread exhibited the lowest percentage among the pretreated samples, as shown in Fig 4.3b. The percentage of closed porosity was statistically significantly lower ($p < 0.05$) than that of MN and UT bread samples, except for the RR bread. No significant difference ($p < 0.05$) was observed in the closed porosity percentage of the MN and UT samples compared to the control bread. The higher closed porosity of the MN bread can be attributed to a greater likelihood of air cell coalescence in the dough. However, previous studies have established that structures with more open pores are considered softer than those with a higher proportion of closed pores (Sozer et al., 2011). According to research by Wang et al. (2011), the hardness of a bread sample increases as the density of closed pores increases.

Fig 4.3c illustrates the crumb thickness as recorded by the µ-CT analysis. A lower value for crumb thickness was observed in the GR bread, which differed significantly ($p < 0.05$) from the other chickpea-enriched breads and the control. This reduced crumb wall thickness could be due to the low starch content in germinated chickpeas, resulting in increased CO_2 release during baking and a thinner wall structure (Scanlon and Zghal, 2001). A lower crumb thickness for RR bread

compared to the MN and UT breads was observed. On the other hand, the MN bread had the highest crumb thickness among the samples studied but was not statistically different ($p < 0.05$) from the UT and control bread. The increase in the bread crumb thickness can be attributed to the micronization of chickpeas, which resulted in a weaker viscoelastic structure and air entrainment during dough kneading. This interference leads to a thicker solid phase for the bread (Van Dyck et al., 2014; Olakanmi et al., 2024).

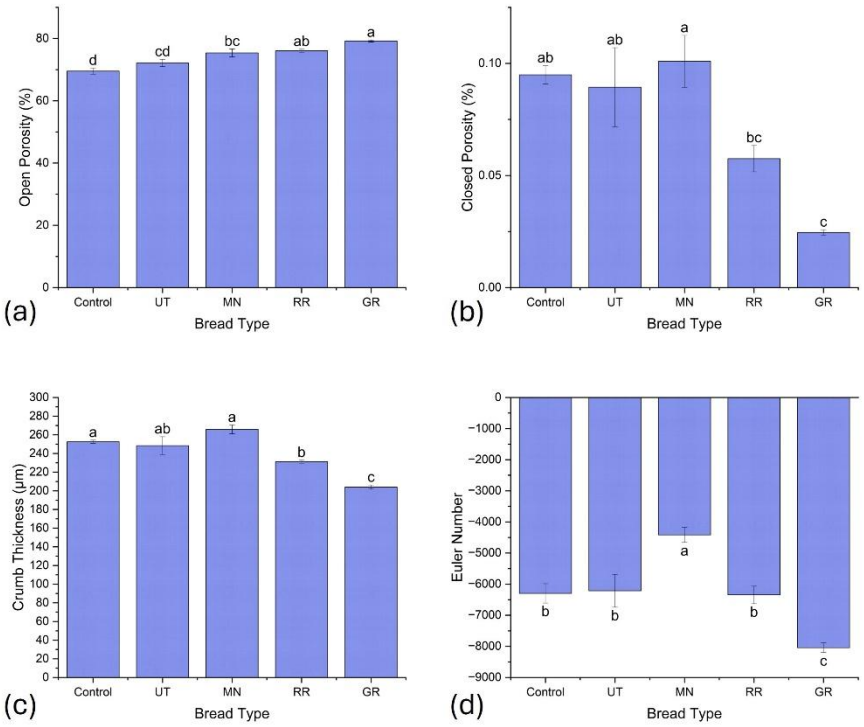


Fig 4.3. Differences in the microstructural parameters of bread samples (a) open porosity, (b) closed porosity, (c) crumb thickness, and (d) Euler number. Control (100% wheat), UT (20% untreated), MN (20% micronized), RR (20% roasted), and GR (20% germinated) chickpea bread.

A properly interconnected 3D structure is desired in bread (Lim and Barigou, 2004; Wang et al., 2011). The Euler value shows the connectedness of the 3D internal structure of the bread studied, with the GR bread sample having the lowest value, as shown in Fig 4.3d. This result entails a better interconnectedness of the internal structure of the bread, which is significantly different ($p < 0.05$) than the other chickpea-enriched breads and the control. The 3D model in Fig 4.4 represents a

section of the crumb solid matrix, pore and combined structure, with the GR bread in Fig 4.4e having more interconnectedness of the pore structure than the other bread samples. This could be attributed to the lower crumb thickness observed in this sample (Fig 4.3c). In contrast, a higher Euler number was observed in the MN bread, as presented in Fig 4.3d. This could indicate cell fragmentation in the bread, leading to less favourable results on the other microstructural parameters studied. This result conforms with a previous study by Gondek et al. (2013) on four bread variates. The authors observed a higher Euler number in the bread variate, which was characterized by an increased closed porosity and structural thickness.

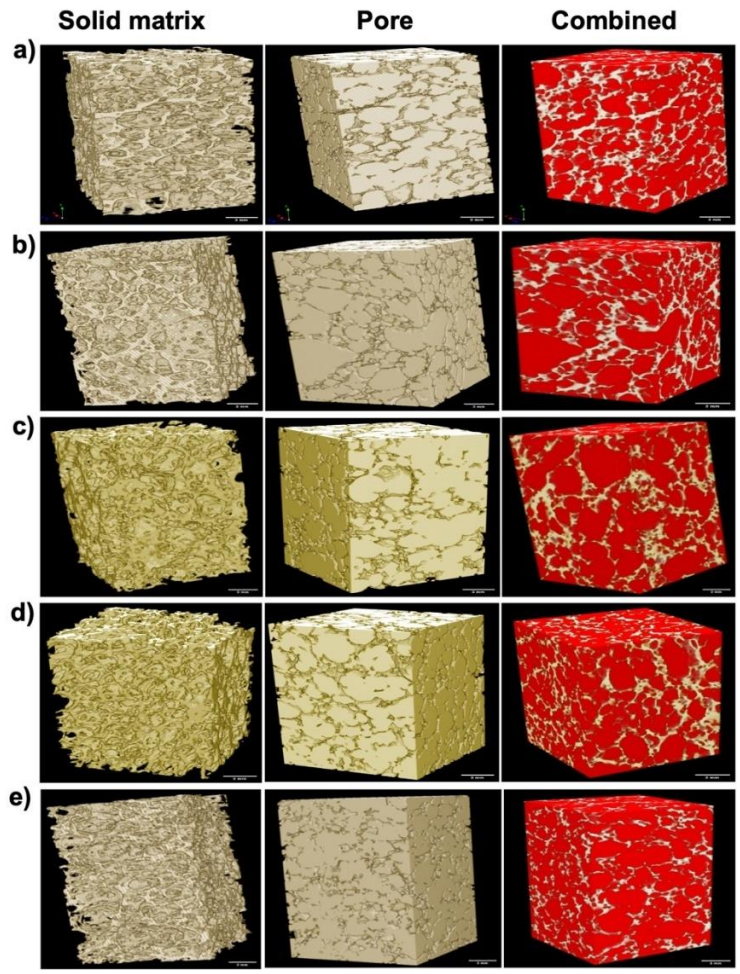


Fig 4.4. 3D model representatives of the solid matrix, pore and combined (i.e., solid and pore (red colour)) structure of bread made with (a) Control (100% wheat) flour, and those enriched with (b) UT (20% untreated), (c) MN (20% micronized), (d) RR (20% roasted), and (e) GR (20% germinated) chickpea flour.

4.6.6. Relationship between microstructure, physical, colour, and C-Cell parameters

This study further investigated whether differences in the bread's physical properties, colour, and C-Cell characteristics are associated with its microstructural features since the intersectionality of these parameters has been least explored (Esteller et al., 2006). Principal component analysis was employed to visualize the relationships among variables (Fig. 4.5), providing insights into how these factors contribute to the observed variations among the bread samples.

The first two PCs explained 80% of the total variance in the data. Bread samples were differentiated as control, UT, GR, RR, and MN samples. PCA plots revealed clear visual distinctions among the bread samples (Fig 4.5). C-Cell parameters, such as cell diameter and wall thickness, correlated strongly with crumb thickness and Euler number (obtained via μ-CT). In contrast, no such association was observed for bread crumb colour. This indicates that the microstructural features of bread are primarily linked to its crumb properties rather than its colour. These findings align with earlier studies (Falcone et al., 2005; Gondek et al., 2013), which also reported that the first PC predominantly represented key microstructural features.

The results further highlighted that the MN bread exhibited higher crumb thickness and Euler number than other chickpea-enriched breads, suggesting a decline in textural properties. This observation is consistent with Gondek et al., (2013), who found that larger Euler numbers and thicker cell walls characterized breads with higher texture. Additionally, the total volume of pores (obtained via μ-CT) was found to relate directly to bread weight, underscoring the role of pore volume as a determinant of bread density and mass.

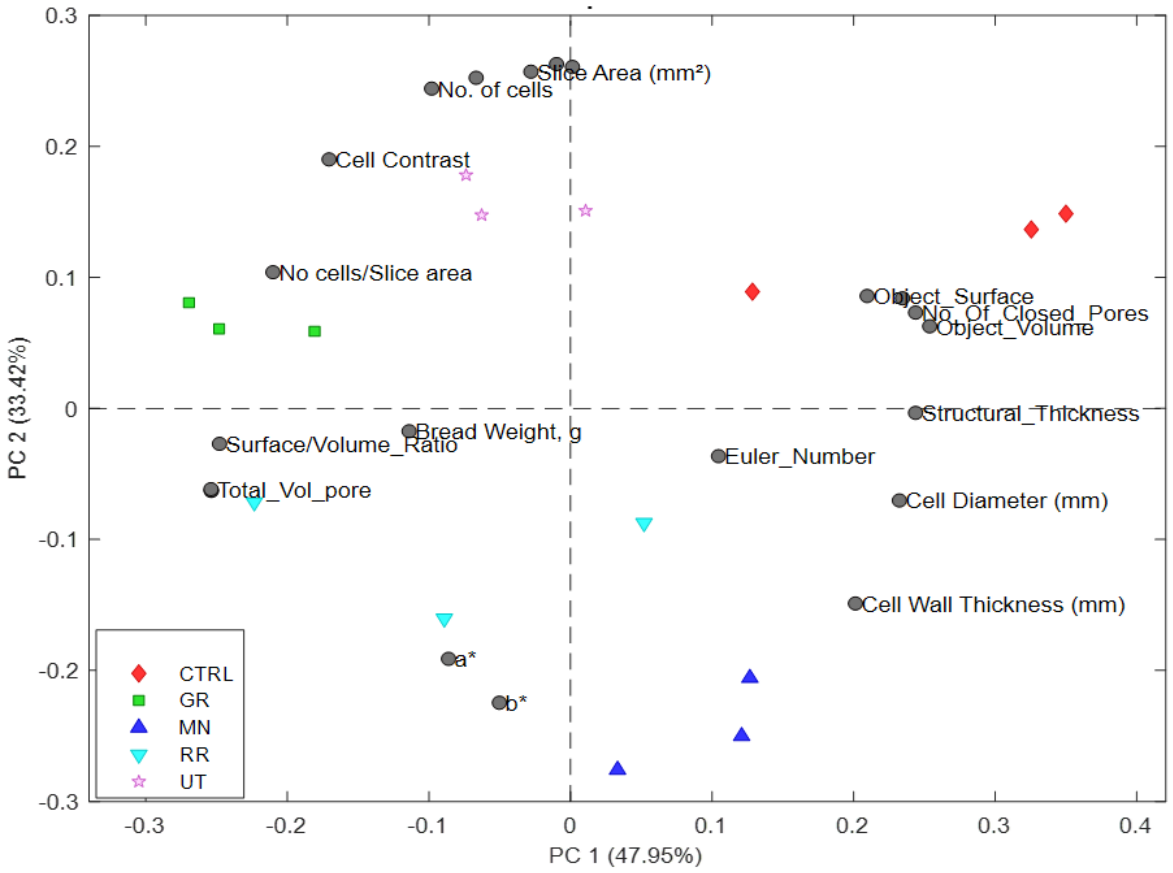


Fig 4.5. PCA biplot of the wheat and chickpea-enriched bread with 80% of the total X-variance explained in the first 2 PCs. CTRL (100 % wheat control), UT (20% untreated), MN (20% micronized), RR (20% roasted), and GR (20% germinated) breads.

4.7. Conclusion

This study investigated the impact of pretreatments on the quality of chickpea-enriched breads. The type of pretreatment imparted to chickpeas influenced key quality attributes, including loaf weight, specific volume, colour, and crumb microstructure of chickpea-enriched bread. Bread made with germinated chickpea flour had a better crumb structure and received higher bread scores. The germinated and roasted chickpea-enriched breads had improved microstructural characteristics compared to the micronized and untreated breads, with their desirable quality attributes surpassing those of the control samples. Overall, the germination of chickpeas resulted in bread with superior quality characteristics compared to bread made with roasted and micronized

chickpea flours. The findings from this study offer valuable insights for the baking industry regarding the quality of nutritionally enriched bread based on different pretreatment options for chickpeas. However, further optimization of germinated chickpeas flour as an ingredient should be explored to develop a more tailored structure-mechanical model for correlating the physical properties of the bread.

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CHAPTER 5

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

5.1 Overall conclusion

X-ray μCT analysis revealed distinct microstructural characteristics among pasta samples made from various chickpea flour blends, including high-protein flour, low-protein flour, reconstituted whole flour, straight-grade flour, and Ferkar-milled flour. Among these, the high-protein flour from the reduction rolls of the roller mill demonstrated superior microstructural stability, reduced cooking loss, and shorter cooking time compared to the 100% wheat control. Although this study focused on a 50:50 chickpea-to-durum wheat semolina substitution ratio, the microstructural parameters such as porosity, structure thickness, and pore connectivity, along with the 3D pasta model, establish a foundation for optimizing chickpea substitution levels in pasta production. These findings indicate that high-protein chickpea flour blends present a promising milling option for pulse flour enrichment in staple foods with minimal microstructural impact.

Beyond milling, this study also investigated the impact of various pretreatment methods on chickpea-wheat microstructural interactions in bread production. Chickpeas were subjected to germination, micronization, and roasting, then milled into straight-grade flour and substituted at a 20:80 ratio (chickpea-to-wheat flour) for bread production. A microstructural evaluation revealed statistically significant differences ($P < 0.05$) in total porosity, open porosity, pore connectivity, and porosity distribution between germinated and roasted chickpea bread samples compared to micronized and untreated chickpea samples. Germinated chickpea bread exhibited improved microstructural characteristics relative to the 100% wheat control. Principal component analysis indicated a correlation between some microstructural attributes and physical properties. These

findings show that germination has the least impact on microstructural mixability while enhancing the nutritional quality of chickpea-enriched bread, making it a promising pretreatment method for pulse-based staple food formulations.

5.2 Future recommendations

- A study combining high-protein flour and germinated chickpea flour should be conducted to develop a single staple product, bread or pasta and establish a relationship between microstructure, functionality and consumer acceptance. The study should additionally consider or establish the cost and energy consumption required to develop these products while comparing them to that of wheat.
- The substitution ratio of the high-protein flour blend with durum wheat semolina should be optimized to produce pasta, and its microstructural stability should be studied to identify a suitable blending ratio.

5.3 Authors' contributions

Chapter 3: The content of this chapter has been published in a peer-reviewed journal with the details below:

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– **Obasi Ukpai Ukoji** (*Thesis author*): Investigation [Lead], Methodology [Lead], Software [Lead], Formal analysis [Lead], Data curation [Lead], Writing – original draft [Lead], Writing – review & editing [Equal].

− **Muhammad Mudassir Arif Chaudhry**: Methodology [Supporting], Validation [Equal], Writing – review & editing [Equal].

− **Lindsey Boyd**: Methodology [Supporting], Resources [Supporting], Writing – review & editing [Equal].

− **Elaine Sopiwnyk:** Methodology [Supporting], Resources [Supporting], Writing – review & editing [Equal].

− **Jitendra Paliwal:** Project administration [Supporting], Conceptualization [Lead], Methodology [Supporting], Funding acquisition [Lead], Supervision [Lead], Resources [Lead], Validation [Equal], Writing – review & editing [Equal].

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− **Lindsey Boyd**: Methodology [Supporting], Resources [Supporting], Writing – review & editing [Equal].

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- **Jitendra Paliwal:** Project administration [Supporting], Conceptualization [Lead], Methodology [Supporting], Funding acquisition [Lead], Supervision [Lead], Resources [Lead], Validation [Equal], Writing – review & editing [Equal].