Faba Bean: A New Zealand Study on an Underutilized Plant-Based Protein Source

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Abstract

Faba bean (*Vicia faba*) is a high protein legume crop that is highly underutilized in most parts of the world, including New Zealand. The objective of this research was to investigate the varieties of faba beans that are native to New Zealand based on their physical and micro-structural features and how they affect cooking properties of the beans. A readily available commercial variety was used for extraction of protein concentrates and an optimum extraction method was researched in this study.

The physical and micro-structural characteristics of the four faba bean native varieties (Early Long Pod, Evergreen, Coles Dwarf and Janet) and their relationship with cooking time were studied. The Janet variety has the highest sphericity, equivalent diameter, thousand kernel weight, seed volume and surface area. The Evergreen variety was the most distinguishable in colour as it presented green hues in comparison to the others which had comparable brown tones. The beans were subjected to boiling and the time taken for appropriate tenderness of the bean was investigated. The Janet variety took the longest cooking time of around an hour and 15 minutes and the least for Evergreen variety. Similarly, beans which had been subjected to prior overnight soaking showed significant decrease in cooking time to around 35 minutes. These results were in accordance with microstructure results which showed that raw beans of the Janet variety had the thickest cotyledon cell wall and the highest degree of surface irregularities suggesting slower water absorption in the seed. Scanning electron microscope showed the presence of large spherical starch globules in a protein granule matrix enclosed in a cell wall. The ground bean flours showed high levels of protein (24-27%) and starch content

(35-39%). Rapid Visco Analyzer presented results for pasting temperature, which was roughly 76-77°C for all varieties, was essential to comprehend the behaviour of starch gelatinization and shed gave light on the minimum temperature required to cook the flour and the functional characteristics of starch in a range of applications.

The high protein concentration of the faba beans presented the opportunity for extraction of protein concentrates. A commercial variety of faba beans was used to extract protein concentrates using aqueous extraction techniques, mainly acidic, alkaline and neutral conditions. The alkaline method produced the highest yield and the purest concentrate among the three methods. Acidic conditions caused significant change in the physical colour of the concentrate after extraction, leading to a bright and fine powder compared to the coarser and darker concentrates extracted by water and alkaline methods. and other functional properties of the concentrate. The concentrates when subjected to protein denaturation using a Differential Scanning Calorimeter, showed that water extracted concentrates had the highest denaturation temperature which represented the denaturation of legumin and the low temperature in acid extraction method represented the denaturation of vicilin. During strain-dependent rheological experiments, the high yield strain values for water extraction method helped explain the flexible and elastic protein network of this concentrate.

Also, protein concentrates were studied using SDS-PAGE to characterize the type of proteins present in the concentrates. Alkaline extraction showed higher amounts of both high and low molecular weight components of convicilin, α-legumin and β-legumin; acid extraction showed higher amount of low molecular weight bands corresponding to α-legumin, β-legumin and a small number of lectins (thin band) with low intensity bands; water extraction showed thick and intense high and low molecular weight bands corresponding to all the native protein constituents including convicilin and α- and β-legumin. Individual protein bands could be seen

clearly on the gel by reducing the disulphide linkages to reveal distinct molecular weight bands using β-mercaptoethanol as a reducing agent.

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Abbreviations

A	Early Long Pod variety
AE	Acid extracted
AL	Alkaline extracted
AOAC	Association of Official Agricultural Chemists
A_s	Seed Surface Area
B	Evergreen variety
BB	Commercial Broad Bean
C	Cole Dwarf variety
D	Janet variety
D_m	Mean Diameter
DSC	Differential Scanning Calorimetry
FAO	Food and Agricultural Organization
FB	Commercial Faba Bean
FC	Foaming Capacity
FODMAP	Fermentable oligo-, di-, and monosaccharides and polyols
FS	Foam Stability
G6PD	Glucose-6-phosphate dehydrogenase
GMO	Genetically Modified Organisms
GOS	Galacto-oligosaccharides
HC	Hydration Capacity
HI	Hydration Index
IBS	Irritable bowel syndrome
L	Length
L-DOPA	L-3,4-dihydroxyphenylalanine
OAC	Oil Absorption Capacity
SC	Swelling Capacity
SDS-PAGE	Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis
SI	Swelling Index
T	Thickness
V	Seed Volume
v-c	Vicine and Con-vicine
W	Width
WAC	Water Absorption Capacity
WE	Water extracted

Chapter 1. Introduction

Plant-based meals have swept the globe in recent years as more individuals than ever choose vegetarian and plant-based diets. Consumers are replacing meat with healthier, ethically sourced options. The meat industry greatly impacts climate change, biodiversity loss, and greenhouse gas emissions. By 2035, it is predicted that the market for these substitute goods will grow to a $290 billion market, resulting in an increase in the consumption of plant-based foods like jams, tofu, miso, mushrooms, legumes, nuts, seeds, cereals, vegetables, and plant protein concentrates and isolates. Most of this demand will be met by these plant-based foods, along with foods made from cells and fermented foods (Penchalaraju & Don Bosco, 2022).

New Zealand has many chances to develop and expand new plant-based protein sources as the world shifts towards plant-based diets and flexible vegetarianism. However, it is challenging to grow the highest protein producers, soy, and lupin, on an as-harvested basis, because of the GM status of soy and the climatic requirements of lupin. New Zealand has great expertise in the isolation and manufacturing of dairy proteins in the dairy industry which can be used to create a new plant-based food industry as many of the requirements are the same (Sutton et al., 2018).

In New Zealand, grain legumes are rotated with other crops such as wheat, barley, grass seed, and white clover seed. Although New Zealand cropping farmers have not advanced as far towards capital-intensive continuous cropping systems as farmers in nations like the United States and Great Britain, there has been a significant emphasis on high input-high yield systems in recent years. Faba bean is an untapped source of sustainable and quality dietary proteins because of the high quantity of protein in it and other agronomic advantages (Martineau-Côté et al., 2022).

There has been a persistent exploration on legumes for their protein extraction, potentiality, anti-nutritional factors, food industry applications and potentiality to provide a sustainable protein-based food (Agrawal et al., 2023; Eze et al., 2022). Pea and soybean are the two most studied and widely grown legumes. It is obvious that the next in the list would be faba bean, being the third-most significant feed grain legume worldwide (Singh et al., 2013; Penchalaraju et al., 2022). It has been observed that cooking legumes takes longer than other foods. There is a lack of research in this literature on the cooking properties of faba beans and how they relate to their physical and microstructural features.

Faba beans being a strong source of carbohydrates (mainly starch ~22-45%), can be eaten raw, dried, or fresh as a vegetable. In Ethiopia, China, the Mediterranean region, and the Middle East, they are frequently eaten for breakfast. Popular faba bean recipes include Nabet soup, Falafel, Bissara, and Medamis. In addition to being added to salads, they are used in Indian soups, porridges, and curries. Additionally, faba beans can be processed into flour for soup powders and baked goods or to make spreads like spreads like hummus (Vishnupriya et al., 2023). They are versatile legumes that can be enjoyed in both savoury and sweet dishes.

As there has not been any recent study on faba beans varieties in New Zealand, it is crucial to discuss varietal differences and how they play a significant role in determining the performance and characteristics of faba beans. Understanding these differences can provide valuable insights into the genetic diversity, agronomic traits, and nutritional composition of the different varieties. This can help establish the foundation for this research by highlighting the importance of selecting the most suitable varieties for specific objectives. There has been a lack of understanding in the longer cooking times of legumes and the factors that affect them. Examining the cooking characteristics could help provide insight into their overall quality and nutritional content. By examining the microstructure and physical characteristics of the

varieties, the differences in cooking time could be discussed which would allow for a deeper understanding of how the microstructural composition impacts the cooking behaviour and overall helps in optimizing their specific utilization in food processing.

Comprehensive assessments on the viability of faba bean protein extraction in New Zealand have indicated that coordinated scaling up across the value chain components—growing, extraction, and go-to-market—is necessary to build a successful faba bean protein market. However, there is a large risk associated with setting up an extraction operation in the event that New Zealand goods or protein extraction fail to find a market. In-depth knowledge is required to completely comprehend the costs associated with joining the industry (FAR Research, 2022). It is therefore essential to select the appropriate and economical extraction techniques during the initial phases. Most studies focused on protein extraction from legumes have utilized either dry or aqueous extraction techniques. Using dry extraction methods are the obvious choice of a sustainable extraction method. This study, however, is focused on various aqueous extraction as it does not utilize certain specific fractionation equipment and can be performed at small scale for individual studies. Selecting the correct aqueous extraction method for protein extraction from faba beans is essential because it directly influences the quality and yield of the extracted proteins. Using an appropriate method ensures that the proteins are extracted efficiently without denaturation or degradation (depending on the pH of the extraction medium-acidic, alkaline, or neutral), leading to accurate results in downstream analyses. Additionally, by choosing the correct extraction technique, researchers can maximize the protein yield, maintain their functionality, and obtain reliable data for further studies. In order to select a potential future supply of plant-based protein from faba beans for New Zealand, this research-based thesis is divided into two parts, one focusing on examining the physical characteristics of native varieties and second on characterizing this plant-based protein from commercial faba beans using various extraction methods.

Chapter 2. Review of Literature

2.1. Faba bean history and worldwide production

Plant-based foods are gaining popularity as more people adopt vegetarian and plant-based diets. The demand for these healthier alternatives to meat is expected to reach $290 billion by 2035, with plant-based foods, cell-based, and fermented foods contributing significantly to this growth (Wood & Tavan, 2022). New Zealand has the potential to develop and expand plant-based protein sources as the world shifts towards plant-based diets and flexible vegan and vegetarianism. However, growing soy and lupin, the highest protein producers, on an as-harvested basis, is challenging due to their GM status and climatic requirements. New Zealand's expertise in dairy protein isolation and manufacturing can help create a new plant-based food industry, as many requirements are similar (Sutton et al., 2018). New Zealand cultivates grain legumes in rotation with wheat, barley, grass seed, and white clover seed.

Table (i). Classification of legumes grown worldwide.

Category	Common Name	Scientific Name	Highest producer (metric tonnes)	References
Pulses	Common bean, dry	*Phaseolus vulgaris*	India (6.6 million)	(Gentry, 1969); (FAOSTAT).
	Chickpeas	*Cicer arietinum* L.	India (11 million)	(Koul et al., 2022); (Çelik, 2022).
	Dry peas	*Pisum sativum* L.	China (13.4 million)	(Thavarajah et al., 2023); (Ambrose et al., 2023).
	Lentils	*Lens culinaris*	Canada (2.3 million)	(FAOSTAT); (Nair, 2018)
	Dry broad beans (faba bean/ horse bean)	*Vicia faba* L.	China (1.7 million)	(Vishnupriya et al., 2023); (Dhull et al., 2021)
Oilseeds legumes	Soybeans	*Glycine max*	Brazil (120 million)	(FAOSTAT); (Brar & Carter, 1992)
	Peanuts	*Arachis hypogaea*	China (18 million)	(Yu, 2022); (FAOSTAT)

Faba bean (*Vicia faba* L.), also known as broad bean, is an annual legume belonging to the family Fabaceae. It is one of the oldest crops in the world, and in terms of area and production, it is the third-most significant feed grain legume worldwide after soybean (*Glycine max* L.) and pea (*Pisum sativum* L.) (Singh et al., 2013). It is a cool-season legume crop that has been grown in the Middle East since ancient times and is traditionally used as a significant source of protein for both humans and livestock (Bangar & Kajla, 2022). Older research shows that they were consumed as food by the Chinese about 5,000 years ago, and were afterward grown by the Egyptians 3,000 years ago, the Hebrews in biblical times, the Greeks, and the Romans a little later (Singh et al., 2013).

Faba beans, also known as field beans, horse beans, or tic beans, are dried beans from the Vicia faba species of the legume genus. They are the ripe dried fruit of smaller seeded types, similar to fresh or frozen green broad beans commonly used in British cooking. Faba beans must mature and senesce before harvesting, while broad beans can be plucked directly from living green bean plants. They are a type of pulse, as they are the dried seeds of a leguminous plant. They can be cooked whole with the skin on or split into cotyledons, like most pulses (Saltmarsh).

The double cropping strategy used in New Zealand is suitable for the annual cool-season legume known as faba bean. When planted in autumn and harvested in the winter or early spring, it can give dairy farmers extra feed when their dairy animals are lactating. Dropping temperatures can have an impact on the final produce, therefore it is vital to determine when to plant in the autumn (Martini et al., 2012). Faba beans can yield well under Canterbury conditions, according to the sole agronomic research published in New Zealand. According to a survey conducted, the seven most significant factors influencing yield in this season were variety, weed dry matter content at harvest, rhizobia seed inoculation, the quantity of beehives per hectare at flowering, number of frosts, and level of water deficiencies (mm and days) that took place throughout vegetative growth, according to an old study conducted on commercial field bean (Vicia faba) varieties in Canterbury during the 1977–1978 season (Newton et al., 1978). Research conducted in New Zealand has demonstrated that seed yields of up to 6 t/ha are possible if disease-free faba bean seed is sown in the autumn months of February at a population of roughly 70 plants/m^2 and the crop is given water as needed. The crop can generate up to 4.3 t/ha of fodder and has also been tested as a winter greenfeed for lambs, by late May (Hill, 1989).

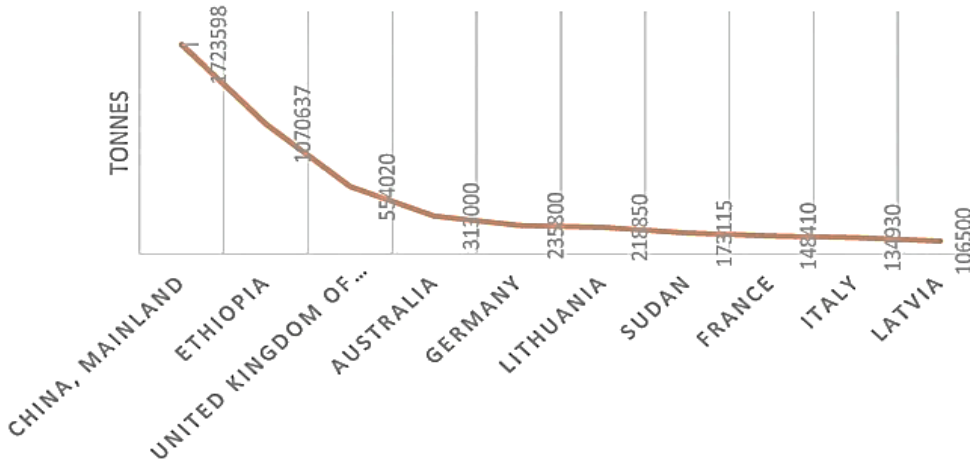


Fig. (i). The top ten nations that produce faba beans globally (FAO, 2021); Reprinted from Bangar & Kajla, 2022, © Springer Nature, with permission from Springer Nature.

2.2. Agrarian conditions for faba bean

The yield of faba beans is significantly influenced by the timing of sowing, harvesting, and irrigation operations. The ideal growing conditions are cool and humid, with early planting and evenly distributed rainfall between 650 and 1000mm. Neutral to alkaline soils with moderate moisture supply and medium texture are essential. Faba beans take 20-25 days to sprout, and the planting-harvest time is 80 to 120 days (Singh et al., 2013). Faba beans, grown at 14-15°C moisture content, offer economic and environmental benefits due to their nitrogen fixation abilities and lower greenhouse gas emissions. They also increase soil fertility and prevent soil-borne diseases. Currently underutilized, faba beans (Vicia faba) are a sustainable plant protein source, as they do not host cereal pathogens and can be dried at a maximum temperature of 32°C (Peterson et al., 2020).

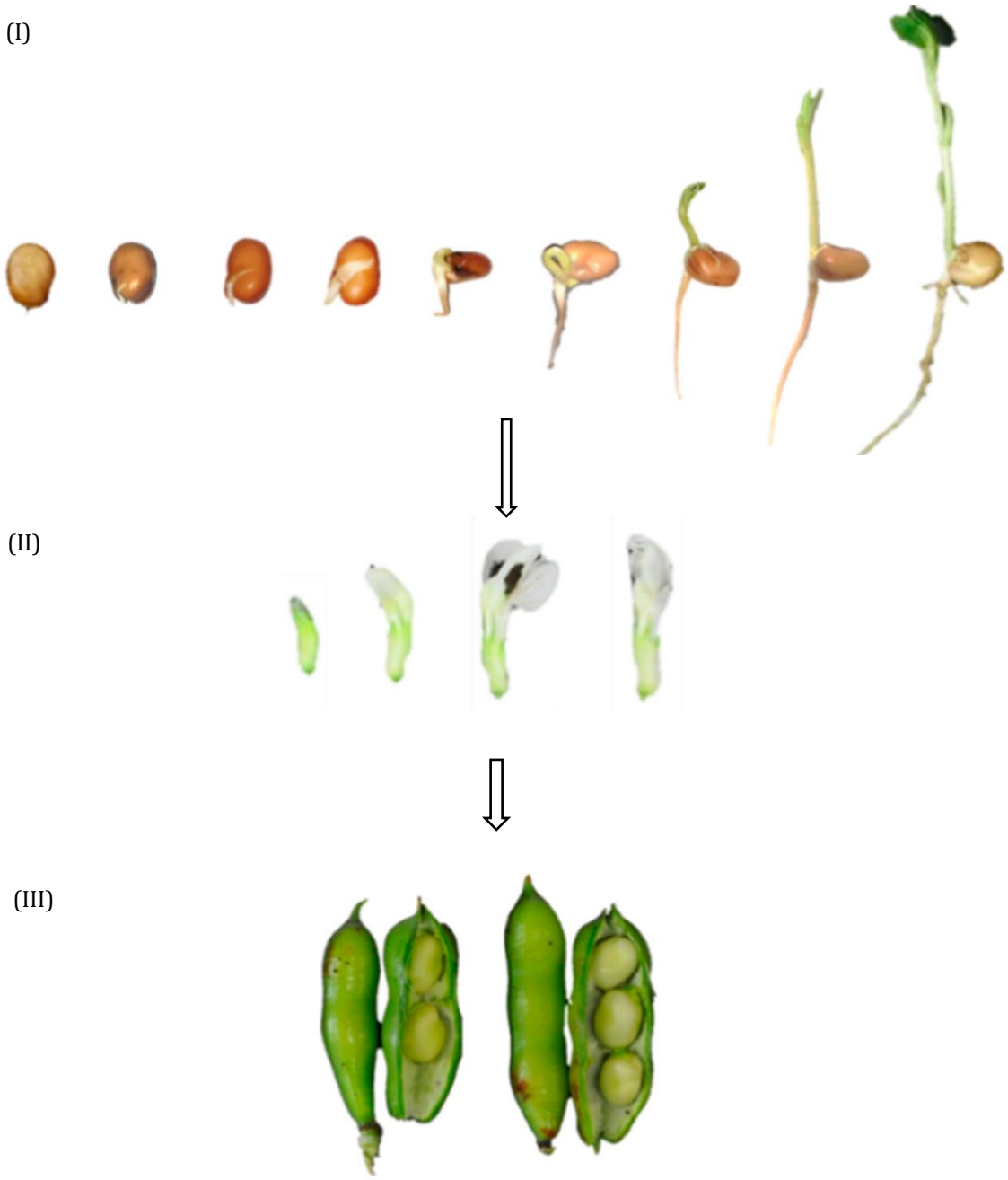


Fig. (ii). Life cycle of faba beans. (I) Growth of faba bean sprouts; (II) Flowering stage of the plant; (III) Growing faba bean pods. Reprinted from Duan et al. (2021), under open access license with no special permission required to reuse all or part of the article published by MDPI, including figures and tables.

2.3. Physical characteristics of faba beans

The physical properties of faba beans are essential for industrial applications, human consumption, animal feed, harvesting, separation, storage, and transportation. These processes depend on their size, shape, volume, density, and porosity. Because of their porosity, faba beans can be utilised in animal feed regimens, and their look satisfies human food standards. Selecting and identifying their possible industrial uses requires an understanding of these features (Efe & Sevdin, 2022).

Faba beans are usually spherical; however, their size and shape can vary depending on their origins and varieties (Efe & Sevdin, 2022). For the purposes of removing foreign materials, screening solids, and calculating heat and mass transfer, seed size is essential. Measurements like length, width, thickness, and equivalent diameter are frequently used. Designing machinery for handling, harvesting, processing, and storing requires understanding physical qualities such as principal dimensions, surface area, projected area, volume, sphericity, true and bulk densities, and porosity. Thousand kernel weight (TKW) is a crucial metric in the food industry since it indicates the typical weight of a crop's thousand kernels. Given that it can reveal details regarding seed size, density, and general regularity, it is a good indicator of seed quality. TKW is frequently used in companies to grade and sort seeds, determine the potential yield of crops, and analyse the quality of grains and legumes. These indicators are useful for evaluating and studying the distinctive characteristics of various crops. Protein concentrates from heavier (and bigger) beans can be extracted to produce a larger volume of extract per bean and a higher yield of overall flour. This can be very advantageous for farmers and producers as it allows them to receive higher profit margins for their crops while requiring less labour, money, and harvest (Wang & Fu, 2020).

Information on physical qualities is essential for engineers, food scientists, processors, and other scientists who might use these resources. Seed volume plays a significant role in various operations like drying, coating, scarification, etc. The surface area of seeds is important to study as it helps us to determine the water uptake and loss during various drying operations used in food industry (Wani et al., 2016).

The following subspecies of the Vicia faba species can be distinguished based on the size of their beans: V. faba var. faba major, (broad bean) which is cultivated for human consumption; V. faba var. equina minor, which is cultivated for animal feed; and V. faba var. minuta, which is a tick bean with the smallest seeds (Vishnupriya et al., 2023).

A study by Svanes et al. (2022), found that when utilized in place of animal protein, faba beans and peas had considerably less of an adverse effect on the environment in Norway than the usual amount of protein consumed there. Environmental effect and nutritional value were independent of one another and no loss in nutrients compared to other protein sources. However, further computations were required to account for the implications on social and economic sustainability. Another study by Sufar et al. (2024), discovered that traditional fertilizing and crop protection increase grain yields in the faba bean output of Northern Britain. It was proposed that increased temperatures would boost yields and that crop protection and fertilization might not have as much of an impact as variety. If verified, breeding and selection for tolerance to both biotic and abiotic stresses could be a valuable strategy to lessen the adverse effects of climate change on yield stability and faba bean yields. Still, there hasn't been much work done to create better genotypes of faba beans.

2.4. Chemistry and nutritional makeup of faba beans

The USDA in 2021 stated that the faba bean is a highly nutritious legume rich in proteins (26.1%), carbohydrates (58.3%), and dietary fiber (25.0%) as well as micronutrients like vitamins and minerals and various bioactive compounds which provide several health benefits (Dhull et al., 2022). Apart from its excellent nutritional content, the faba bean has an advantage as it is not a regulated allergen (unlike soy) and is not genetically modified (non-GMO) (Martineau-Côté et al., 2022).

2.4.1. Protein

The protein level of faba beans is considerable, ranging from 20% to 41%, which is about twice as much as that of other cereal grains. Most legume proteins are classified on the basis of their solubility in various solvents as – globulins, prolamins, albumins and glutelins. 60% of the protein fractions are globulins (soluble in salt solution), 20% are albumins (soluble in water and salt solution), 15% are glutelins (soluble in acid/alkali), and 8% are prolamins (soluble in alcohol) (Eze et al., 2022). Numerous factors, including source type, growing season, and variety, might affect this broad range of protein content. The essential amino acids isoleucine, leucine, lysine, methionine, tyrosine, phenylalanine, valine, histidine, tryptophan, and threonine, as well as the non-essential amino acids aspartic acid, glutamic acid, alanine, arginine, glycine, proline, and serine, are all present in faba beans (Dhull et al., 2022).

Globulins, a type of salt-soluble protein, are high in aspartic, glutamic, leucine, and arginine and 85% salt-soluble. Based on the sedimentation coefficient, they are divided into 11S proteins (legumin), which are conserved in faba beans, and 7S proteins (vicilin-30% and convicilin-3.2%), which have polymorphic subunits and multigene families (Bangar & Dhull, 2022).

While glutelins are soluble in sodium hydroxide solution and contain larger levels of glycine, methionine, and histidine than prolamins, prolamins are alcohol-soluble proteins rich in leucine, proline, and glutamic acid. Albumins are primarily metabolic proteins with greater sulphur content and may have enzymatic properties like lectins and protease inhibitors (Bangar & Dhull, 2022).

2.4.2. Carbohydrates

Faba beans contain somewhere between 51% and 68% of carbs, of which 41% to 58% are starches. Under a scanning electron microscope, the starch granules are visible to have cavities on the surface and round, oval, or irregular forms. The remaining carbohydrates consist of soluble sugars, primarily oligosaccharides like raffinose, stachyose, and verbascose, and dietary fiber, which makes up 15% to 30% of the total carbohydrates and is found in the bean's seed coat as both soluble and insoluble dietary fiber (Dhull et al., 2022). Important components of starch that affect the characteristics and digestion of food are amylose and amylopectin. The starch and fiber content of the beans may contribute to its distinctive texture, health advantages, and sensory qualities; however, the raffinose family of oligosaccharides (RFO) may counteract these advantages (Badjona et al., 2023). Because of their anaerobic fermentation, RFOs can induce stomach discomfort such as gas production and flatulence. By increasing the relationship between proteins, carbohydrates decrease the thermodynamic affinity of proteins for water molecules, hence increasing gelling capacity (Badjona et al., 2023).

2.4.3. Minerals and vitamins

Minerals including calcium, iron, magnesium, phosphorus, potassium, sodium, and zinc, as well as vitamins like vitamin C, niacin, folate, and vitamins A and K, are all present in faba beans. Some vitamins and minerals in high and low quantities have various positive effects on the body. For those with hypertension, a high potassium range (1062mg/100g) and low

sodium range (13mg/100g) are ideal which can be met by consuming faba beans (Dhull et al., 2022). Whole faba bean has higher mineral content than dehulled samples, but its bioavailability is often low due to antinutritional substances like condensed tannins and phytates. The reason being their numerous acidic functional groups, phytic and oxalic acids which can bind minerals to produce insoluble ions in the intestine (called oxalate and phytate, respectively), which reduces the absorption of vital minerals (Martineau-Côté et al., 2022). Iron and zinc are the key minerals present in deficient amounts in plant-based diets, which is a cause of concern especially for vegetarians. Among 15 faba bean varieties studied, 14 showed poor bioavailability of Phy:Zn and Phy:Fe. Further research is needed to understand the impact of processing activities on these minerals' bioavailability and the variables influencing their accumulation (Badjona et al., 2023).

2.5. Microstructure of legumes

Pulse seeds have starch granules embedded in a protein matrix as part of their microstructural characteristics, and their entire structure is covered in a fibrous cell wall (Ajala et al., 2023). The spatial arrangement of cells and the intercellular spaces in food materials is referred to as food microstructure. Food quality enhancements and the development of new products in this century will mostly centre on microscopically sized manipulations because the majority of components essential for transport characteristics, rheological and physical behavior, textural qualities, and sensory aspects are located under the range of 100μm (Aguilera, 2005).

The three main components of a pulse seed are the cotyledon, embryonic sac, and seed coat, which make up 80–90%, 1%, and 16% of the entire seed, respectively. The outer coating that protects the pulse seed is the seed coat, also called the hull or testa. The pulse seeds' germination and metabolism, mechanical characteristics (such as permeability, hardness, and

porosity), biochemistry, and chemical exchange (such as water and gas) are all greatly impacted by their seed coats (Zhong et al., 2018). The embryonic axis consists of two main parts: embryonic roots and shoots, and cotyledon tissue is primarily composed of large parenchyma cells enclosed by a cytoplasmic network fill, containing subcellular organelles. Each parenchyma cell's cell membrane encloses subcellular organelles such as protein bodies, oil bodies, and starch granules via a cytoplasmic network fill (Ajala et al., 2023).

A product's microstructure greatly affects its mechanical characteristics, such as its texture and firmness, and if it is seriously damaged, it can have a negative effect on the product's quality (Karim et al., 2017). A crucial tool for researching foods and food items is scanning electron microscopy, or SEM. In the study of seed microstructure, SEM has proven especially helpful (Swanson et al., 1985).

2.6. Bioactive composition of faba bean

According to Dhull et al. (2022), the faba bean contains a number of biochemical substances, including phenolic compounds, lignans, flavonoids, and terpenoids. Flavonoids in faba beans have anti-inflammatory and diabetes-fighting properties, while phytochemicals, including polyphenolic and non-phenolic compounds, have antioxidant, antibacterial, and anticarcinogenic properties. Plant maturity, genotype, and environment influence antioxidant qualities (Valente et al., 2019).

2.6.1. Phenolic compounds

Plant items such as fruits, vegetables, and cereals naturally contain substances called polyphenols, which include flavonoids, phenolic acids, lignin, tannins, and coumarins (Luna-Guevara et al., 2017). According to a study, the phenolic compounds in legume seeds have been shown to have good effects on biomarkers associated with cardiovascular disease, which

makes them appropriate for use as dietary supplements. These compounds can help prevent the deposition of triglycerides and lower levels of free radicals (Singh et al., 2017). Processing beans improves the way polyphenols are absorbed and metabolized, preserves their antioxidant potential, and changes the gut bacteria, all of which increase the nutritional value of the beans (Nicolás-García et al., 2022).

2.6.2. Lignans

Lignans are naturally occurring molecules that can be found in edible and plants. Because of their capacity to coordinate divalent transition metal ions and scavenge free radicals, they have demonstrated favourable benefits for a variety of diseases (Álvarez-Caballero et al., 2021). Like lignin, lignans are three-dimensional polymers that make up the cell walls of plants. Because of its insecticidal, antifungal, antibacterial, and antiviral qualities, it is thought that they defend plants from diseases and pests (Yoder et al., 2014).

2.6.3. Possible treatment for Parkinson’s

Parkinson's disease is a brain condition causing movement, mental health, sleep, and pain related issues with no cure; however, treatments and medications can lessen symptoms (WHO, 2023).

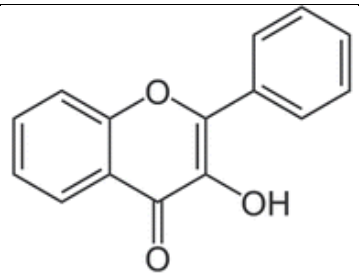
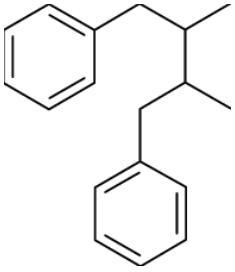
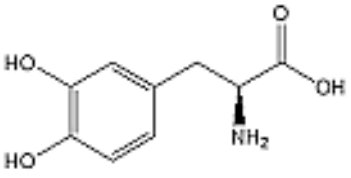
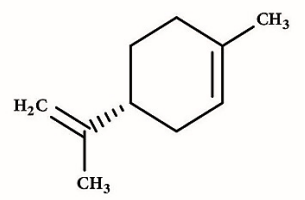
Faba beans are a good source of levodopa (L-DOPA), the precursor to dopamine, and a rich source of lysine-rich protein, which may be utilized as a medication to treat Parkinson's disease (Singh, et al., 2013). Parkinson's disease is caused by dopamine deficiency, and faba bean is used for treatment due to its ability to produce L-DOPA, which improves motor function. Cooked faba bean seedlings or pods have been found to improve motor cell symptoms, possibly linked to C-DOPA. Different processing methods affect L-DOPA content,

with the highest to lowest content found in fresh, frozen, oven-dried, air-dried, and boiled materials (Ofoedu, et al., 2022).

2.6.4. Terpenoids

The compounds known as terpenes have basic hydrocarbon structures, whereas terpenoids are altered terpenes that have different functional groups and methyl groups that have been oxidized and relocated or eliminated at different points (Masyita et al., 2022). Terpenoids possess various health benefits, including anticancer, anti-inflammatory, antibacterial, antiviral, antimalarial, hypoglycaemic, cardiovascular disorder prevention, treatment, immunoregulation, antioxidation, and neuroprotection, according to previous research (Yang et al., 2020).

Table (ii). Chemical structure of some bioactive compounds found in faba beans.

Name of bioactive compound	Chemical structure	References
Phenolic compounds (flavonoids)		(de Souza et al, 2018)
Lignans		(Johnsson, 2004)
L-Dopa		(Tesoro et al., 2023)
Terpenoid (limonene)		(Soundharrajan et al., 2018)

2.7. Cooking characteristics

Since cooking eliminates anti-nutritional elements and assures sensory quality, preparing beans to the right consistency is essential for customer appeal. The quality of starch and amylose content determine most of the cooking properties of cereals; contents of cell walls, proteins, and lipids also play an important part (Dharmaraj). Legumes vary widely in quality depending on their physicochemical and sensory characteristics, which makes cooking time a critical component in determining bean quality. Long cooking periods, however, are unsuitable for regions with low incomes since they can result in increased energy costs. As such, choosing

bean varieties for production and consumption requires careful consideration of fast boiling rates (Mwangwela, et al., 2021).

Hydration capacity is the percentage increase in a legume's weight after soaking, which indicates how long it takes to cook. It depends on the chemical composition of the seed's cotyledons and seed coat. Cooking time is the time it takes to cook a legume, which can vary between varieties and is influenced by the seed's size and hardness. Factors affecting a legume's hydration, swelling capacity, and cooking time include seed size, seed coat thickness, chemical composition, temperature, pH level, and presence of solids in the water used to soak or cook the legume. Soaking legumes in water for a longer period can reduce their cooking time (Wood et al., 2006).

The amount of water that one gram of food material can absorb under particular circumstances is known as its swelling capacity (SC). It gauges the capacity of starch to swell and absorb water, depicts non-covalent bonding between starch molecules, and displays synergistic interactions in granules. SC affects the ratios of amylopectin and α-amylose as well (Awuchi et al., 2019). In order to shorten the cooking time of pulses, which need more time to cook, they are soaked overnight to enable hydration, swelling, and softening. The steady development of the cotyledon and seed coat is another outcome of this process. Pulses differ in how much they swell and hydrate, and this is correlated with how well they cook. Pulse seeds are frequently, though not always, soaked before cooking (Yadav et al., 2018).

Cooking processes change nutritious elements like proteins and improve the digestion of starches. Macroscopic texture changes, such as hydration, solubilization, expansion of starch granules, and disintegration of cell wall and cotyledon lamellar polymers, are brought about by thermal processing of bean seeds (Perucini-Avendaño et al., 2024). Cooking and hydration are two different but connected processes. For a seed to soften and the starch to

gelatinize, hydration must take place either before or during cooking (Wood et al., 2006). Cooking time is also related to the pulse seed weight and hydration level. Cooking quality is influenced by the physical characteristics of grains, such as their size, weight, volume, hull, and cotyledon. It is easier to choose appropriate canning methods when you are aware of these qualities. Pulses that are less hard and prone to splitting, along with a greater capacity for hydration, are more appropriate (Yadav et al., 2018).

2.8. Effect of processing on the composition of faba bean

The main purposes of food processing are to turn raw materials into edible forms and to guarantee product quality and safety. Processing faba beans enhances their palatability and increases the quality and bioavailability of their nutrients (Oyeyinka et al., 2022).

2.8.1. Dehulling and soaking

The procedure of dehulling improves the colour of protein flour by removing antinutrient components that lead to bitterness or astringency (Eze et al., 2022). Dehulling boosts protein and amino acid content in faba beans, while decreasing fat, crude fiber, sugar, tannin, polyphenols, and phytic acids. However, trypsin inhibitory activity and ash content decrease after soaking. Faba bean phenolic content and antioxidant activity decrease significantly after dehulling, possibly due to leaching bioactive components (Dhull et al., 2022).

2.8.2. Cooking, autoclaving, and irradiation

The conventional method of preparing faba beans involves boiling them at atmospheric pressure, which preserves proteins and carbs while reducing anti-nutritional components. A 2012 study found that boiling dehulled faba beans decreased vicine levels, but increased con-

vicine levels (Oyeyinka et al., 2022). According to earlier research by Kmiecik et al. (2000), boiling, blanching, and cooking faba beans significantly reduces ash content and protein content without altering mineral composition. Cooking removes tannins and phytic acid, while longer autoclaving processes increase protein digestibility. However, high-heat processes eliminate bioactive molecules like L-Dopa (Dhull et al., 2022). The study's findings by Osman et al. (2014), showed that the chemical composition and mineral content of faba bean seeds remained unchanged after heating or low gamma irradiation doses, but antinutritional components like tannin and phytic acid decreased.

2.8.3. Fermentation

According to various tests performed by El-Moghazy et al. (2011), The fermentation of faba beans alters the amino acid pattern, lowering the pH, and altering the nutritional properties, with a slight increase in ash content and a decrease in fat, primarily due to phytic acid. Fermentation in faba beans enhances mineral accessibility, releases bioactive peptides, lowers glycaemic index, breaks down anti-nutritional components, and increases antioxidant activity, while maintaining protein and starch levels (Agrawal et al., 2023).

2.8.4. Enzyme treatment

The Faba bean's composition is affected by enzyme treatment, which lowers substrate components. Exogenous enzymes like phytase are used in food processing to hydrolyze phytates, reducing phytic acid levels and promoting controlled reactions. The highest activity enzyme pH may influence anti-nutrient composition. To lessen flatulence, α-galactosidases have also been employed to lower the galacto-oligosaccharide content of faba beans. It is advised to do more research on the sustainable usage of enzymes in the processing of faba beans (Rahate et al., 2021).

2.8.5. Germination

During germination, storage proteins are hydrolysed, which raises the number of amino acids in pulses (Badjona et al., 2023). Germination reduces starch, phytates, and α-galactosides, improves dietary fiber, and enhances calcium bioavailability. Phosphorus bioavailability is boosted by decreased levels of phytic acid and hemicellulose (Dhull et al., 2022). Another study by Rahate et al., 2021, showed a notable decrease in condensed tannins was observed following a 24-hour germination period. Germination increases folate content by 40%, improves organoleptic properties, and reduces vicine by 86%, balancing decreased ANF with nutrient loss during respiration, despite not eliminating lectins (Sharan et al., 2020).

2.8.6. Extrusion

Complex molecular structures can be broken down by extrusion, which can affect the physicochemical characteristics of flour and its different fractions (L'Hocin et al., 2020). High-temperature and pressure extrusion improves plant-based protein digestibility by disintegrating anti-nutrients, reducing condensed tannins and phytic acid, improving protein functions and starch digestibility (Agrawal et al., 2023).

2.8.7. Blanching, freezing, and frozen storage

Blanching is a process of briefly scalding food in water to soften it and inactivate enzymes, particularly peroxidase. It is best for faba beans, which can be damaged by chlorophyll degradation, either through the breakdown of chloroplasts in cells or the conversion of chlorophyll to phaeophytin (Rahate et al., 2021). Blanching causes starch gelatinization, Ca2+ ions leach out, and half-folate reduction in faba beans, but little is known about its impact on anti-nutrients (Oyeyinka et al., 2022).

2.8.8. Other thermal pre-treatments

It has been demonstrated by Anderson et al. (1994), that Roasting is an effective thermal pre-treatment to decrease trypsin inhibitor activity and phytic acid content in faba beans. Dry roasting significantly reduces antinutritional components and nutrients. Long roasting at 150°C increases antioxidant capacity by creating new phenolic compounds (Dhull et al., 2022; Vidal-Valverde et al., 1998; Siah et al., 2014). The unpleasant beany flavour of faba beans is caused by lipoxygenase and peroxidase in untreated whole beans, which can be prevented by heat pre-treatment using microwave heating (Rahate et al., 2021).

2.9. Applications of faba bean flour and protein

Faba beans, plant-based protein sources, are versatile in various foods like bread, pasta, tofu, yogurt, and meat substitutes. Their physicochemical, functional, and technical properties depend on storage proteins like 7S and 11S (Agrawal et al., 2023). Traditional ingredients in various cuisines are being replaced with faba bean proteins, serving as active ingredients in dairy-free food products (Dangi et al., 2022).

2.9.1. Extruded products

A study found that semolina pasta fortified with faba bean flour improves dry matter loss, cooking time, protein content, glycaemic index, and dietary fiber levels (Agrawal et al., 2023). Faba beans are used in extruded breakfast cereals to enhance protein content and provide more fiber and protein than corn-based snacks (Rahate et al., 2021). The spaghetti made with 30% maize flour and 70% faba bean flour demonstrated excellent product quality and good characteristics at 100°C and 28% moisture level (Agrawal et al., 2023).

2.9.2. Bakery products

The global demand for gluten-free breads is boosting the popularity of faba bean flour (Ofoedu, et al., 2022). Faba bean flour bread offers a higher nutritional index, improved protein digestibility, and longer shelf lives compared to soy flour bread, with improved amino acid content (Agrawal et al., 2023). The sensory analysis revealed that the fermentation of faba beans used in bread manufacturing did not significantly impact its crumbliness, pores size, or sponginess (Hans et al., 2022). Dextran, an exopolysaccharide with colloid properties, enables the incorporation of up to 43% faba bean flour in bread without compromising volume or texture compared to wheat bread (L'Hocine et al., 2020).

2.9.3. Tofu and yoghurt

The production of yogurt-like plant-based products is challenging because plant proteins are distinct and reactive to acidity. Conventional yogurt, which is prepared by fermenting cow's milk, generates a coherent protein network (Goldstein et al., 2022). A study found that faba bean tofu and yogurt have lower gel strength, water retention, and a firm texture due to higher ascorbic acid levels (Agrawal et al., 2023). Yogurt stored at room temperature for up to three days can have its oxidation reduced by adding red kidney bean protein hydrolysate, according to another study (Ferreira et al., 2021).

2.9.4. Protein isolates and concentrates

The protein content of the faba bean protein isolates that are commercially purified exceeds 90% (Badjona et al., 2023). Protein concentrates and isolates enhance functional properties like emulsifiers, water and oil absorption, and combat protein deficiency (Chaudhary et al., 2022). Faba bean protein's functional characteristics suggest it could be used in egg yolk powder in

mayonnaise, and its addition to durum wheat pasta increased protein, resistant starch, and dietary fiber content (Agrawal et al., 2023).

2.9.5. Meat analogues

Flexitarians are increasingly adopting plant-based protein products, including meat alternatives made from faba bean concentrate, which enhances texture, sensory qualities, nutritional value, and shelf life (Agrawal et al., 2023). The source of plant-based protein, influencing its functional qualities like water-holding, fat absorption, solubility, foaming, and gelling, significantly impacts the final structure and textural characteristics of meat analogues (Penchalaraju & Don Bosco, 2022).

2.9.6. Beverages

COVID-19 pandemic shifts market preferences from carbonated beverages to functional drinks, with apple fruit juice with faba bean hydrolysates offering higher amino acids and antioxidants, while malted barley and faba bean beer tasted similar (Agrawal et al., 2023). Faba bean protein concentrate (FBPC) or hydrolysates show superior stability in high-protein beverages compared to native FBPC or hydrolysates, potentially serving as a viable soy substitute for plant-based milk beverages (Agrawal et al., 2023).

2.10. Extraction techniques of faba bean proteins

The capacity to extract faba bean proteins into enriched flours, concentrates, or isolates at a lesser cost makes them commercially significant. Protein extraction is influenced by variables like pH, temperature, salt type, ionic strength, solvent to four ratio, and particle size. While other processing techniques have little impact, dehulling dramatically raises the protein content (Bangar & Dhull, 2022). To isolate bean proteins, wet processing techniques such as

water extraction, alkaline extraction, acid extraction, and salt extraction are frequently utilized. Since spray drying is an extremely effective and affordable approach to set up large-scale production of protein powders, it is mostly used to dry protein extracts. Additionally, research has shown that the produced protein powers have good functional and mechanical qualities (Penchalaraju & Don Bosco, 2022).

2.10.1. Dry extraction techniques

Dry extraction of faba bean protein is accomplished using pin milling and air classification. Using cyclones with a classifier wheel or restriction valve, air classification is a sustainable process. Based on variations in size and density, protein and starch fractions are separated. These two fractionates are separated by spiral air streams. Strong protein and starch connections become looser because of cell breakdown caused by repeated milling. Larger starch grains or fiber-rich particles can be easily separated from smaller protein-rich particles by milling, which releases the starch components and breaks up the protein bodies into smaller particles (less than 10μm) (Eze et al., 2022). Dry extraction is cost-effective and environmentally friendly, but its main drawback is a reduced protein yield because it cannot grind protein from the chloroplast membrane and stroma (Bangar & Dhull, 2022). Another drawback is that when extracting protein from legumes, anti-nutritional factors may adhere to the fine protein fraction (Eze et al., 2022). With present technology, it is not yet possible to produce high-purity isolates; but, by reusing fractions and combining several separation processes in series, one can enhance product concentration (Assatory et al., 2019).

2.10.2. Aqueous extraction techniques

Proteins are extracted with wet extraction techniques using aqueous solvents or chemicals like water, acid, or alkali, and the protein is then precipitated or recovered. The main solvent for extraction is usually water and the flour to water ratio ranges from 1:5 to 1:20.

When a protein's net charge in a solution reaches zero, it is said to be at the isoelectric point (pI) (Cruz-Solis et al., 2023). Extraction methods involving aqueous or alkaline pH ranges, proteins are most soluble at pH levels that are higher or lower than the pI, because of the electrostatic repulsion caused by the positive or negative charges interacting with other proteins (Dangi et al., 2022).

2.10.2.1. Alkaline extraction. Alkaline extraction is widely used method for protein extraction that entails adjusting the pH of the extractable material's solution using an alkali like sodium or potassium hydroxide to sustain a basic pH of 8-11. Such high pH ranges are necessary for protein solubilization in which disulphide linkages in the proteins are broken which improves protein yield and recovery. The pH is then brought down to roughly 4-4.8 using an acid like hydrochloric acid to precipitate the protein as no more solubilization occurs because most proteins reach their isoelectric point at this pH range. The alkaline solution helps break down the cell walls of the legumes, releasing the proteins. A separation technique like centrifugation, screening or filtration is employed to subsequently separate the supernatant mostly consisting of insoluble materials like starch and insoluble fibres from the precipitated protein. The recovered protein may be then washed and resolubilized at a neutral pH to enhance the quality of the extracted protein. This extract is then dried to form a powder using freeze drier (laboratories) or spray driers (industrial). Large quantities of alkali have detrimental effects as well, such as amino acid racemization, lower protein digestibility, and lysine and cysteine breakdown, even if they can break down the cell membrane and encourage protein release (Eze et al., 2022).

2.10.2.2. Acidic extraction. The protein solution's pH is lowered below the protein's isoelectric point throughout this process. Because the proteins in pulses and legumes have a net positive charge that increases their solubility at such low pH levels, this approach works

especially well for them (Eze et al., 2022). Proteins have different solubility characteristics at different pH levels, and the acidic pH provided by hydrochloric acid aids in dissolving the proteins effectively. Compared to alkaline extraction, this approach has demonstrated improved sensory characteristics and the inactivation of lipoxygenase, the enzyme responsible for the beany flavours, in the majority of protein products based on legumes (Eze et al., 2022). However, using low pH ranges have a significant impact on the yield and purity of protein extracted due to which this approach is used less frequently (Dangi et al., 2022).

2.10.2.3. Water extraction. In this method, the plant material is mixed with water to create a protein-rich liquid. This mixture is then agitated or blended to help release the proteins from the plant cells. After that, the liquid is separated from the solid parts, usually by straining, filtering, or centrifugation. This method distinguishes between protein and other non-protein substances such as fiber and carbs by using solubility differences (Onyango, 2022). This leaves with the protein-rich liquid which can be further processed or dried to be used in various ways (Penchalaraju et al., 2022). This method is a gentler way to obtain proteins from legumes while maintaining their natural properties.

2.11. Functionality of faba bean proteins

Functional qualities are crucial for assessing and predicting the behaviour of novel proteins, lipids, fibre, and carbohydrates in specific systems and determining their potential use as supplements or replacements (Chandra et al., 2015). The physicochemical and functional properties of the food systems can be changed by changing the conformational structure of the protein and their interactions. Their behaviour is also influenced by environmental factors such as pH, salt content, solvent proteins' viscosity, denaturants, and surfactants. Proteins are vital components in food processing because they have a substantial impact on physical properties such as cohesiveness, emulsification, hydration, water retention, gel formation, viscosity,

foaming ability, and colour effects (Eze et al., 2022). Legumin and vicilin are the two main legume storage proteins, and their composition affects protein functionality. The size, structure, and amino acid makeup of these proteins vary, giving rise to unique functional characteristics. Higher polarity of amino acids and reduced molecular weight make vicilin-rich proteins more soluble. Poor foaming and emulsifying qualities are frequently associated with high legumin concentration because of the structural restrictions imposed by disulphide bonds, which limit interfacial interactions (Shi et al., 2022).

2.11.1. Water absorption capacity

One important metric for assessing whether protein isolates are compatible with fluid-like food items is their water absorption capacity (WAC) (Brishti et al., 2017). The total quantity of water which is capable of being absorbed per gram is determined by a protein powder's absorption capacity (Aryee et al., 2017). Taste, mouthfeel, and texture of proteins are all influenced by their water absorption capacity, which was observed to be a pH-dependent property (Dangi et al., 2022). Because proteins can have both hydrophilic and hydrophobic properties, they are susceptible to interaction with water in food. Because faba bean flour contains hydrophilic components, it has a high potential for water absorption (Chandra et al., 2015). A significant capacity for water absorption is produced by polar side chains at the key locations of the protein-water interface (Yang et al., 2023).

2.11.2. Oil absorption capacity

An essential functional characteristic of food is oil absorption capacity (OAC), which influences taste, flavour, texture, and product yield. It is based on how much oil is absorbed by a substance and is affected by noncovalent bonding and the physical trapping of oil in proteins (Wang et al., 2020). The conformational shift of the faba bean protein exposes hydrophobic

and non-polar groups on its surface, which can interact with oil molecules to increase the protein's ability to absorb fat. This ability to absorb is also influenced by the molecular weight of the protein components; smaller peptides have a lower ability to capture oils (Dangi et al., 2022). For food structure interaction, taste retention, improved palatability, and longer shelf life—especially in meat or bakery products—a high oil absorption capability is essential (Sirivongpaisal, 2008).

2.11.3. Protein solubility

A crucial functionality test in food systems is the solubility of a protein, which influences other functional characteristics like gelling and emulsification of the protein fractions (Du et al., 2018). The percentage of protein in solution relative to the total protein in the source substance under particular circumstances, such as pH and ionic strength, is referred to as protein solubility (Shi et al., 2022). Proteins must be soluble in order for the food chain to function, and pH has an impact on surface charge and hydrophobicity, which in turn alters the balance between electrostatic repulsion and protein-solvent and protein-protein interactions. Because smaller molecular weight hydrolysates of faba bean proteins are able to create stronger hydrogen bonds with water, they exhibit better solubility (Dangi et al., 2022). Because of electrostatic repulsion from positive or negative charges, proteins are most soluble at higher pH values and least soluble at their isoelectric point (pI; pH 4-5). Below pH 4, carboxyl groups in their non-ionic form decrease proteins' ability to absorb water (Ferreira et al., 2021).

2.11.4. Foam capacity and foam stability

The amount of interfacial area formed when whipping a protein determines its foaming capacity, and the amount of time it takes for the foam to lose half of its volume determines its foam stability (Mauer, 2003). For optimal foam production, proteins should have a high

solubility in water, be flexible, and form a cohesive film at the water-air interface with enough viscosity to avoid amalgamation and burst (Cano-Medina et al., 2011). Food items with bubbles add to their texture and look, giving commercial goods like ice cream, bread, mousse, and meringues their distinct appearance. They are also an essential component of foamy goods like milkshakes, whipped cream, and cappuccinos (Ellis et al., 2018).

Table (iii)

Factors determining the foaming properties of plant proteins; Reproduced from Amagliani et al. (2021) © Elsevier, with permission from Elsevier.

Factors	Protein source	Composition	Protein extraction method	Physicochemical properties	Extrinsic Factors
Properties	Pulses Oilseeds Cereals Tubers	Protein profile Macronutrients Micronutrients	Wet extraction Dry extraction	Solubility Surface charge Surface hydrophobicity Molecular flexibility	pH Temperature Other components

Because legume flours have higher total protein content and soluble albumin- and globulin-type proteins, they generate more foam than cereal flours. Bean proteins exhibit a greater charge, which might potentially reduce hydrophobic interactions therefore enhance the solubility of protein. This would enable the proteins to move rapidly over the air-water interface, encasing air bubbles and promoting the production of foam (Goldstein et al., 2022).

2.11.5. Process of analysis using SEM

A high-energy electron beam between 100 to 30,000 volts is applied to an object under a scanning electron microscope (SEM), usually from a heat source. SEMs use lenses to compress the spot and focus concentrated electrons on the specimen since the spot size created by the gun is too large to produce a sharp image. The working distance determines the necessary magnification, which is resolved by automatic adjustment in modern SEMs.

Electrons from the scanned material are detected by the electron detector; these electrons might be either backscattered electrons (BSE) or secondary electrons (SE) (Mohammed et al., 2018).

The operator adjusts the brightness and strength of the signals until a clear image is obtained when they are displayed on the viewing screen. Magnification up to 10,000x is used for fine details. The details that are delivered depend on the electron voltage mode; richer surface information is provided by low accelerating voltages, while comprehensive internal information is provided by high accelerating voltages. The visualization of the topography of the sample, which is dependent on the quantity of BSE and SE signals, produces the partially three-dimensional image that is acquired from SEM (Mohammed et al., 2018).

2.11.6. Denaturation of protein

Protein molecules can undergo denaturation, a biological process that breaks covalent bonds and modifies the molecule's secondary, tertiary, or quaternary structure. Understanding the distinct features and attributes of denatured proteins is essential for comprehending the mechanism of folding and stability of proteins (Acharya et al., 2021). Denaturation occurs when proteins undergo a transition from their native state to a more disordered arrangement due to the breakdown of inter- and intramolecular bonds during heating (Lefèvre et al., 2022). Protein denaturation is a crucial process influenced by amino acid sequence and extraction method, often irreversible and can be detected using differential scanning calorimetry (DSC) (Badjona et al., 2023). As the denaturation temperature of the protein isolate is shown as an endothermic peak on the thermogram in DSC, it must be determined prior to texturization or heat treatment (Brishti et al., 2017). While proteins can denature as a result of temperature changes, pressure changes, pH changes, freezing and thawing processes, and freeze-drying, they are rather stable at neutral pH. This is accomplished via hydrogen bonding, sulfhydryl-disulfide exchanges, and molecular cross-linking. Denatured proteins precipitate and lose

solubility when they form cross links with molecules of higher molecular weight. Pharmaceutical and commercial food manufacturing both frequently use this procedure (Yousefi et al., 2022).

Using a controlled temperature increase or drop, DSC is a thermodynamic technique that evaluates the amount of heat energy absorbed by a sample. In order to estimate the thermal transition temperatures in solution, solid, or mixed phases, it is frequently utilized in the study of biological reactions. A sample cell and a reference cell are given energy in a simple DSC experiment, and both cells gradually raise their temperatures to the same level. The amount of heat that is not absorbed or released by the sample's molecule is equal to the difference in input energy needed to bring the temperature of the sample to that of the reference (Gill et al., 2010). An empty pan served as a reference when samples were weighed in an aluminum pan. To find the compound transition temperature, both pans were heated across a predetermined temperature range in DSC equipment furnaces and covered with metal lids (Ghanbari et al., 2023).

2.11.7. Rheological properties

The study of flow and deformation in food systems is known as rheology. Structures derived from food components through intricate physical, chemical, and biological alterations during processing and storage give foods their characteristic feel. Properties that humans can feel, such stickiness, elasticity, and hardness, are determined by these structures (Zhong & Daubert, 2012). The rheological properties of faba bean ingredients refer to how their behaviour under different conditions like flow, deformation and stress. These properties include viscosity, elasticity and flow behaviour (Żmudziński et al., 2021).

The pasting properties of starch present in faba bean flour can be studied using the Rapid Visco Analyzer (RVA). During cooking or other food processing procedures, the RVA monitors

changes in paste viscosity. These measurements can provide insight into the degree of interaction between a variety of food ingredients as well as the impact of modifications to physical processing conditions on the functionality of proteins (Onwulata et al., 2013). The linear viscoelastic region (LVR), a range where repeatable oscillatory testing may be carried out without structural degradation, is used to assess the viscoelastic character of hydrogels using rheometric experiments such as amplitude/strain sweep tests. The elastic and viscous moduli are measured in these tests as a function of applied strain (γ) (Budai et al., 2023).

2.12. Importance of this study

Despite adequate evidence supporting their various applications, faba beans are mostly underutilized, according to the literature review mentioned above. Nonetheless, not much research has been done on how these beans' microstructural traits relate to their cooking qualities. Research on certain faba bean varieties that are appropriate for the people of New Zealand has been scarce, and studies on sustainable farming methods are also lacking. These gaps show where more study may be done to improve faba bean production methods and add to New Zealand's agricultural knowledge base. An examination of the literature revealed that there is a growing interest in using faba beans as a plant-based protein source. However, there has not been enough research done to determine the best and most suitable way to extract this protein from faba beans.

Therefore, to systematically study the potentiality of faba beans to be used as a plant-based protein source, this study was divided into two parts. Part I examined the effect of physical and microstructural characteristics on the tenderisation/cooking time of faba beans. As was learnt from the literature, cooking helps eliminate major anti-nutritional factors which are one the main causes of underutilization of these beans. The objective of this part was to find the relationship between the seed microstructure and physical structure and the time taken

to achieve satisfactory tenderisation. Four faba bean varieties native to New Zealand were chosen for this study and the best variety based on these characteristics was identified.

Part II examined the protein extraction methods from a commercial faba bean variety. The literature explained that both dry and aqueous fractionation methods are suitable for extraction of protein from legumes. However, this study made the use of aqueous methods due to the lack of proper air classification systems needed for dry fractionation. The methods used in this study may not be as environmentally friendly as the dry methods, however, detailed information was provided for each wet method that can provide more pure protein concentrations with specific benefits for each method. It has also been studied that faba bean flour and proteins have immense applications in the food industry, so studying the functional properties of each extract helps to understand the variability in products that can be improved using a certain protein concentrate. This study could help improve the feasibility of legume protein extraction in New Zealand and help reach its goals for a more sustainable and plant-based future.

Chapter 3. Physical properties and cooking characteristics of New Zealand faba bean varieties

3.1. Introduction

Plant-based foods are gaining popularity as more people adopt vegetarian and plant-based diets because of various health and environmental problems associated with meat diets. Legume crops, a sustainable source if high-protein food grown throughout the world, among which faba bean (*Vicia faba* L.), also known as broad bean, is an annual legume belonging to the family Fabaceae. (Singh et al., 2013; Penchalaraju et al., 2022).

The mature seeds of the faba bean are high in dietary fiber (25.0%), proteins (26.1%), and carbs (58.3%) in addition to other bioactive ingredients (Dhull et al., 2021). As faba beans contain a high percentage of starch, other researchers investigated characteristics of starch, such as granule size, shape, and chemical makeup, and how it may have a major impact on the functional behaviour of bean flours. High gelatinization temperatures, limited swelling, and minimal breakdown viscosity values are the outcomes of high amylose concentration of the bean starch (Romero et al., 2019). Prior studies have found a positive, linear relationship between physical properties (seed dimensions, surface area, seed volume, etc.) and moisture content (Matouk et al., 2018). Jeganathan et al. (2021), examined the topography of the faba bean's abaxial, adaxial, and cross-sectional surfaces and verified varietal differences linked to their micromorphological traits.

To the best of the authors' knowledge, there is no published information on New Zealand-grown varieties of faba beans, and there is limited knowledge on the inter-relationship of physical and microstructural parameters to the overall quality and acceptability of faba beans as a potential plant-based food source. Examining the microstructure can also assist explain the

relatively long cooking time and provide insight into the cell makeup that affects cooking quality. It would be possible to assess the cooking properties of the varieties and select one that is appropriate for their specific use by looking at variations in cell wall thickness and surface topography. Considering the above, this study aimed to understand mainly the relationship between the physical and microstructural properties of four faba bean seed varieties and the cooking time for appropriate tenderness. The experimental design for this study involved: a) studying the dimensional characteristics, colour, and weight of the beans, as well as b) the microstructural parameters to study seed topography and bean cotyledon structures using a scanning electron microscope. The second objective was to study the nutritional components of the bean flours and how changes in viscosity were influenced by temperature fluctuations and shear force in the flour suspensions. This study will assist in identifying the best variety out of the four, which may be helpful for New Zealand industries to proceed further to create a plant-based product that would benefit from these beans' high protein content. In this study, the terms broad bean and faba/broad bean have been used interchangeably.

3.2. Material and methods

3.2.1. Materials

Four locally grown whole dry broad bean seed varieties, named Early Long Pod (A), Evergreen (B), Coles Dwarf (C), and Janet (D) were procured from Morton Smith-Dawe Ltd., Christchurch, New Zealand. Each whole dry broad bean seed variety was stored in separate bags in a cool and dry place (21°C) under further studies. The four varieties were grown in Canterbury, which is known as the certified seed growing area of New Zealand. Early long pod (A) was sown in autumn/winter and reached maturity at 11-13 weeks, Evergreen (B) was sown in autumn-spring and reached maturity at 11-13 weeks, Coles dwarf (C) was sown in

autumn/winter and reached maturity at 11-13 weeks and Janet (D) was sown in winter-spring and reached maturity at 10-12 weeks.

(A) Early Long Pod (B) Evergreen (C) Coles Dwarf (D) Janet

Fig. 1. The four varieties of broad beans used: Early long pod (A), Evergreen (B), Coles dwarf (C), and Janet (D).

3.2.2. Methods

3.2.2.1. Physical measurements

The whole dried broad bean varieties were randomly selected and measured for length (L), width (W), and thickness (T) using a digital calliper (Product Code: 11787105, Fisherbrand ™ Traceable Digital Carbon Fiber Callipers, Thermo Fisher Scientific, Massachusetts, United States) with a 0.1 mm resolution and ± 0.2 mm accuracy using 8 replications for each variety. These dimensions were used to measure the equivalent diameter (D_m), sphericity (**Φ**), seed volume (V) and surface area (A_s) of the seeds, which were determined based on the following relationship (Matouk et al., 2018; Wani et al., 2016):

$$D_m \text{ (mm)} = (LWT)^{1/3} \quad (1)$$

$$\boldsymbol{\Phi} = D_m / L \quad (2)$$

$$V \text{ (mm}^3\text{)} = \boldsymbol{\pi} b^2 L^2 / 6(2L - 3), \text{ where } b = (WT)^{1/2} \quad (3)$$

$$A_s \text{ (mm}^2\text{)} = \boldsymbol{\pi} b L^2 / 2L - b \quad (4)$$

3.2.2.2. Colorimetry

Using a colorimeter (Chroma Meter CR-400, Konica Minolta, Inc., Tokyo, Japan), the entire bean of each variety was analysed to examine colour differences among them, and this measurement was based on the L*, a*, and b* coordinates. The colorimeter consisted of a measuring head and a data processor A random single bean from each variety was placed on the measuring head with a white background. A total of 8 replicates on a different bean were performed for each variety for the purpose of precision in the measurements. The L*, a*, and b* coordinates were selected on the data processor and on a scale of zero to one hundred, L* denoted lightness from black to white, whereas a* and b* denoted chromaticity without numerical bounds. It is known that negative a* is associated with green, positive a* with red, negative b* with blue, and positive b* with yellow (Konica Minolta, 2006-2023). The white calibration plate was used to calibrate the system to obtain accurate results. A total of 8 replicates were performed for each variety for the purpose of precision in the measurements. The total colour was calculated as a range of dark to light, more green or red and more blue or yellow according to these values.

3.2.2.3. Thousand kernel weight

A digital electronic balance with a 0.001 g precision was used to calculate the weight of the thousand kernels. In a pre-weighed beaker, ten seeds of each variety were weighed at a time. The measurements were made using ten replicates of the sample, and the average of one bean was obtained. One bean's weight was used to determine how much one thousand kernels weighed (weight of one seed x 1000) (Matouk et al., 2018).

3.2.2.4. Cooking characteristics

Cooking characteristics were analysed using the methods established by Wani et al. (2016), but with 50 g of beans.

3.2.2.4.1. Swelling capacity and swelling index. 50 g of beans of each variety were added in a graduated cylinder and the volume was noted. The number of beans was counted and then transferred into a 100 ml beaker. The beans were submerged in water and covered with aluminium foil and kept soaking overnight at room temperature. The water was then drained, and the beans were pressed gently between paper towels to soak any remnant water on the surface. The beans were then transferred back into a measuring cylinder and the volume was measured after soaking. The swelling capacity and swelling index of beans of each variety were measured using (Adebowale et al., 2004; Wani et al., 2016):

Swelling Capacity, (SC) = (Volume after soaking – Volume before soaking) / No. of seeds (5)

Swelling Index, (SI) = Swelling Capacity / Volume of 1 Seed (6)

Where Volume of 1 seed = Volume before soaking / No. of seeds (7)

3.2.2.4.2. Hydration capacity and hydration index. Like swelling capacity, beans of each variety were added in a pre-weighed measuring cylinder and weighed to measure around 50 g of each. The number of beans making 50 g was noted for further calculations. The beans were similarly submerged in water overnight at room temperature. The water was then drained, and the beans were patted dry and weighed again to note the increase in weight after swelling. The hydration capacity and hydration index of beans of each variety were measured using (Adebowale et al., 2004; Wani et al., 2016):

Hydration Capacity, (HC) = (Weight after soaking – Weight before soaking) / No. of seeds (8)

Hydration Index, (HI) = Hydration Index / Weight of 1 Seed (9)

Where, Weight of 1 seed = Weight before soaking / No. of seeds (10)

3.2.2.4.3 Cooking time. Four 400 ml glass beakers were filled with distilled water and placed on a hot plate. The water was boiled, and around 20 g of beans of each variety were added to a marked beaker and made to cook. The timer was started as soon as the beans were added. A bean was taken from the boiling water at various times, starting from 10, 20, 30, 40 minutes and so on. It was then pressed between two glass slides gently until the appropriate softness of each variety was achieved, where the seat coat breaks easily and the beans are tender all the way through, without being mushy (Lange, 2022). The time at which each variety was cooked sufficiently, till the centre of the bean, was noted as the cooking time for the variety (Adebowale et al., 2004; Wani et al., 2016). A similar method was followed for beans that had undergone prior soaking for 8 hours at 25℃ in beakers containing 20 g of beans with 400 ml distilled water and the time was noted for them as well to compare the difference in cooking time for raw and soaked beans. All the above procedures were done in triplicates for each variety to obtain a more accurate recording.

3.2.2.5. Microstructure analysis

Using methods as used in previous studies by Ajala et al. (2023), the microstructure of the protein and starch granules in the whole beans of each variety was characterized using a scanning electron microscope (SEM). Using a sharp blade, beans from each type were carefully sliced into flatter pieces to maximize the surface area for imaging. The beans were then fixed, dried, and gold coated at the Manawatū Microscopy and Imaging Centre (MMIC), Massey University, Palmerston North, New Zealand. Each variety's sliced pieces were fixed for 48 hours using an electron microscope fixative that contained 3% glutaraldehyde and 2% formaldehyde in 0.1M phosphate buffer. Following a 20-minute dehydration period with standard ethanol, the surplus fixative was rinsed with distilled water. After being cleaned three times in 100% ethanol for half an hour, the samples were dried with a critical point drier

(Polaron E3000 series II, Quorum Technologies, England) (Ajala et al., 2023). To maximize the surfaces available for efficient findings, each type was positioned on a stub that faced either upward or downward, accordingly. Gold coating was applied to the dried and fixed samples using a gold sputter coater (Baltec SCD 050 sputter coater, New York, USA). The samples were examined with an SEM (SU3800 Scanning Electron Microscope, Hitachi High-Tech Corporation, Tokyo, Japan) at the Robinson Research Institute, Victoria University of Wellington, for imaging. Each faba bean sample's top surface was imaged in three separate sites at 400x and 1000x magnifications. To observe the starch globules and protein granules in the bean cotyledon, cross-sectional pictures of each faba bean sample were captured at a magnification of 800x. The number of starch granules, cotyledon cell diameter, thickness of cell wall, diameter of starch granules and the length of surface ridges were calculated using ImageJ software. The SEM images were loaded onto the software and calculated under the 'Analyze Particle' setting, which provided data for length measurements used for diameter, thickness and surface length as well as counting particles helped to quantify the number of starch granules. A series of 9 measurements were taken for each parameter using 3 cells of each variety. The mean of these measurements was taken using Minitab.

3.2.2.6. Preparation of bean flour

The broad beans were weighed, ground (Coffee Grinder Model BCG200, Breville, Pty Ltd., Sydney, Australia) and sieved through a fine-mesh sieve (B.S. 410/I.S.O. 3310-1:2016, Glenammer Sieves, Ayrshire, United Kingdom) to remove the bigger husk components and collect the bean flour. This flour was weighed and stored in a zip-lock bag, away from sunlight, at 21°C until further processing.

3.2.2.7. Protein content

The crude protein and nitrogen content of the bean flours was estimated using the Kjeldahl method (Kjeltec 2100 System, Tecator, Sweden), using 6.25 as the conversion factor from nitrogen % to protein %. Each sample was tested in triplicates in addition to two blanks. The protein content was calculated from the nitrogen % using the following equation (Vogelsang-O'Dwyer et al., 2020):

Protein % = Nitrogen % x Conversion Factor (11)

3.2.2.8. Pasting properties

The pasting properties of the broad bean flours were determined using a Rapid Visco-Analyzer (RVA) (Rapid Visco-Analyzer 4500, Perten Instruments, New South Wales, Australia), using methods from Romero and Zhang (2019). To obtain 3.5 g of bean flour sample, each flour sample was precisely weighed using a digital electronic scale with a 0.001 g precision. The flour was added and combined with about 25 g of water in the canister to create a suspension. The paddle was inserted in the canister and assembled in the instrument. The dispersions were allowed to acclimate to 50°C for one minute. They were then heated to 95°C at a rate of 6°C per minute and maintained there for five minutes. Lastly, they were cooled back to 50°C at a rate of 6°C per minute and held there for two minutes. A spindle speed of 160 rpm was selected. The total time of a test run was 21 minutes. Each variety was run in the RVA thrice for a more accurate recording.

3.2.2.9. Statistical analysis

The physical and cooking measurements for each variety were performed in triplicates and the colorimetric measurements were performed on 8 beans of each variety. Using Minitab 21 statistical software (Minitab LLC, Chicago, USA), all the data for average mean and

standard deviation were statistically analyzed by one-way analysis of variance (ANOVA) at $p \leq 0.05$. The microstructural images obtained in triplicates for each parameter from the SEM were precisely measured using ImageJ software (ImageJ 0.5.8, National Institutes of Health and the Laboratory for Optical and Computational Instrumentation, University of Wisconsin, USA). ANOVA was used for analysis to obtain the mean of these measurements from ImageJ. Pearson's Correlation Coefficients (MS Excel, Microsoft 365, Version 2202) were calculated to study the correlation among various factors.

3.3. Result and discussion

3.3.1. Physical properties of faba bean seeds

Common physical attributes that were examined, including the surface features, size, colour, texture, and shape, can differ amongst various varieties of the same bean and offer important insights into their quality and their suitability for protein extraction and other functional properties. This is because knowing the principal dimensions like length, width and thickness controls the surface area for cooking characteristics of the beans. The D variety has the largest equivalent diameter, sphericity, seed volume, and surface area, based on the results shown in **Table 1** but none of the values differed significantly among the different varieties

The variations in seed variants that result in distinct plant growth patterns could be the origin of the physical differences between the bean varieties. Such size variations can also result from variations in growing conditions, such as temperature, soil type, and harvesting time. However, there was, no significant difference in the physical characteristic values among the varieties. A large set of data was presented for the physical characteristics of each bean variety, and therefore, averages show no big trends related to variety. Foods are graded, and their quality controlled based on their size and form, which are significant physical characteristics that are

employed in screening to remove extraneous objects. A physical characteristic like sphericity also comes in handy for calculating heat and mass transfer and fluid movement during cooking for example (Sahin & Sumnu, 2006).

Table 1. Physical characteristics of whole dried varieties of faba beans.

Parameter	Faba bean variety			
	Early long pod (A)	Evergreen (B)	Coles dwarf (C)	Janet (D)
Length L (mm)	19.88 ± 1.20[a]	18.18 ± 0.90[a]	20.74 ± 1.90[a]	19.42 ± 1.50[a]
Width W (mm)	14.88 ± 1.00[a]	13.72 ± 0.80[a]	15.84 ± 1.70[a]	14.84 ± 0.80[a]
Thickness T (mm)	6.02 ± 0.90[a]	6.66 ± 0.80[a]	6.26 ± 1.30[a]	7.74 ± 1.20[a]
Equivalent dia. Dm (mm)	12.08 ± 1.40[a]	11.61 ± 0.60[a]	12.44 ± 1.10[a]	12.55 ± 0.80[a]
Sphericity Φ	59.71 ± 2.70[a]	63.85 ± 1.70[a]	59.46 ± 2.50[a]	66.17 ± 4.10[a]
Seed vol. V (mm^3)	514 ± 186[a]	449.6 ± 74[a]	551.8 ± 135.6[a]	568 ± 121.1[a]
Surface area A_s (mm^2)	390 ± 94.6[a]	356.7 ± 39.9[a]	412 ± 71.9[a]	418.4 ± 60[a]
1000 kernel weight (g)	1456 ± 213.6[a]	1188.8 ± 223.1[a]	1457.3± 293.1[a]	1552 ± 319[a]

All values are reported as the mean ± SD, where N=8 (8 replicates with 8 measurements from each variety). Values within a row not having a common superscript differ significantly ($p<0.05$).

3.3.2. Thousand Kernel Weight

Weight variations between varieties can also be caused by growing conditions and agricultural techniques. **Table 1** showed that, the TKW of the varieties did not statistically vary significantly from each other.

3.3.3. Colorimetry

In food systems, colour measurement is an important technique for researching consumer acceptability of products, market performance and statistics, and post-harvest losses resulting from quality degradation that causes colour variations (Dutta & Nath, 2024). The bean

variety with the lightest colour among the others is variety D, which has the greatest value of L* (i.e., 52.35). The darkest bean among the others is variety B, as shown by the lowest value of L* (50.09). These values were not significantly different from each other (**Table 2**). The maximum value of a*, 11.88, indicated that variety C has reddish hues, while the lowest value, 1.82, which was significantly lower than variety A, C and D, indicated that variety B has higher levels of green hues. Variety C, which had more yellow hues, according to the high values of b* (i.e., 28.65) with blueish hues. Overall, variety B is significantly greener than all varieties, variety C is yellower than all the other types and variety D is the lightest variety overall, redder than A and B, and slightly yellower than every other variety except C.

When comparing various types, the colorimeter readings for the beans are a useful tool for indicating quality and diversity in the colours of the different varieties. As expected, the results show that variety C (being Evergreen) stays green in colour even upon complete maturity and drying which was unlike the other three varieties. This property could play part in explaining some other important characteristics mentioned later in this paper. Despite having a similar overall look, beans range widely in their flavour and aroma profiles, which gives each variety a distinct place in the food industry. A sample's average of several colorimeter readings gives enough information to evaluate difficult samples in a meaningful manner, permitting natural variation without compromising the quality of the final result (Phillips, 2024).

Table 2. Colorimeter results for whole faba bean seeds.

Parameter	Faba bean variety			
	Early long pod (A)	Evergreen (B)	Coles dwarf (C)	Janet (D)
L*	51.55 ± 9.58^{a}	50.09 ± 2.67^{a}	51.28 ± 3.01^{a}	52.35 ± 4.13^{a}
a*	8.11 ± 3.35^{a}	1.82 ± 2.16^{b}	11.88 ± 3.05^{a}	9.60 ± 2.55^{a}
b*	26.12 ± 4.59^{a}	28.19 ± 1.65^{a}	28.68 ± 1.14^{a}	28.55 ± 1.67^{a}

All values are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements from each variety). Values within a row not having a common superscript differ significantly ($p<0.05$).

3.3.4. Cooking characteristics

We can learn more about how much water beans can absorb, how much they swell, and how their size and texture change when soaked by investigating their hydration capacity. Because it can affect the final texture, flavour, and appearance of foods developed from these beans, this knowledge is helpful for food processing and cooking (Wood et al., 2016). According to the mentioned data in **Table 3**, variety A had the highest swelling capacity, measuring 2.67 ml/ seed which varied significantly from B, C and D. Variety B and C did not differ significantly. This demonstrates that variety A can absorb large amounts of water and grows considerably in volume when submerged. This trait can be advantageous in food processing since it can result in foods made of this variety having better mouthfeel, more volume, and better texture. The reason behind the rise in volume and weight of some beans after soaking and water absorption can be attributed to their hydration and swelling capacities. (Wood et al., 2016) found that the swelling capacity of chickpeas ranged from 1.33 to 1.60 ml/ seed, which is significantly less than the swelling capacity of all four of the faba bean varieties investigated in this study (2.12 to 2.67 ml/ seed). This might be because, in comparison to chickpeas, faba beans can swell more when exposed to water because of their bigger size and cellular structure, resulting in a lesser number of seeds required to uptake the same volume. When employing these distinct bean varieties, the variations in their tendency to swell can affect how different meal preparations and finished products turn out. Therefore, for this study, swelling and hydration capacities were directly linked to the difference in seed volume and weight upon soaking as well as the number of beans amounting to the same weight.

Determining the ideal cooking time for the varieties helps in ensuring the beans are cooked to the desired texture and taste. By understanding these characteristics, one can achieve optimal results in their dishes and avoid overcooking or undercooking the beans. As per the results below, the variety D had the maximum cooking time of about an hour and fifteen

minutes to reach the favourable softness of the beans (**Table 5**). A high swelling index in beans means that a higher amount of water is absorbed by each bean in particular, which softens the beans, making them tender and shortens the time it takes to cook them. However, there was no significant difference in swelling and hydration capacities among the varieties, which could explain the varying cooking times.

Soaking the beans overnight prior to cooking has been shown to dramatically reduce (almost 1/3rd) the cooking time, and thus, the energy consumption to cook them is reduced as well. A noticeable swelling of the beans and softening of the seed coat was observed upon prior soaking resulting in a dramatic decrease in cooking times (**Table 4**, **Table 5**). The variety B took the least amount of time to cook, and this time was reduced even further when using pre-soaked seeds. It was observed that A took longer to soften than C under no soaking conditions but cooked faster than C upon prior soaking. Cooking results in permanent modifications to proteins and middle lamella pectin, giving food a mushy feel. Intrinsic characteristics such as size, thickness of the seed coat, cotyledon composition, size of the hilum and micropyles, temperature, pH level, and presence of particles in the soaking media affect how hydrated the beans are (Perera et al., 2023).

Table 3. Swelling and cooking capacities of the four varieties of faba beans.

Faba bean variety	Swelling capacity, (ml/seed)	Swelling index	Hydration capacity, (g/seed)	Hydration index
Early long pod (A)	2.67 ± 0.03^{a}	0.51 ± 0.04^{a}	1.52 ± 0.02^{a}	0.51 ± 0.03^{a}
Evergreen (B)	2.12 ± 0.11^{b}	0.52 ± 0.03^{a}	1.17 ± 0.05^{b}	0.50 ± 0.01^{a}
Cole dwarf (C)	2.19 ± 0.04^{b}	0.49 ± 0.03^{a}	1.11 ± 0.02^{b}	0.45 ± 0.01^{b}
Janet (D)	2.51 ± 0.04^{c}	0.49 ± 0.03^{a}	1.43 ± 0.01^{c}	0.49 ± 0.007^{a}

All values are reported as the mean ± SD, where N=3 (3 measurements from each variety). Values within a column not having a common superscript differ significantly ($p<0.05$).

It has been determined that the disintegration of the central lamella in some bean varieties results in simple cell separation, which softens the beans during cooking and explains that some bean varieties require shorter cooking times than others because of their inherited morphological traits (Wani et al., 2016; Perera et al., 2023).

Table 4.

The four varieties of faba bean seeds before and after soaking.

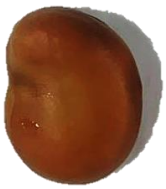
Faba bean variety	Before soaking	After soaking
Early long pod (A)		
Evergreen (B)		
Coles dwarf (C)		
Janet (D)		

Table 5. Cooking time of the four varieties of faba beans before and after soaking.

Faba bean variety	Cooking time before soaking (min)	Cooking time after soaking (min)
Early long pod (A)	66.67 ± 5.77^{a}	25.00 ± 0.02^{a}
Evergreen (B)	56.67 ± 2.89^{b}	20.00 ± 0.10^{b}
Coles dwarf (C)	61.67 ± 5.77^{b}	26.67 ± 5.77^{a}
Janet (D)	73.33 ± 2.89^{a}	36.67 ± 2.89^{c}

All values are reported as the mean ± SD, where N=3 (3 replicates from each variety). Values within a column not having a common superscript differ significantly ($p<0.05$).

3.3.5. Faba bean seed microstructure

Faba bean seeds were imaged cross-sectionally using a scanning electron microscope. The starch granules in the beans, regardless of variety, were comparatively large and spherical/oval in shape, with a diameter ranging from 16.02 to 35.48 μm (**Table 6**). These granules were embedded in a protein body matrix, which was completely encased in a cell wall with a thickness of 0.64 to 2.06 μm. The four faba bean varieties when studied under SEM with no significant difference in the number of starch granules per cell (**Fig. 2**., **Table 6**). The diameter of the typical cell varied between 97.27 to 161.9 μm, with each variety containing approximately 6–10 starch granules. Only the thickness of the cell walls in variety A and B varies significantly. Variety D was not significantly different than A and the values for variety C were not significantly different from both A and B. Other physicochemical properties suck as cooking time of different varieties of beans may vary due to differences in the cotyledon microstructure. The overall integrity and hardness of the beans are influenced by their cell wall. Variety D beans may take longer to cook and soften because of their thicker cell walls, which may hinder the beans' ability to absorb water and develop the right texture when cooked (Wani et al., 2016).

To investigate the microstructural traits of the four faba bean types, the surface of the cotyledons of mature seeds was imaged. **Fig. 3.** reveals the top surface of each of the four varieties. It was discovered that the beans' surface had ridges, grooves, and wrinkles. The length, width, structure, and arrangement of the ridges on the surface vary depending on the type of bean. Ridges could indicate how smooth or rough the surface of the bean is, which can affect the texture, absorption of water, milling ease, and even the efficiency of protein extraction methods (Wood et al., 2016).

The broad ridges on variety A's surface formed symmetrical rectangular cell wall connections and measured around 30-36 μm in length (33.58 ± 3.60), as seen at x1000 magnification. These strong lines indicated a more pronounced and long-lasting ridge pattern, which may affect the texture and water-absorbing capacity of the beans. This configuration may prolong the cooking time because the rectangular ridges and bigger borders on the bean surface produce uneven heat dispersion during cooking. As seen at x1000 magnification, the B variety had irregular thinner ridges with smoother troughs that averaged roughly 29-35 μm in length (32.27 ± 3.44). A smoother surface texture was indicated by flatter and thinner ridges. This kind of ridge pattern suggested a smoother, less-pronounced surface, which could result in a more balanced diffusion of heat when cooking. This might have been another factor in the consistent cooking and short cooking time when compared to varieties with more structural ridges and thickness. With uneven patterns and thin connections between the ridges, the C variety had narrow Y- or T-shaped ridges of approximately 50-60 μm length (54.24 ± 5.30). The D variety had the highest degree of surface irregularities and the most distinct surface topography due to tapering crests that were about 29-36 μm long (32.11 ± 4.89) instead of distinct ridges. The values for these lengths did not vary significantly in varieties A, B and C; C variety was however, significantly different than the rest.

Wood et al., (2016) assumed that the surface ridges marked the locations of the underlying cell wall connections. Longer boiling durations were needed to soften the beans because thicker ridges, as seen in A and D, may contain a higher concentration of cellulose in the cell walls, which is resistant to breakdown during cooking because of its strong and rigid structure.

Table 6. Cross-sectional and surface properties of the four faba bean varieties observed through SEM.

Faba bean variety	Number of starch granules/cell	Cotyledon cell diameter, μm	Thickness of cell wall, μm	Average dia. of starch granule, μm	Length of surface ridges, μm
Early long pod (A)	6.33 ± 1.52^{a}	107.74 ± 16.97^{a}	1.47 ± 0.12^{a}	25.97 ± 3.03^{a}	33.58 ± 3.60^{a}
Evergreen (B)	7.00 ± 2.65^{a}	101.24 ± 3.97^{a}	0.99 ± 0.13^{b}	26.15 ± 4.16^{a}	32.27 ± 3.44^{a}
Coles dwarf (C)	7.33 ± 2.31^{a}	121.51 ± 8.56^{a}	1.07 ± 0.37^{ab}	22.72 ± 6.96^{a}	54.24 ± 5.30^{b}
Janet (D)	8.00 ± 1.00^{a}	125.60 ± 36.30^{a}	1.71 ± 0.31^{a}	28.79 ± 5.12^{a}	32.11 ± 4.89^{a}

All values are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements for each parameter for every variety). Values within a column not having a common superscript differ significantly ($p<0.05$).

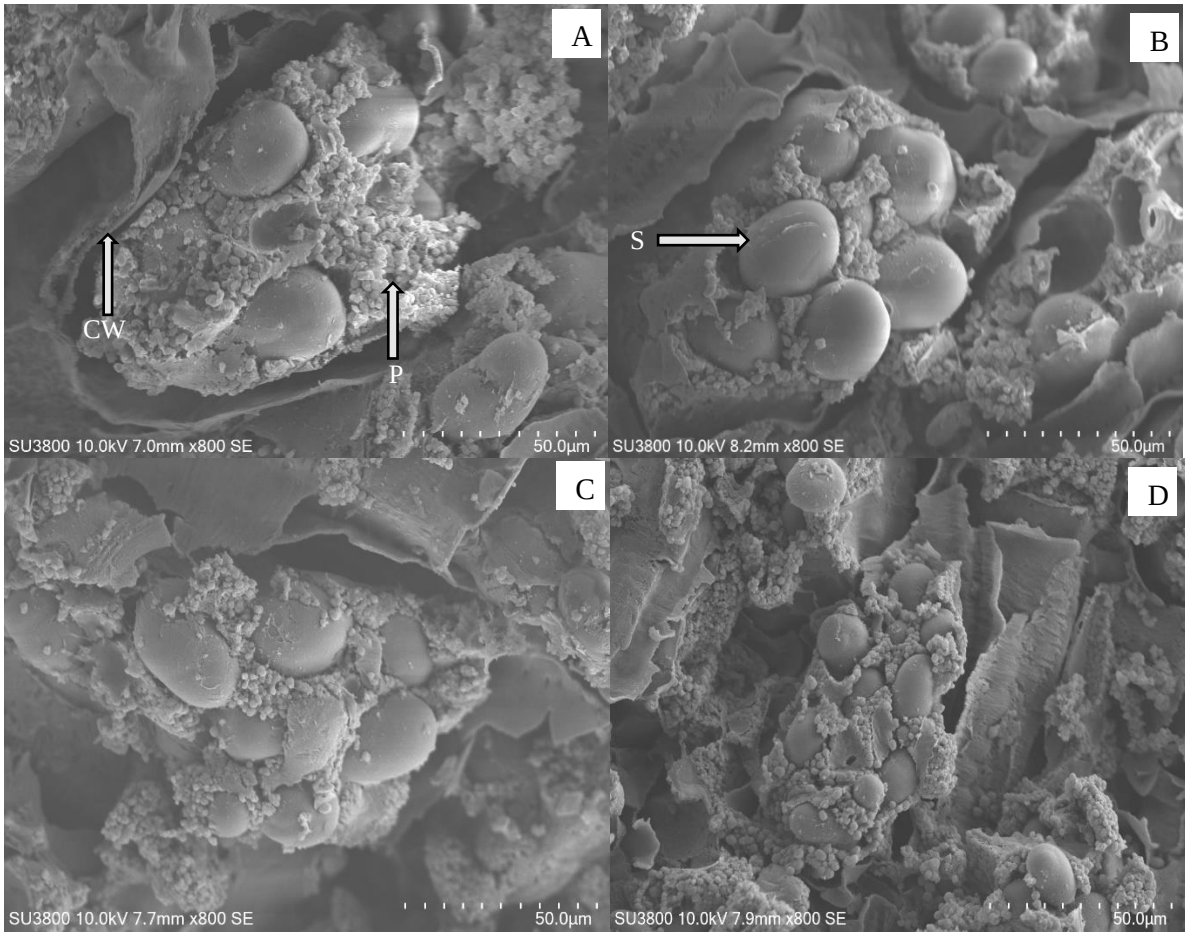


Fig. 2. Cross section of the four faba bean seed varieties through SEM at x800 magnification. A, B, C and D represent Early long pod, Evergreen, Coles dwarf and Janet varieties, respectively. Note: S: starch granule, P: protein bodies, and CW: cell wall. Image J software was used to study the images for the reported values of cross-sectional of the faba bean samples.

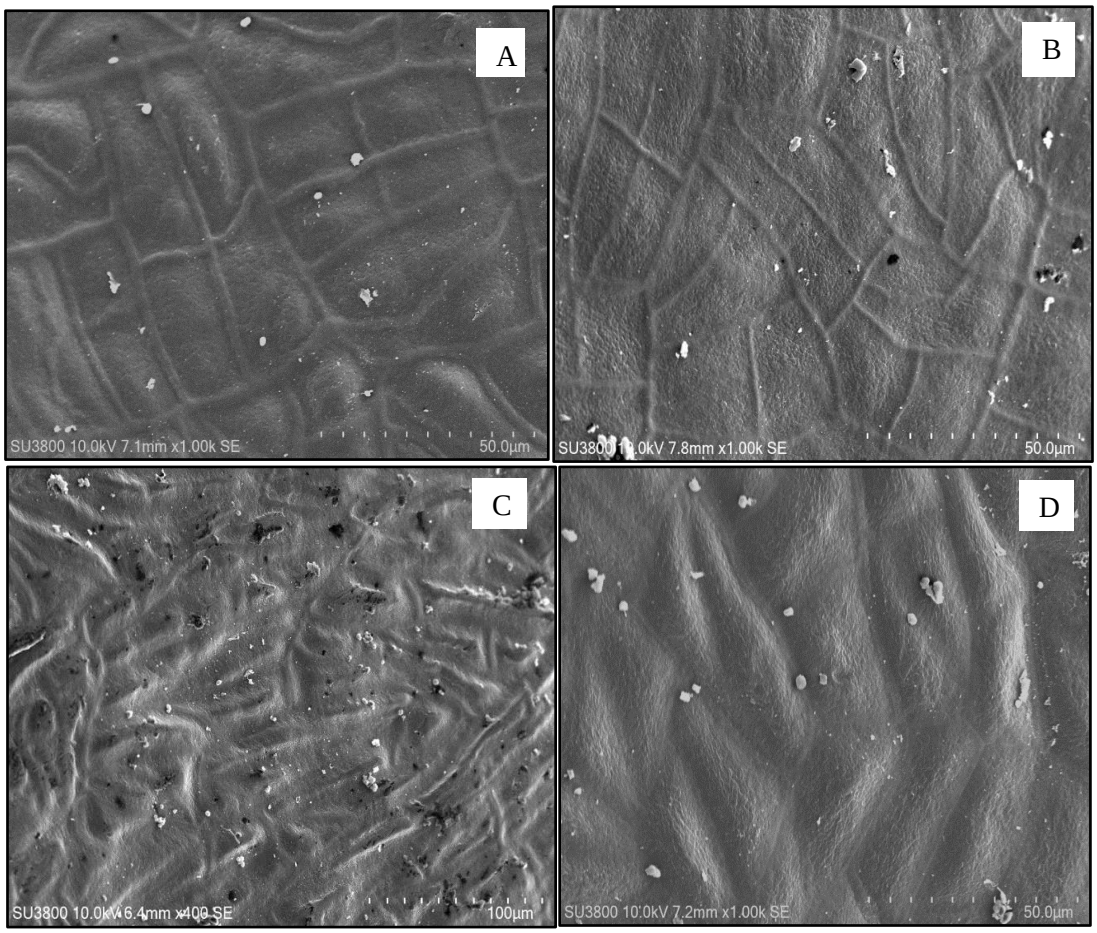


Fig. 3. Cotyledon surface topography of the four faba bean seed varieties. A, B, C and D represent Early long pod, Evergreen, Coles dwarf and Janet varieties, using SEM at x1000, x1000, x400 and x1000 respectively.

3.3.6. Protein content

The protein content of the four varieties was quantified as shown in **Table. 7**, the C variety had the highest protein percentage (27.49%), which was significantly higher than D (25.38%), A (24.81%), and B (24.81%), all of which had no significant difference in protein contents. These results for protein content were within the range reported by (Dhull et al., 2022). The morphological traits unique to a given species of broad bean may be connected to the variance in protein content among several varieties. The generation of protein in beans can also be influenced by the growth environment, including temperature, soil composition, and genetic diversity. Fertilization and irrigation are two agricultural methods that can affect

protein levels (Walter et al., 2022; Martineau-Côté et al., 2022). It would be preferable to use a bean variety with a higher protein content as a plant-based protein source. As the beans were ground manually and not traditionally dehulled, it was necessary to quantify the amount of starch and insoluble dietary fibre in the flours. When tested for carbohydrate content of the flours, the varieties showed insoluble dietary fibre in the range of 9.7-10.2% and starch in the range of 35.3-39.1%, which was in the range as discussed by (Badjona et al., 2023). Temperature, water availability, and soil fertility can all affect how much protein and starch are in broad beans. While the protein level may somewhat drop as the beans grow the starch amount usually increases. For some food processing applications, like flour or protein isolates, a lower starch content can be advantageous. Because faba beans have a high starch content and give livestock energy, they have long been an essential component in animal feed. The seed coat of faba beans contains over half of the dietary fibre content, which is crucial for gut health and general wellbeing. It supports a healthy digestive system, lowers blood sugar levels, and may even lower the chance of developing some chronic illnesses (Chaudhary et al., 2022). To conclude, one may evaluate other functional characteristics of the variety and observe whether they support its intended uses.

Table 7. Important nutritional values for the four faba bean flours varieties.

Faba bean variety	Nitrogen %	Crude protein %	Starch %*	Insoluble dietary fibre %*
Early long pod (A)	3.96 ± 0.07^{a}	24.81 ± 0.51^{a}	39.10	10.20
Evergreen (B)	3.96 ± 0.03^{a}	24.81 ± 0.22^{a}	37.80	9.70
Coles dwarf (C)	4.39 ± 0.007^{b}	27.49 ± 0.06^{b}	35.30	10.30
Janet (D)	4.06 ± 0.01^{c}	25.38 ± 0.07^{a}	38.40	10.20

All values for nitrogen% and crude protein % are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements from each variety). Values within a column not having a common superscript differ significantly ($p<0.05$). Starch %* and insoluble dietary fibre* values were not tested in replicates. 6.25 was used as factor for coverting nitrogen % to crude protein % (x 6.25).

3.3.7. Pasting properties

Variety D had significantly higher peak viscosity (693cP) at a pasting temperature of 77.11°C and peak time of 8.33 minutes, as seen in the below **Fig. 4**. Peak viscosity is a sign of the starch's high water-absorbing and holding capacity, which causes the gel to become thick and viscous when heated and cooled. The temperature rises above the starch's gelatinization point, causing the starch granules to swell and the viscosity to rise quickly (creating a peak). The term "pasting temperature" refers to the temperature at which viscosity begins to develop and paste forms as the amylose leeches out of the starch granules (Liang & King, 2003; BeMiller, 2011). This pasting temperature, which is roughly 76-77°C for all varieties, is essential to comprehend the behaviour of starch gelatinization and can give light on the minimum temperature required to cook a flour and the functional characteristics of starch in a range of applications. At the holding stage a decrease in viscosity was found as the melting of the crystalline regions of starch granules enabled rapid water movement into the granules causing a breakdown viscosity. A reduced breakdown value was detected since the peak did not decline at a rapid rate. This could mean that the sample's starch was resilient enough to endure the continuous high heat and shear stress and led to a decrease in the rate of rupturing of starch granules (Balet et al., 2019). The re-association of amylose with the cooled sample as the starch granules were cooled and retrogradation occurred which caused a gel to develop because of recrystallisation of amylose chains and a sharp rise in viscosity that resulted in the final viscosity. Variety A, had a significant low peak time, indicating a faster rate of thickening/pasting, and the thickest gel (1078.70 cP as final viscosity) at the end of the run compared to other variations which was caused due to disintegration of the granules. A higher setback value for variety A, C and D indicated a higher tendency of the starch to retrograde (Sandhu & Singh, 2006).

Table 8. RVA analysis results for the four faba bean flour varieties.

Parameter	Faba bean variety			
	Early long pod (A)	Evergreen (B)	Coles dwarf (C)	Janet (D)
Peak 1 (cP)	620 ± 13.11[a]	591 ± 15.72[b]	584 ± 15[b]	693 ± 28[c]
Trough 1 (cP)	592 ± 18.3[a]	573 ± 19.1[ab]	546 ± 23.80[b]	663 ± 43.7[c]
Breakdown (cP)	28 ± 5.29[a]	17.67 ± 7.57[a]	38 ± 15.87[a]	29.67 ± 16.80[a]
Final Viscosity (cP)	1079 ± 71.8[a]	962 ± 51.2[a]	1045 ± 39.8[a]	1138 ± 109.5[a]
Setback (cP)	487 ± 64.6[a]	389 ± 32[b]	499 ± 16.82[a]	475 ± 65.8[a]
Peak Time (min)	7.82 ± 0.04[a]	8.22 ± 0.08[b]	8.02 ± 0.08[b]	8.33 ± 0.26[b]
Pasting Temp (°C)	76.21 ± 0.03[a]	76.83 ± 0.54[a]	77.11 ± 0.03[a]	77.11 ± 0.83[a]

All values are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements from each replicate). Values within a row not having a common superscript differ significantly ($p<0.05$).

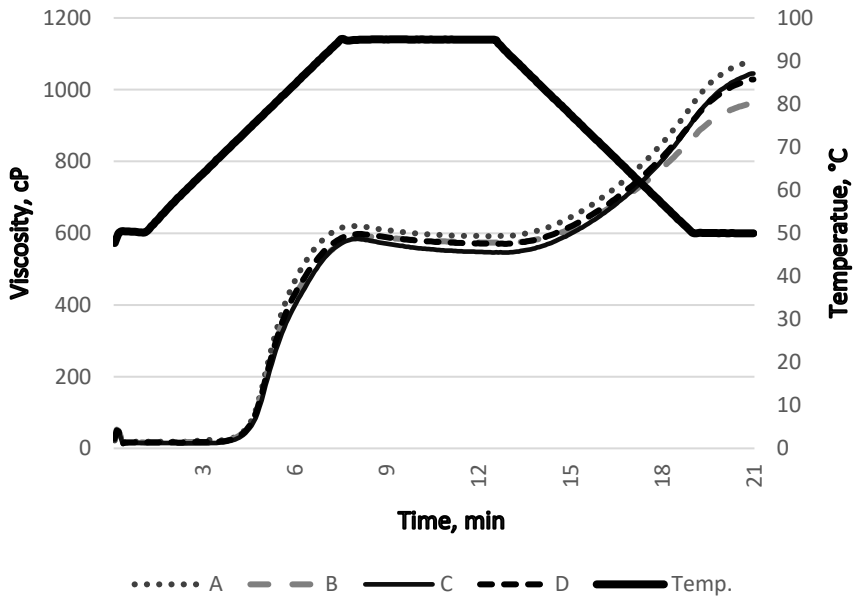


Fig. 4. RVA pasting graphs for the four faba bean flour varieties. A, B, C and D represent Early long pod, Evergreen, Coles dwarf and Janet varieties, respectively.

The results of the RVA tests conducted on the faba bean flour samples provided insight into the fact that the starch did not experience adequate breakdown or viscosity reduction throughout the holding period, which could have resulted in a greater final viscosity and affected the overall viscosity profile (Balet et al., 2019). In many industries, such as food

processing, where the ideal viscosity level is critical to the quality of the finished product, this could have an impact on the texture, consistency, and performance of the starch.

3.4. Conclusion

The physical and micro-structural properties of the four native faba bean varieties—Early long pod, Evergreen, Coles dwarf, and Janet—were examined. The Evergreen variety displayed a noticeable colour difference (green) from the other types (brown) however, the physical dimensions of the various varieties did not differ considerably from one another. When the beans were cooked, the Evergreen and Coles dwarf varieties required a far less cooking time than the Janet and Early long pod varieties. Soaking the beans ahead of time cut down on cooking time by nearly a third. Positive Pearson correlation coefficient values were observed between seed weight, volume, cotyledon cell wall thickness, number of starch granules per cell with the cooking time (**Table iv.**) (**Appendix C)**. Coles dwarf had significantly lower cell wall thickness which could explain the low swelling and hydration capacity. Coles dwarf also had higher protein content than the other varieties. The bean flours when subjected to cooking temperatures and shear conditions implied that higher gelatinization of starch occurred at peak pasting temperatures. The pasting temperatures did not vary significantly among the varieties; however, Janet showed a significantly higher peak viscosity which could be explained by the higher hydration and swelling capacities resulting in a thicker and viscous gel during cooking.

Chapter 4. Extraction and characterization of faba bean protein concentrates

4.1. Introduction

Because of the accompanying health and nutritional benefits, interest in plant-based diets has been steadily rising among consumers, scientists, and other organizations. In addition to these advantages, the manufacturing of plant proteins uses less energy and resources than that of animal proteins. Many extraction strategies have been developed for different protein sources with the goal of improving our understanding of the physical and chemical properties of faba beans and increasing the availability and efficacy of the crops of significant interest (Sharan et al., 2020). According to their solubility in various solvents, the proteins found in faba beans can be categorized as follows: albumins (20%), globulins (salt soluble) (60%), glutelins (soluble in NaOH solution) (15%), and prolamins (alcohol soluble) (8%). The globulins have a significant role in the storage proteins found in legumes, influencing the rheological and textural characteristics of the proteins (Dangi et al., 2022).

With a primary goal of extracting protein concentrates from bean flour, this study examined the commercial variety of faba/broad beans that are widely available in the New Zealand market. As the literature mentioned above indicates, faba beans, a significant source of plant-based protein, provide advantages that almost entirely outweigh the drawbacks of animal proteins (Eze et al., 2022). The initial objective of this research was to provide a focused overview of the various wet fractionation techniques used to extract protein concentrates from broad beans and highlight the differences between them. These techniques include neutral or water extraction, acid extraction, and alkaline extraction. This study's second objective was to characterize the extracted protein concentrates according to their functional features, variations

in denaturation temperatures, colorimetric disparities, and the effect of strain on the protein dispersions. The protein concentrates were also studied to characterize the proteins present in the concentrates and their variations depending on extraction methods and the addition of certain reducing agents. The primary objective of this section of the study was to determine the optimal technique for extracting legume protein concentrates based on these factors of yield, protein concentration, and functional characteristics. Additionally, other experiments were conducted to gain insight into potential uses for these protein concentrates, with the goal of promoting their future widespread use and scaling up in the food industry.

4.2. Material and methods

4.2.1. Materials

The commercial varieties of the faba beans were procured from Davis Trading Co., Palmerston North, New Zealand. The dry broad bean seeds were stored in a cool (21℃) and dry place under further studies. The broad beans were weighed, ground (Coffee Grinder Model BCG200, Breville, Pty Ltd., Sydney, Australia) and sieved through a fine-mesh sieve (B.S. 410/I.S.O. 3310-1:2016, Glenammer Sieves, Ayrshire, United Kingdom) to remove the bigger husk components and collect the bean flour. This flour was weighed and stored in a zip-lock bag, away from sunlight at 21°C until further processing.

BB

Fig. 5. Commercial variety of broad bean (BB).

4.2.2. Methods

4.2.2.1. Protein extraction methods

Methods for protein extraction from faba beans were followed using methods established by (Penchalaraju et al., 2022).

4.2.2.1.1. Water extraction method. 25g of whole seeds were combined with 250ml of water and blended using a blender (Zip Blender White 500W, Model Number: Zip231) to form a slurry. The slurry was stored at 4-8°C overnight. The slurry was then centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) at 4°C, 2000 x g for 15 minutes, the supernatant and pellet were collected, and the pellet was resuspended in water and centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) again at 4°C, 2000 x g for 15 minutes. The pellet was discarded, while the supernatant was collected to be freeze dried to form a powder. The powder obtained was then stored in a vacuum package, air-tight container, or zip-lock bag at 4°C (Penchalaraju et al., 2022). To obtain a fine powder, the sample was then finely ground using a mortar and pestle before use for any further experimentations.

4.2.2.1.2. Alkaline extraction method. This method involved weighing 10g of bean flour, adding 100 ml water to create a suspension (1:10 flour to water ratio), adjusting the pH to 9.0 with 1M NaOH, and keeping the suspension in a 250 ml beaker at room temperature for 3 hours. The suspension was then transferred to 50 ml centrifuge tubes and centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) at 4°C, 2000 x g for 15 minutes, and the supernatant was collected in a 250 ml beaker. The pellet was collected in a separate 250 ml beaker and resuspended in distilled water and adjusted to pH 9.0 for alkaline extraction with 1M NaOH, kept for 30 minutes at 25°C and then centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) at 4°C, 2000 x g for 15 minutes. The pellet is discarded, and the total supernatant collected was then adjusted to pH of 4.5 for isoelectric precipitation with 1N HCl,

and centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) at 4°C, 2000 x g for 15 minutes. The supernatant was discarded, and the pellet was then washed with water and freeze dried to form powder. The powder obtained was then stored in a vacuum package, air-tight container, or zip-lock bag at 4°C (Penchalaraju et al., 2022). To obtain a fine powder, the sample was then finely ground using a mortar and pestle before use for any further experimentations.

4.2.2.1.3. Acid extraction method. This method involved weighing 10 g flour, stirring it with 100ml water to create a suspension and adjusting the pH to 2.0 for acid extraction using 1N HCl. The suspension was kept at room temperature overnight and centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) at 4°C at 2000 x g for 15 minutes. The supernatant and pellet were collected. The pellet was collected in a separate 250 ml beaker and resuspended in distilled water, following which, the pH was adjusted to 2.0 for acid extraction, kept for 6 hours and centrifuged again under the same conditions. The pellet was discarded, and the supernatant collected and freeze dried to form a powder which was stored at 4°C (Penchalaraju et al., 2022). To obtain a fine powder, the sample was then finely ground using a mortar and pestle before use for any further experimentations.

4.2.2.1.4. Protein content. The crude protein and nitrogen content of the faba bean protein concentrate obtained from the three methods was estimated using Kjeldahl system (Kjeltec 2100 System, Tecator, Sweden), using 6.25 as the conversion factor from nitrogen % to protein %. Each sample was tested in triplicates in addition to two blanks. The protein content was calculated from the nitrogen % using the following formula:

Protein purity % = Nitrogen % x Conversion Factor (12)

Protein recovery % = Mass of protein extracted (g) / Total mass of protein in starting material (g) x 100 (13)

Protein yield % = Mass of protein extracted (g) / Total mass of flour for extraction (g) x 100 (14)

4.2.2.2. Colorimetry

The faba bean protein concentrates were interpreted in a chromameter (Chroma Meter CR-400, Konica Minolta, Inc., Tokyo, Japan) to study the measurement of colour and their differences based on the L*, a*, b* coordinates. The colorimeter consisted of a measuring head and a data processor. The beans were placed on the measuring head with a white background. The L*, a*, b* coordinates were selected on the data processor and on a scale of zero to one hundred, L* denoted lightness from black to white, whereas a* and b* denoted chromaticity without numerical bounds. It is known that negative a* is associated with green, positive a* with red, negative b* with blue, and positive b* with yellow. Three replicates were performed on each protein concentrate to obtain accurate results. The total colour was calculated as a range of dark to light, more green or red and more blue or yellow according to these values.

4.2.2.3. Functionality tests

Methods for performing various functionality tests were followed using methods established in a recent study on functionality of faba bean protein isolates by Shi et al. (2022), with some variations as mentioned below.

4.2.2.3.1. Water absorption capacity. In centrifuge tubes that had been previously weighed, 1.5 g of protein isolate, and 50 mL of distilled water were combined by vortex for two minutes at maximum speed of 500 rpm. Subsequently, the tubes underwent a 30-minute centrifugation (Multifuge X Pro Series, Thermo Scientific, NZ) at 3000 × g to separate the protein from the supernatant (Badjona et al., 2024). The protein isolate-containing centrifuge tubes were reweighed after the supernatant was carefully decanted following centrifugation.

Grams of water absorbed per gram of sample on wet basis was the unit of measurement for water absorption capacity, or WAC, expressed as g water/g protein (%). The following formula was used to determine the WAC:

$$WAC\ (g\ H_2O) = (W_2\text{-}W_1)/W_0 \times 100 \quad (15)$$

Where, W_2 = Weight of tube plus the sediment (g), W_1 = Weight of the tube plus dry sample (g), and W_0 = Weight of the dry sample (g)

4.2.2.3.2. Oil absorption capacity. An empty 10 mL centrifuge tube was weighed which was then filled with 500 mg of protein isolate and 5 mL of rice bran oil. To ensure the completer dispersion of the sample in the oil, the tubes were shaken in a vortex for two minutes, at max speed and then left at room temperature for 30 minutes. Finally, they were centrifuged (Multifuge X Pro Series, Thermo Scientific, NZ) for 30 minutes at 3000 × g (Badjona et al., 2024). The clear oil (supernatant after centrifugation) was decanted by inverting the tubes for twenty-five minutes. The tubes were then reweighed on wet basis. The amount of oil absorbed per gram of protein isolate is expressed in terms of grams. The following equation was used to calculate the Oil Absorption Capacity (OAC) expressed as g oil/g protein (%):

$$OAC\ (g\ Oil) = (O_2\text{-}O_1)/O_0 \times 100 \quad (16)$$

where O_2 = Weight of tube plus the sediment (g), O_1 = Weight of the tube plus dry sample (g), and O_0 = Weight of the dry sample (g)

4.2.2.3.3. Protein solubility. The protein solubility was tested at a neutral pH according to the methods established by Shi et al. (2022) and Vogelsang-O'Dwyer et al. (2020) on faba bean protein ingredients and with some modifications as shown below. A protein concentrate of 0.2 g was mixed with 19 ml of water and the pH was adjusted to 7.0 using 0.5 N NaOH. Similar protein dispersions were made with acid and basic pH ranges from 3.0 to 8.0 with 0.5

intervals using 0.1 N NaOH and 0.1 N HCl. A magnetic stir plate was used to agitate the solutions for one hour at room temperature at 500 rpm. To encourage precipitation, the whole solution volume was raised to 20 g with water and let to rest for 10 minutes. The solution above the precipitate was separated into an aliquot (~10 g) for each sample, which was centrifuged at 4000 x g for 10 minutes at room temperature using a centrifuge (Multifuge X Pro Series, Thermo Scientific, NZ). Using a Kjeldahl system (Kjeltec 2100 System, Tecator, Sweden), the protein concentration of the supernatant (~5 g) was determined. The protein concentration in the supernatant was divided by the protein content in the original sample (×100%) to get the percent protein solubility (Shi et al., 2022).

4.2.2.3.4. Foam capacity and foam stability. The protein concentrates 1% (w/v) (500 mg in 50 ml water) were dispersed into 50 mL of distilled water using a Waring blender (Zip Blender White 500W, Model Number: Zip231, Australia) at the maximum speed (~10000-15000 rpm) for 5 min and then immediately transferred into a graduated cylinder. The sample volume was recorded before and after whipping. The foaming capacity was determined with the equation given below:

$$FC = (V_2\text{-}V_1)/V_1 \times 100 \quad (17)$$

Where, V_1 = volume of protein solution before whipping, and V_2 = volume of protein solution after whipping

The foam stability was determined by measuring the change in foam volume after 10, 20, 30, 40 and 0 min of standing time at room temperature. The stability was calculated by using the following equation:

$$FS = (V_t/V_0) \times 100 \quad (18)$$

Where, V_t = foam volume at time t, and V_0 = initial volume of the dispersion

4.2.2.4. Differential scanning calorimetry

The sample preparation for Differential Scanning Calorimetry (DSC) involved hydrating the samples with water according to their concentration. The acid extracted protein concentrate (AE) was hydrated using 300 mg of protein concentrate in a 5 ml beaker and adding 0.2 ml of distilled water to make a paste and kept for a few minutes to hydrate the sample completely. The same is done for other protein concentrates extracted by alkaline extraction (AL) using 970 mg in 2.85 ml distilled water and water extraction methods (WE) using 500 mg in 0.3 ml distilled water. The DSC used 2 aluminium pans, one standard pan and one pan containing the sample. The reference pan and lid were weighed using an analytical balance and noted before preparing the sample pans. The sample pan and lid were weighed, and the empty pan mass was noted. The sample pastes were stirred thoroughly using a micro-spatula to ensure complete hydration and homogeneity of the sample. 9-14 mg of the hydrated samples were weighed in the pan and the total weight was noted before the run. The aluminium lid was placed on the pan with the help of tweezers and sealed shut using a Tzero Press. The QDSC instrument (Alphatech QDSC 2000, Alphatech Systems Limited, Auckland, NZ) was setup by turning on the N_2 gas and set to a pressure of 120 kPa. The instrument was turned on according to the QDSC Quick Start Guide. The experimental method was setup in the software on the system with the weights of the empty pan, the sample weight and the temperature range and rate of heating (i.e., 25 to 120°C @ 5°C/minute). The lid was opened using the controls on the software and the reference and sample pans were placed in the chamber of the QDSC. The lid was closed, and the sample was made to run with the set parameters. The resultant graph could be seen after the run was complete with specific peaks for the samples. The lid was opened from the controls and the first sample pan was removed and weighed again to measure the weight lost after denaturation to run further samples.

4.2.2.5. Rheology

Protein samples were prepared using 500 mg of water extracted protein concentrate mixed in 0.3 ml of distilled water, 900 mg of acid extracted protein concentrate mixed in 0.6 ml of distilled water, and 970 mg of alkaline extracted protein concentrate mixed in 2.85 ml of distilled water. The concentrations of the protein samples were similar to those for used in section 4.2.2.4. The samples were defrosted to room temperature prior to use after being hydrated over night at 4℃. With a cone and plate geometry (CP10-4) and a gap size of 0.05 mm, the rheometer (Model MCR 301, Anton Paar, NZ) was used to evaluate the strain sweep studies on the protein dispersions. Additionally, the rheometer had a water bath with precise temperature control. The variations in the elastic (G′) and viscous (G") moduli as a function of strain (0.1% to 100%), providing 41 measuring points, were measured at a constant frequency of 1 Hz and temperature of 25°C in order to calculate the linear viscoelastic region (LVE). The The sheer stress at which the moduli are independent of the increasing stress is known to understand the LVE (Stojkov et al., 2021). The discussion will be based on the corresponding maximum strain value over which the curve drops (referred to as the "range of LVE" in the following) in order to enable quantitative comparison of the strain sweeps. Higher LVE range values signify a more elastic network, meaning the material is more resilient and strain resistant. Each sample was measured in triplicates to get precise results (Wittek et al., 2021).

4.2.2.6. Tricine-sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The protein concentrates extracted from section **4.2.2.1** were used to carry out Tricine SDS-PAGE. The sample solutions were prepared using 100 mg of concentrate in 1 ml of distilled water. These solutions were diluted using 5 µL of the sample with 95 µL to achieve 100 µL solution. These were further diluted with equal volumes of sample buffer (Tricine

sample buffer, Bio-Rad Laboratories Pty Ltd., NZ) with a set including 2% β-mercaptoethanol and a set of samples without β-mercaptoethanol. This was followed by heat treatment at 60°C for 10 minutes. Samples were stored in the freezer until electrophoresis was performed.

20 µL of samples and 10 µL of the prestained protein standard (Precision Plus Protein™ all blue prestained protein standards, Bio-Rad Laboratories Pty Ltd., NZ) were loaded into a pre-cast gel (4-20% Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 50 µL, Bio-Rad Laboratories Pty Ltd., NZ). Tricine SDS-PAGE was carried out with a 1x running buffer (10x Tris/Tricine/SDS buffer, Bio-Rad Laboratories Pty Ltd., NZ), at 125V for about 45 minutes until the blue colour was almost about to run out of the gel. Afterwards, the gels were rinsed with RO water and stained with Coomassie stain (Bio-Safe Coomassie G-250 Stain, Bio-Rad Laboratories Pty Ltd., NZ) for one hour, followed by rinsing with RO water for 30 minutes until the bands were clear. The gels were kept in RO water at 4°C until observation. The images of the gels were captured and quantified using a gel scanning densitometer (Molecular Imager Gel Doc XR, Bio-Rad Laboratories Pty Ltd., NZ) and the bands were analyzed using Image Lab™ software version 6.0.0 (Bio-Rad Laboratories, Hercules, CA, USA). The densitometer results for molecular weight and band intensity were used to study the protein characterisation among the extracted protein concentrates for both reducing and non-reducing conditions.

4.2.2.7. Statistical analysis

The protein content, functional properties, denaturation, rheological and SDS-PAGE experiments were run in triplicates for each protein concentrate extracted. Using Minitab 21 statistical software (Minitab LLC, Chicago, USA), all the data for average mean and standard deviation were statistically analyzed by one-way analysis of variance (ANOVA) at $p \leq 0.05$. Image Lab™ software version 6.0.0 (Bio-Rad Laboratories, Hercules, CA, USA) was used for the analysis of the bands after the SDS-PAGE runs.

4.3. Result and discussion

4.3.1. Protein purity, yield, and recovery after extraction

Using the Kjeldahl method and a conversion factor of 6.25 to obtain the protein percentage from the nitrogen percentage, the protein content in the protein concentrates recovered from the commercial broad bean (BB) samples by three extraction methods was determined. The findings revealed a significant variation in protein purity levels between the water extraction (57.37%), acid extraction methods (55.63%) and the alkaline extraction (67.98%) ($p < 0.05$). The yield of protein after extraction ranged between 16.13% and 20.57%. The lowest was observed in acid extraction followed by water extraction methods and the highest yield in alkaline extraction. Similar trends followed for protein recovery as the highest amount of protein was recovered from alkaline extraction (55.27%) and the lowest from acid extraction (**Table 9**). These findings suggest that the alkaline extraction technique was superior in terms of separating and purifying the proteins from the sample (Penchalaraju & Don Bosco, 2022). Protein structure, amino acid concentration, processing conditions, and environmental factors all affect studies on bean protein extraction, solubility, and yield. Higher extraction yields are obtained from alkaline environments because they promote the breaking of chemical bonds, which increases protein dispersion and segregation (Cruz-Solis et al., 2023). Water, a relatively mild solvent that can dissolve some proteins, is used in the water extraction procedure. However, it does not break the protein-protein connections that keep the protein in the broad bean matrix as well as alkaline conditions do. Acidic extraction conditions have the potential to denature proteins, causing them to unfold and perhaps impair their structural integrity. In acidic environments, certain proteins can precipitate and solidify into clumps, which can further limit the amount of protein that can be recovered (Mesieres et al., 2021) These findings clearly indicate that the alkaline extraction method should be used to extract the

protein concentrate from the New Zealand variety for functional research. The aforementioned findings have also been confirmed by other investigations. Badjona et al. (2023) concluded that although the protein content of faba bean concentrates was typically 56%, alkaline extraction techniques have been known to increase it by roughly 10-15%.

Table 9. Protein purity, recovery, and yield of the faba bean protein concentrates by three extraction methods.

Method of extraction	Nitrogen %	Protein purity %	Protein yield %	Protein recovery %
Water extraction	9.17 ± 0.04^{a}	57.37 ± 0.24^{a}	18.69 ± 0.58^{a}	42.38 ± 1.45^{a}
Acid extraction	8.89 ± 0.05^{b}	55.63 ± 0.34^{b}	16.13 ± 0.55^{b}	35.47 ± 1.28^{b}
Alkaline extraction	10.87 ± 0.05^{c}	67.98 ± 0.34^{c}	20.57 ± 0.38^{c}	55.27 ± 1.03^{c}

All values are reported as the mean ± SD, where N=3 (3 replicates from each extracted concentrate). Values within a column not having a common superscript differ significantly ($p<0.05$).

4.3.2. Colorimetry

The protein concentrations that were extracted using various extraction methods produced powders with a range of colour variations. Colorimetric assays provide the measurement of colour intensity resulting from particular protein reactions. The amount of protein contained in the concentrates can be estimated by measuring the colour intensity, which is useful for evaluating the nutritional value and quality of the protein concentrates as well as for comparing various extraction techniques. Based on the results, the acid extracted concentrate has the lightest, green hues (a* of 0.15, b* of 12.88, L* of 58.44). While the water extracted concentrate has the reddest hues (a* of 1.77), the alkaline concentrate has somewhat deeper colour. The a* values were all significantly different from each other ($p < 0.05$), whereas no significant difference was found in L* for alkaline and acid extraction methods, and b* values for all three methods (**Table 10**). However, L* values for acid extraction show significant difference implying the lighter hues of the final extracted protein concentrate. In

food systems that can employ specific colour grades as a crucial quality control parameter throughout manufacturing, assuring consistency and fulfilling regulatory requirements, colorimetry of protein concentrates is vital. More proteins become soluble at low pH levels during acid extraction, which also lightens the extract's colour (Mesieres et al., 2021). The light colour of the protein concentrates may be result of the inherent light colours of the beans or due to the various chemicals used during the extraction process. An increase in pH results in an increase in the extract's protein production. Dark-colour protein fractions can be produced when phenolic compounds interact with protein leading to the formation of brown pigments (Yu et al., 2023).

Table 10. Colorimeter results for the faba bean protein concentrate obtained from three extraction methods.

Method of extraction	Acid extraction	Alkaline extraction	Water extraction
L*	58.44 ± 0.84^{a}	55.65 ± 1.31^{b}	56.94 ± 2.40^{b}
a*	0.15 ± 0.19^{a}	1.04 ± 0.24^{b}	1.77 ± 0.11^{c}
b*	12.88 ± 0.62^{a}	14.21 ± 1.28^{a}	14.51 ± 1.20^{a}

All values are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements from each concentrate). Values within a row not having a common superscript differ significantly ($p<0.05$).

4.3.3. Functionality tests

4.3.3.1. Water absorption capacity

The protein concentrate extracted using the alkaline extraction method has the maximum Water Absorption Capacity (WAC) at 111.40, followed by that of the water extraction method at 77.29, and the lowest in the acid extraction method at 40.80 (**Table 11**). It seems reasonable that the bean proteins would be more effectively broken down by the alkaline extraction process, enabling the beans to absorb more water and increasing their capacity to do so (Cruz-Solis et al., 2023). The protein structure may be impacted by the acid

employed in the acid extraction process, which would reduce the protein's ability to absorb water. This would explain the lower water absorption. The water extraction method allows for better water absorption than acid extraction since it does not use extremely high or low pH levels. It also helps to preserve more of the native protein structure. The overall functionality of the protein concentrate can still be enhanced by using the water extraction approach. The final food product's texture can be greatly influenced by the WAC of food ingredients, such as broad bean protein powder. Higher WAC protein powders tend to absorb more water, which raises the moisture content of the product and makes it softer and more elastic (like bakery goods). Conversely, a lower WAC may lead to a texture that is drier. As a result, the WAC influences how the food tastes and behaves altogether (Miedzianka et al., 2023). Therefore, when selecting the extraction process, it is crucial to take the intended functionality and use of the protein concentrate into account.

4.3.3.2. Oil absorption capacity

The protein concentrate extracted by the alkaline extraction method has the highest Oil Absorption Capacity (OAC) at 178.50, which is the most among the three methods (and is significantly different from the acid extraction method with the lowest value being 58.17, and the medium value at 65.99 for the water extraction method, $p < 0.05$) (**Table 11**). According to these findings, alkaline extracted protein concentrates can absorb large amounts of water and oil. This could be because the alkaline environment helps improve hydration and emulsifying qualities. As a result, the proteins have a high capacity for absorption and can bind to both water and oil efficiently (Cruz-Solis et al., 2023). Since the acidic environment can lead to protein denaturation and aggregation, acid extracted concentrates typically have the lowest OAC levels. Low OAC may arise from this reduction in the protein's capacity to bind with oil, because protein solubility declines at low pH, making it difficult for the oil and aqueous phases

to interact effectively (Lima et al., 2023). Water extraction methods may not effectively solubilize and extract all the protein from the source sample, which can lead to a lower protein content and less potential for oil binding, resulting in a mediocre OAC. The OAC aids in determining the amount of oil that a protein or ingredient can absorb, influencing the texture, stability, and flavour of food, among other aspects. OAC is especially helpful in applications where the interaction of oil and protein is critical, such as batters, coatings, and emulsions (Miedzianka et al., 2023). Because of their superior absorption capacities across a range of media, alkaline extracted protein concentrate is therefore a better choice for use in food product formulations, as these data indicate. In food systems, the texture and general quality of food can be impacted by the use of acid extracted protein concentrates with low OAC. Food with low OAC may not be as crisp or have an even flavour distribution, which affects the food's overall acceptability to the senses (Chandra et al., 2014).

4.3.3.3. Protein solubility

The maximum protein solubility (63.75%) was observed in the protein concentrate recovered by acid extraction, followed by alkaline extraction (55.42%) and water extraction (49.49%) at pH 7, which were all significantly different from each other (**Table 11**). A similar trend was observed at other low and high pH ranges (**Fig. 6.**). The minimum solubility was observed at pH ~4.5, which can be corresponded as the isoelectric point. It can be stated that the solubility increased for the three samples at pH ranges before and after this isoelectric point. This can be explained by the increased electric charge repulsion, an increased protein-protein interaction and an increased interaction of water with the protein (Pelegrine et al., 2005; Ma et al., 2022). Additional findings about the behaviour of the protein extracts in different solvents can help to explain this. The majority of the protein concentrate solubilizes in the solvent and absorbs a small quantity during acid extraction, resulting in a high solubility, which can be

explained by the low water and oil absorption capacity (De Paiva et al., 2023). A more soluble protein and finer particle size, which promotes a stronger air-water interaction, may be the reason why acid extracted protein concentrates have the maximum volume and capacity of foams, as demonstrated by the foam capacity. During acid extraction, the protein's structure disintegrates at low pH levels, rupturing the links that keep the protein together and increasing its solubility (Mesieres et al., 2021). Since all the extracts used to measure protein solubility were brought to the same pH ranges, any disturbance of the protein during the actual protein extraction process could only produce inconsistent solubility results. According to studies by Cruz-Solis et al. (2023), alkaline extraction under high pH conditions led to the formation of insoluble protein aggregates, which could absorb more solvents and decrease protein solubility. This process resulted in larger particle sizes (Deleu et al., 2024). High solubility has general uses that could be advantageous for products that need protein to be easily absorbed or to improve texture. Furthermore, improved protein functionality—which is essential in food processing to achieve desired qualities like emulsification, foaming, and gelation—can be indicated by increased solubility (Pelegrine et al., 2005).

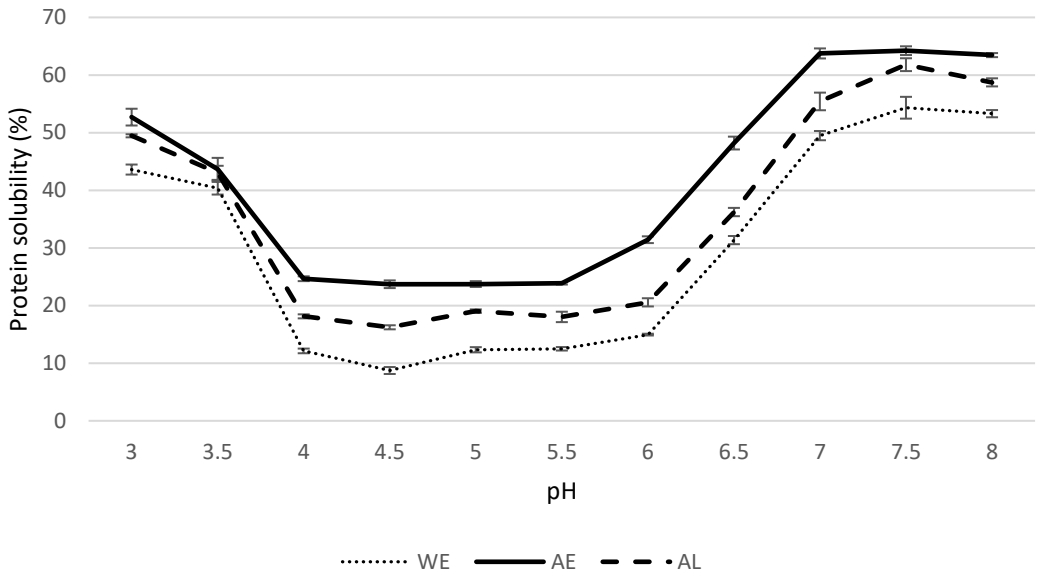


Fig. 6. The protein solubility (%) of faba bean protein concentrates obtained by three methods across a pH range of 3-8. Note: WE: water extracted, AE: acid extracted, AL: alkaline extracted.

4.3.3.4. Foam capacity and foam stability

The protein concentrate that was extracted utilizing the alkaline extraction method had the highest foam stability and the lowest percentage of foam capacity (8.83%) as well as the lowest average rate of collapse (0.08). The stability of foam can be impacted by the protein extraction method. Naturally, the intrinsic foaming characteristics of proteins vary. The protein structure and solubility can be greatly impacted by the pH conditions at the time of extraction (high solubility of protein concentrate extracted by acid extraction method, for example) (Moll et al., 2022). Because water and acid extraction procedures may preserve the ability of the protein to interact with water and air, which is essential for producing and stabilizing foam, they are more likely to produce higher foam capacities but also higher average rates of collapse. The proteins in these procedures might not aggregate or precipitate as much as they would in the alkaline extraction method, which would lead to a less dense and less stable foam and a higher collapse rate. A reduced rate of collapse is also typically associated with increased

protein concentration (Mesieres et al., 2021). For a variety of reasons, the alkaline technique produces foam that collapses less quickly, which is very desired in food systems. A less stable foam would collapse fast, losing its structure and volume, rendering the product less aesthetically pleasing and unusable. Certain foams, including whipped cream and meringues, provide food an appealing appearance for consumption. Additionally, mouthfeel and texture are enhanced by a consistent foam (i.e., light, and airy instead of flat and watery which is undesired). Stable foams in food systems saves manufacturers from significant costs due to less food spoilage (Deotale et al., 2023).

Table 11. Results for various functionality tests on faba bean protein concentrate extracted by the three methods.

Method of extraction	Water absorption capacity (g H_2O per unit 100g of protein conc.)	Oil absorption capacity (g Oil per unit 100g of protein conc.)	Protein solubility (at pH 7), %	Foam capacity, %	Average rate of foam collapse, ml/min
Water extraction	77.29 ± 14.60^{a}	65.99 ± 5.04^{a}	49.49 ± 0.80^{a}	19.94 ± 0.12^{a}	0.14 ± 0.04^{a}
Acid extraction	40.80 ± 3.35^{b}	58.17 ± 1.70^{b}	63.75 ± 1.53^{b}	29.83 ± 0.76^{b}	0.28 ± 0.05^{b}
Alkaline extraction	111.40 ± 20.20^{a}	178.50 ± 20.40^{c}	55.42 ± 0.87^{c}	8.83 ± 0.76^{c}	0.08 ± 0.02^{a}

All values are reported as the mean ± SD, where N=3 (3 replicates from each extraction method). Values within a column not having a common superscript differ significantly ($p<0.05$).

4.3.4. Differential scanning calorimetry (DSC)

The thermal stability of different proteins can be efficiently studied using DSC. The denaturation temperature was represented by the peak temperature (**Table 12**). The results show that water extraction method yielded protein concentrates with the highest denaturation temperature (peak temperature) of 106.16°C, followed by 91.61°C for alkaline extracted and

the lowest temperature of 75.76°C for acid extracted concentrates. These values differed significantly from each other ($p < 0.05$) (**Fig. 7(a), (b), (c)**). The stronger protein thermal resistance is demonstrated by the higher temperatures for denaturation. The larger difference of a high denaturation temperature of water extraction method can be explained by the fact that using water might have preserved the protein structure in its native state better than the acid or alkaline methods. Water extraction is a milder process that may result in less disruption to the protein's conformation, making it more stable and requiring a higher temperature for denaturation. The lowest denaturation temperatures in acid extracted faba bean protein concentrate could be due to alteration in the protein conformation and stability during extraction, making it more susceptible to denaturation at a lower temperature (De Paiva Gouvêa et al., 2023). In faba beans, the proteins legumin and vicilin denature at different temperatures. Legumin denatures at a temperature above 85°C, while vicilin denatures at a lower temperature, around 75-80°C. This could conclude that the peak temperature in acid extraction method represented the denaturation of vicilin, while the same in water and alkaline extraction method represented the denaturation of legumin (Bühler et al., 2021). The enthalpy of the reaction was obtained by integrating the area under the peak. A high or low enthalpy refers to the amount of heat absorbed or released during a phase transition or a chemical reaction. A low enthalpy suggests a shorter peak and a smaller amount of heat involved in the process (Ghanbari et al., 2023). In the context of protein denaturation, having low enthalpy can be beneficial in processes where controlled unfolding or alteration of protein structures is desired without excessive energy input. The enthalpy values varied significantly between the extraction methods, whereas no significant difference was observed between the offset temperatures between the three methods (**Table 12**). This controlled denaturation with low enthalpy can help in achieving specific modifications to proteins while minimizing unwanted side effects (Ghanbari et al., 2023; Bühler, et al., 2021).

Table 12. Thermal properties of the faba bean protein concentrate extracted by the three methods.

Method of extraction	Onset temperature, (°C)	Peak temperature, (°C)	Offset temperature, (°C)	Enthalpy, ΔH, (J/g)
Water extraction	97.23 ± 0.47[a]	106.16 ± 0.92[a]	113.86 ± 3.64[a]	0.6620 ± 0.0186[a]
Acid extraction	75.74 ± 0.97[b]	75.76 ± 0.96[b]	106.02 ± 5.54[a]	0.4704 ± 0.0048[b]
Alkaline extraction	75.29 ± 2.03[b]	91.61 ± 0.17[c]	108.46 ± 7.09[a]	2.3320 ± 0.1980[c]

All values are reported as the mean ± SD, where N=3 (3 replicates with 3 measurements from each replicate).

Values within a column not having a common superscript differ significantly ($p<0.05$).

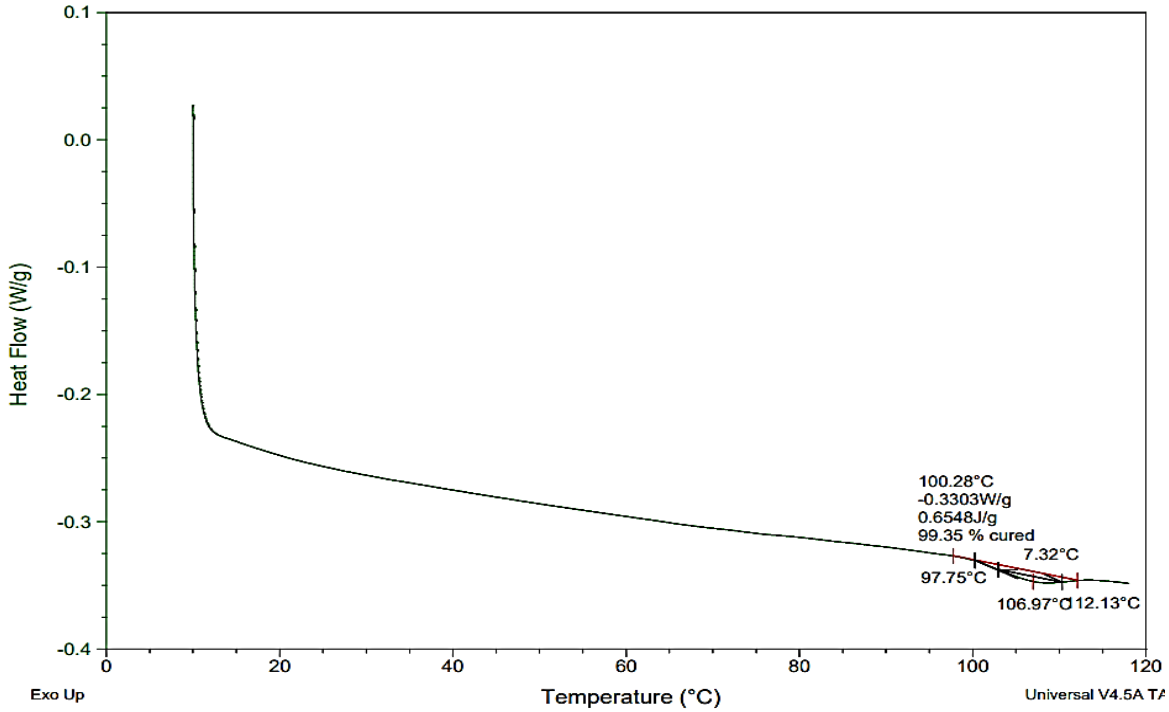


Fig. 7(a). DSC thermograph of water extracted (WE) faba bean protein concentrate.

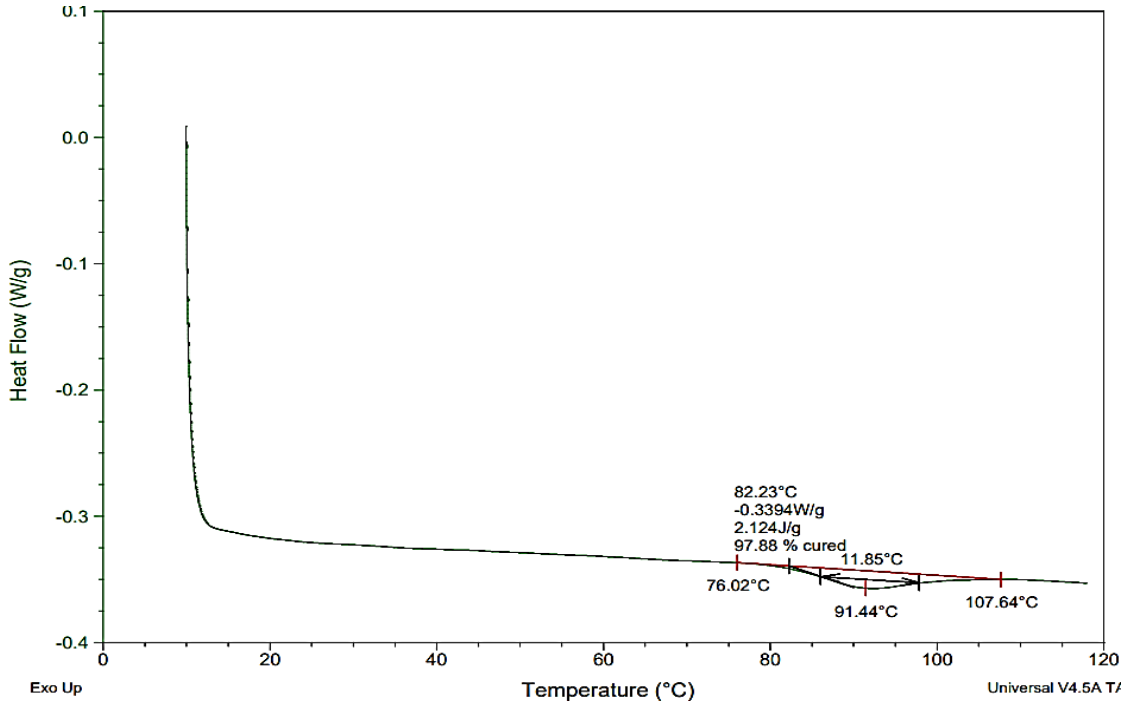


Fig. 7(b). DSC thermograph of acid extracted (AE) faba bean protein concentrate.

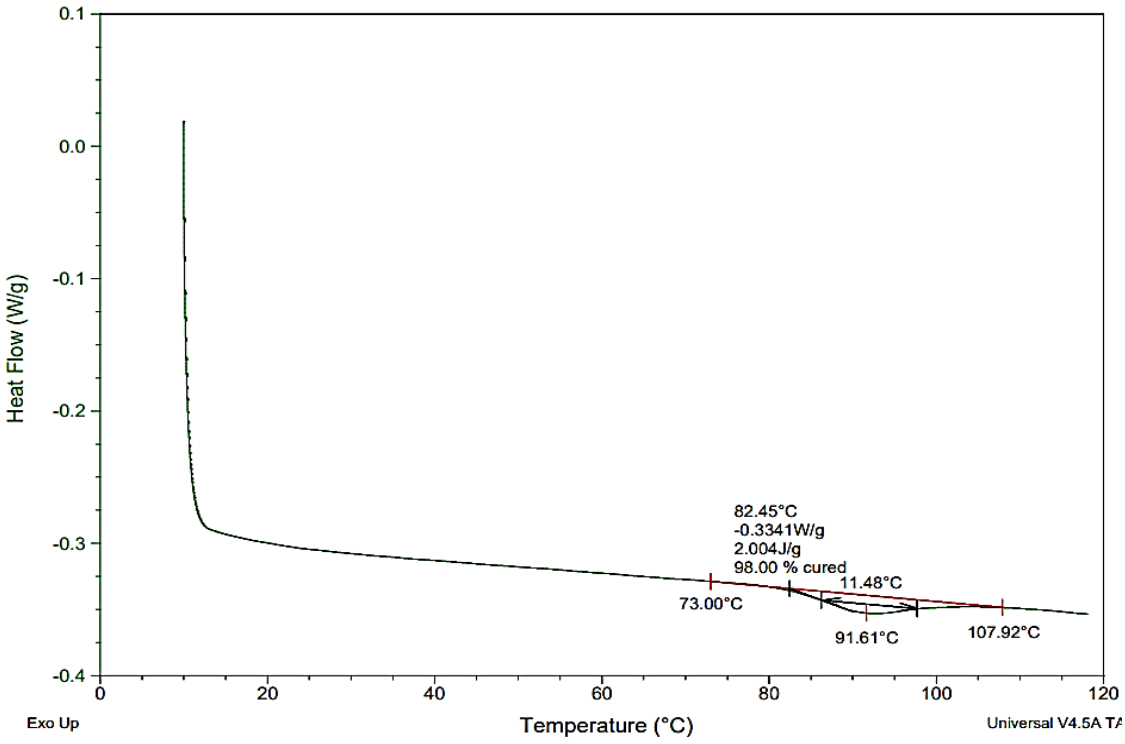


Fig. 7(c). DSC thermograph of alkaline extracted (AL) faba bean protein concentrate.

4.3.5. Rheology

At a fixed frequency of 1.0 Hz at 25°C, the strain-dependent rheological behaviour of the faba bean protein concentrates extracted by three different procedures was compared using strain sweep methods over a strain of 0.1% to 100%. **Fig. 8** showed the variation in the storage (G′) and loss modulus (G") with strain. The data for G′, G″ and the yield strain were provided by the rheometer, which were used to calculate the loss tangent which is a measure of the ratio of loss modulus to storage modulus (G″/ G′). The behaviour of the material at both the non-viscoelastic, or larger strains, and the linear viscoelastic, or small strains, could be studied by conducting strain sweep tests. Starting at extremely low shear stresses, the linear viscoelastic (LVE) area was identified. This region exhibits gel-like behaviour when storage modulus G′ is greater than loss modulus G" and both G′ and G″ values were significantly different for each extraction method ($p < 0.05$). G′ showed minimal to no dependence on strain in this area. The G′ sharply drops at high strain regions beyond the yield point, suggesting strain-thinning behaviour in the nonlinear viscoelastic range (nLVE) (Frihart & Gargulak, 2022). According to the results in **Table 13**, The pseudo-solid or gel-like structures for the three protein suspensions were explained by the G' value being higher than the G" value. The most flexible and elastic protein network was identified by the concentrates that were extracted using water, since they exhibited the highest range of LVE (29.90%). It was observed that both G′ and G″ were the highest for alkaline extraction followed by that of water extraction and the least for acid extracted faba bean protein concentrates. The alkaline extracted protein concentrate was shown to be both stiff and viscous under the applied strain conditions by a larger G′ and G″ value. In another study by Rafe et al. (2022), at greater concentrations, a shear thickening effect resulted from a rise in both G' and G" with the protein concentration. Concentrates with both acid and alkaline extractions demonstrated consistent elastic and viscous characteristics, displaying LVE behaviour. For water-extracted concentrates, however, this was not the case.

Fig. 8 depicts a small slope in the water extracted concentrate's G' and G" up to a specific yield point, beyond which there is a noticeable decline. It implied that the response of the material might not be exclusively LVE. The upward slope suggested that the water-extracted concentrate was departing from the LVE region, which might be the result of structural alterations in the concentrate under the applied strain conditions, such as molecular rearrangements, or nLVE effects (Rafe et al., 2022). While water extracted concentrate presented a higher yield strain value (point of decrease in G', G"), indicating that the concentrate can deform significantly before yielding, acid extracted concentrates showed a low yield strain, meaning that the concentrate deformed or flowed very little before reaching its yield point. Similar results for the ranges of LVE and G' values ranging from 500-1000 Pa were found in studies on soy, pea, potato, oat, rice and other plant-based protein isolates by Wittek et al. (2021). Understanding the yield strain value can help us better understand the mechanical properties of the concentrates and how they behave under stress (Wittek et al., 2021).

Table 13. Storage modulus (G'_{LVE}) and loss modulus (G''_{LVE}) values for the faba bean protein concentrate extracted by the three methods during strain-sweep experiments.

Method of extraction	Storage modulus (G'_{LVE}), (Pa)	Loss modulus (G''_{LVE}), (Pa)	Loss tangent (Tan δ)	Yield strain (λ), (%)
Water extraction	711.17 ± 6.76^{a}	478.90 ± 1.96^{a}	0.67 ± 0.02^{a}	29.90 ± 0.03^{a}
Acid extraction	476.23 ± 1.26^{b}	236.97 ± 0.55^{b}	0.49 ± 0.04^{b}	2.60 ± 0.06^{b}
Alkaline extraction	855.90 ± 8.78^{c}	372.43 ± 1.89^{c}	0.43 ± 0.02^{b}	2.27 ± 0.10^{c}

All values are reported as the mean ± SD, where N=3 (3 replicates from each extracted concentrate). Values within a column not having a common superscript differ significantly ($p<0.05$).

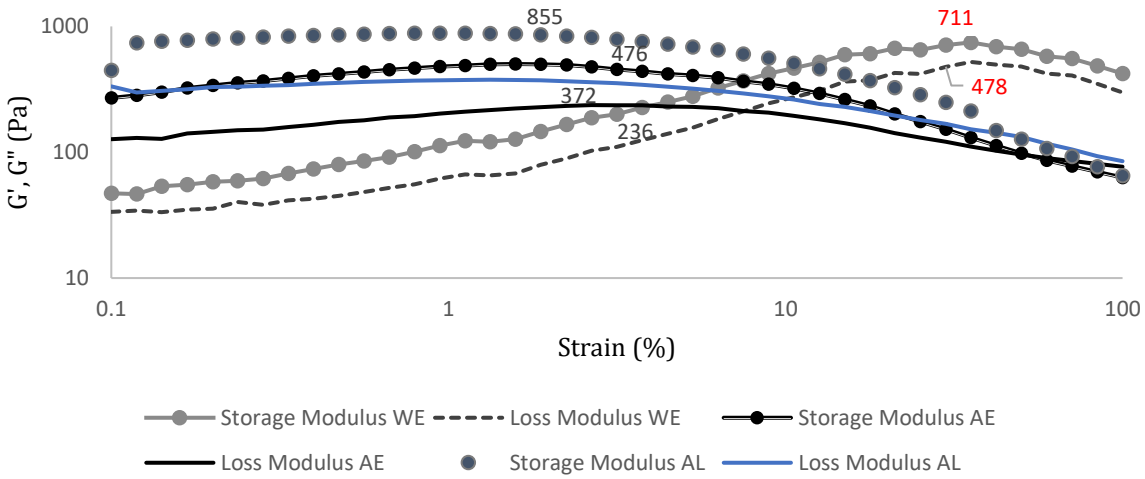


Fig. 8. The storage (G′) and loss (G″) moduli changes of the faba bean protein concentrate extracted by the three methods as a function of strain. Note: WE: water extracted, AE: acid extracted, AL: alkaline extracted. The highest values of G′ and G″ are marked before visible decline in the slope for the three methods as per data from strain-sweep experiments.

4.3.6. Protein characterization

The protein profiles of three protein concentrates were determined using reducing and non-reducing conditions through tricine-SDS PAGE. **Fig. 9.** shows the SDS profile of the three extracted protein concentrates under reducing conditions (with 2% β-mercaptoethanol). The profiles exhibited a wide variety of proteins and polypeptide subunits with molecular weights (MW) ranging from 150 kDa to 10 kDa. Most bands attributed to the protein components of legumin, vicilin and convicilin. These are the constituents of globulin protein fractions, which take up the majority (70-80%) of the total faba bean protein content (Stone et al., 2024). The bands observed in the gel were identified by MW, as bands A-D correspond to convicilin (~65-250 kDa), bands E-F correspond to vicilin (~45-65 kDa), band G corresponds to α-legumin (~37-45 kDa), band H-I might correspond to lectins which are phytohaemagglutinins (PHA) present in faba beans in higher concentrations than any other legumes (~29-34 kDa) and bands J-M correspond to β-legumin (~11-29 kDa) (Ladjal et al., 2015; Stone et al., 2024).

For **t**he SDS profile of the three extracted protein concentrates under non-reducing conditions (without β-mercaptoethanol), the bands observed in the gel were identified by MW, as bands A-E correspond to convicilin (~65-250 kDa), bands F-H correspond to vicilin (~45-65 kDa), band I corresponds to α-legumin (~37-45 kDa), band J-K might correspond to lectins (~29-34 kDa) and bands L-Q correspond to β-legumin (~11-29 kDa) (Ladjal et al., 2015; Stone et al., 2024). WE and AL methods showed thicker, more intense bands at a higher MW of ~50-100 kDa (bands C, E and F), as was supported by densitometer results for intensity with respect to relative front, corresponding to higher concentrations of convicilin and vicilin and a distinct thin lower MW band of ~15 kDa (band Q). AE method was however showed the least intense bands with distinct bands thick bands (K and P) at ~32 kDa and ~17 kDa corresponding to lectins and β-legumin, respectively.

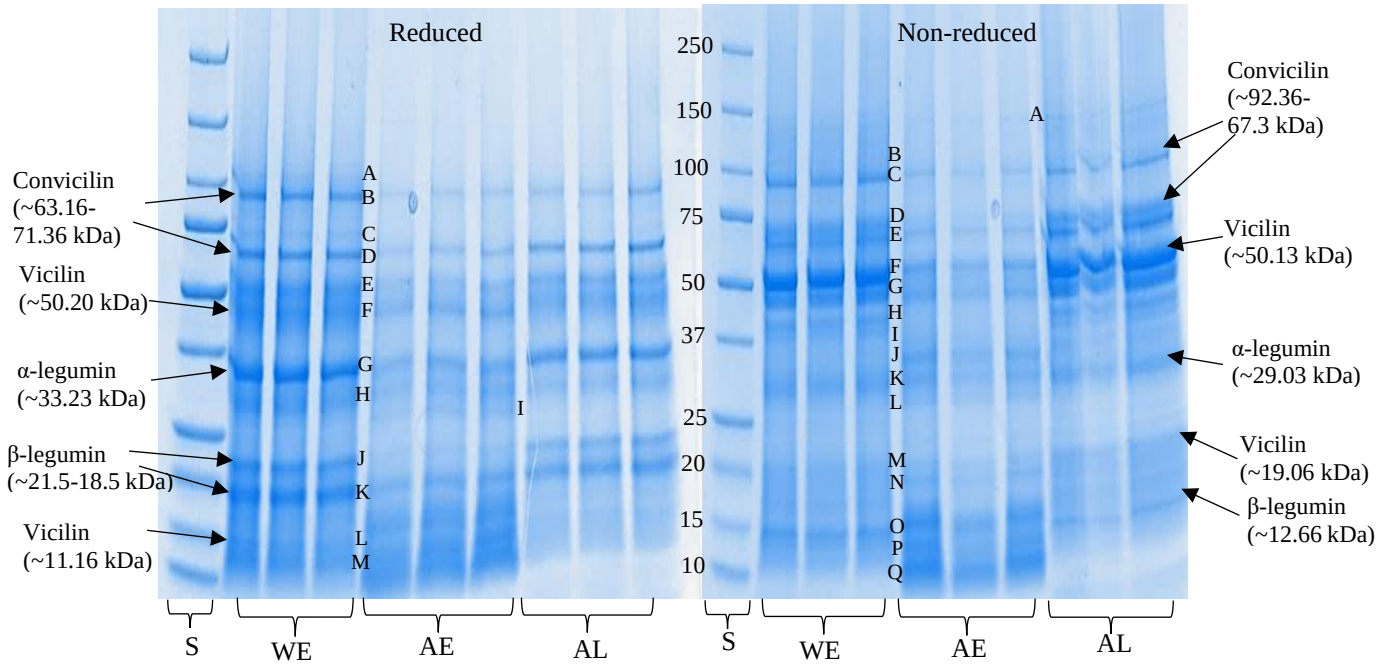


Fig. 9. Comparison of SDS-PAGE of faba bean concentrates under reduced (with β-mercaptoethanol) and non-reduced conditions (without β-mercaptoethanol). Lanes were loaded in triplicates as follows, S: standard; WE: water extracted; AE: acid extracted; AL: alkaline extracted. A-Q denote bands which correspond to the various polypeptide subunits of molecular weight between 10 and 250 kDa. The protein bands were quantified, and the molecular weight of each band was denoted using a gel scanning densitometer.

The differences observed in protein extracted by different extraction methods can be attributed to the impact these methods have on the protein structures. Under reducing conditions, AE method extracted more vicilin fractions, while AL and WE methods showed the α- and β- legumin fractions. As per results in sections **4.3.3.** and **4.3.4.**, the low denaturation temperatures of AE concentrates established the denaturation of vicilin fractions. These vicilin fractions had high solubility and foaming capacity, while the legumin fractions (AL and WE) had higher foaming stability and thermal stability (higher denaturation temperatures than AE). Similar results were concluded in a study by Chang et al., (2022), on the extraction of plant proteins from green pea and chickpea, where the lower molecular weight and less rigid

conformational structure and lower disulphide bond content of vicilin fractions when compared to the larger molecular weight of legumin fractions (α-legumin + β-legumin) helped explain the differences in functional properties. The sulphydryl groups and disulphide linkages are highly correlated with the stability of tertiary structure of the protein. The extreme pH exposure on the faba bean proteins during extraction, disturb the native structure of proteins and the folded protein structure destabilizes resulting in cold denaturation (Guckeisen et al., 2021; Chang et al., 2022).

The low pH values during AE, resulted in the cleavage of electrostatic interactions between amino acids, which can affect the stability of α-helices and β-sheets in proteins, which overall led to changes in the conformation and functionality of the protein. The higher pH levels during the AL and WE methods present with a more rigid conformation due to the stronger disulphide linkages between the α- (acidic) and β- (basic) legumin fractions. Studies show that lentil legumin-like proteins have an isoelectric point around 4.6. Lower pH values (~ pH = 2.0 for AE) may be related to lower net surface charge, leading to low molecular weight aggregates (Jarpa-Parra et al., 2015).

The difference between reducing (with β-mercaptoethanol) and non-reducing conditions can be explained by comparing the band intensity and thickness of the bands in the two gels. Both the gels showed distinctive bands throughout the MW range (20-250 kDa). Under non-reducing conditions, thicker bands were observed at high MWs while a decrease in intensity of the bands were observed at lower MW ranges. The concentration of the target protein has been directly associated with the signal strength of a band (LabXchange, 2021). Under reducing conditions, distinct separate bands were observed at both higher and low MW ranges. The key difference between these conditions lied in the reduction of disulphide bonds. β-mercaptoethanol is a reducing agent that breaks disulphide bonds in proteins, leading to the denaturation of the tertiary and quaternary protein structure. This resulted in the separation of

disulphide linked dimers or higher order proteins into subunits which are monomeric protein units, allowing a clear visualization of individual protein bands on the gel (Foroumadi & Saeedi, 2013). Upon comparison, no significant difference was observed in bands for AE in both reducing and non-reducing conditions. For AL and WE methods, under reducing conditions, the legumin subunits are distinctly present, however, under non-reducing conditions, only part of the legumin was present in subunits (Chang et al., 2022). Therefore, when β-mercaptoethanol was used, the protein bands appeared more distinct and separated compared to the gel without it.

4.4. Conclusion

The commercial variety of faba bean was used to extract protein concentrates using three aqueous fractionation methods, namely, acid, alkaline and water methods. The protein purity, yield and recovery of the protein concentrates differed significantly among the three extraction methods. Acid extracted yielded overall lighter coloured extracts and alkaline extracted yielded the darkest coloured extracts which were in direct relation to the protein concentration. The three methods were compared based on their functional, denaturation and rheological properties. Upon testing for various functional properties, alkaline method showed significant high values for oil and water absorption capacities. The protein solubility was highest for acidic methods, followed by alkaline methods which were explained by the fact that using pH values lower and higher than the isoelectric point (pH of 4.5) resulted in an increased protein solubility. Acid extracted method also resulted in a high foam volume, however, with a higher rate of collapse. The concentrates were subject to denaturation temperatures, which varied significantly with the highest denaturation temperature for water extracted concentrates followed by that of alkaline and acid extractions. It could be concluded form these results that acid extracted concentrates are more susceptible to denaturation at a lower temperature and

water extracted concentrates denatured at significantly higher temperatures. Upon strain sweep experiments, water extracted concentrates showed the highest yield strain concluding the high limits of deformation for these concentrates. Gel electrophoresis experiments on the concentrates under reducing and non-reducing conditions showed that reducing conditions showed distinct bands due to the disruption of high molecular weight proteins to their monomeric units and water and alkaline extracted concentrates showed intense bands of both high and low molecular weight, while acid extracts had the least intense bands. The alkaline and water extraction methods showed majorly the presence of legumin protein fractions, whereas acid extraction method majorly showed the presence of vicilin protein fractions. Therefore, vicilin fractions had higher solubility, and foaming properties but lower thermal properties compared to legumin fractions due to the weaker structural conformation of vicilin.

Chapter 5. Conclusions and future recommendations

The present study examined the many characteristics of faba beans as a promising crop for New Zealand, with the aim of providing a highly nutritious plant-based or legume source to cater to the growing number of individuals adopting vegetarian and vegan diets. Given the similarities in needs, New Zealand's experience in the production and isolation of dairy protein can be beneficial in establishing a new plant-based food sector. The first section of this study provided sufficient demonstration of how the physical attributes and seed microstructure of four New Zealand faba bean varieties affected their cooking capabilities. This study found a strong relationship between the faba bean seed parameters such as cooking time with cotyledon cell wall thickness, number of starch granules per cell, as well as, volume, and weight of the seed. The bean flours when subjected to cooking temperatures and shear conditions implied that higher gelatinization of starch occurred at peak pasting temperatures. The extraction of faba bean concentration from a commercial faba bean variety using three aqueous methods and characterisation of the protein concentrates were studied in the second part of this study. In order to investigate the potential of faba beans as a plant-based protein source, researchers and innovators in the food industry can benefit from the functional features of the concentrates, which were best displayed by the alkaline extraction process. However, upon denaturation, rheological and protein characterisation experiments, water extracted concentrates showed the best behaviour with high denaturation temperatures, and high yield strain values. SDS-PAGE experiments explained the composition of the proteins present in each concentrate with water extracted concentrates containing most of the native high and low molecular wight subunits (convicilin, vicilin and legumin), while acidic extracted concentrates showed low molecular weight bands with the least intensity. Reducing conditions helped in segregating the thick bands to distinct thin molecular wight bands.

A recent study in the realm of food packaging technologies has examined another fresh potential of faba beans. Researchers at the University of Saskatchewan created a novel plant-based film derived from faba beans that may be used in place of plastic wrap to preserve the freshness of meat items. Food may be preserved, and food waste can be decreased with the help of this ecological and biodegradable packaging option. Additionally, this film might be improved by adding components that have antimicrobial capabilities to stop microbial deterioration and that, when broken down in soil, would enrich the soil with nutrients and proteins (CBC, 2024). The findings of this thesis demonstrated that the highly elastic qualities required to create this biodegradable film were present in the water-extracted concentrates. This approach is even more environmentally friendly because the extraction procedure used is the most natural and does not require any additional chemicals.

Furthermore, this study proved the feasibility of using faba beans as a considerably larger source of protein in the daily diet than has previously been the case. The possibility of faba bean protein extraction and its uses in the food business are already beginning to be understood by FAR New Zealand. This study helps to improve the selection of native varieties and their qualities to help choose the best for the required purpose, which may be cost, yield, sustainability, or other seed properties. It also offers useful information on other properties of this protein extraction based on the extraction techniques.

Considering this, more study on faba beans and their extracted components would be extremely beneficial to the global cause of food security as well as to the development of a plant-based food source that would aid in achieving the UN's sustainable future goals of increased agricultural productivity, better nutrition, enhancing the lives of rural residents, and supporting global economic growth.

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Appendix C

Table (iv). Pearson's coefficient values for correlation of seed parameters for the four varieties with cooking time.

	Seed parameters	**Correlation coefficient**
Cooking Time	Thickness of cell wall	0.97474
Cooking Time	No. of starch granules	0.43037
Cooking Time	Seed volume	0.77602
Cooking Time	Thousand kernel weight	0.88349