Canola Protein Isolate: Physicochemical, Thermal and Rheological Characteristics of Canola Protein Isolate

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Abstract

This thesis investigates functional and nutritional properties of CanolaPRO, a commercial canola protein isolate. CanolaPRO is made from canola meal, a protein-rich byproduct of canola oil extraction. Canola meal derived products often remain underutilized for human consumption due to its historical anti-nutritional factors and extraction difficulties.

Several analyses were conducted to characterize CanolaPRO, including proximate analysis, water holding capacity, foaming capacity, thermal stability (DSC), amino acid profile, particle size distribution and rheology. The results show a high protein purity (89%), low fat and ash and 7% moisture content with water holding capacity measuring at 3.85g water/ g protein.

Foaming testing showed initial capacity at 141.7% and stability of 76.8% after 120 minutes, which shows better performance than other plant protein isolates. Thermal analysis shows a denaturation temperature of 89.2°C indicating high stability suitable for food processing. Amino acid profile indicated a balanced and nutritionally rich profile with a high glutamic acid content and other essential amino acids as well as a finer particle size than soy or pea protein isolates.

Rheological studies indicate good gel formation with the addition of a moderate amount of hydrocolloid. The overall high purity, and nutritional quality makes CanolaPRO a sustainable alternative plant-based protein isolate.

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1. Introduction

1.1 Background information

The international food industry has seen rapid growth in plant-based alternatives, which was brought on by concerns of animal welfare, sustainability and global health. The global plant-based food market is estimated to be valued at USD 14.2 billion in 2025 and USD 44 billion by 2035 (FMI, 2025). This exponential increase is closed related to the rising consumer awareness of animal agriculture on the environment and the need to reduce greenhouse gas emissions, deforestation and water pollution (Alae-Carew et al., 2022). Plant based diets have been known to reduce the risk of heart disease, certain varieties of gastrointestinal cancers. Due the lower saturated fats, high fiber, and presence of antioxidants and other essential nutrients it lower the risk of diabetes and obesity (Kubala, 2025). The increase in production and demand of plant-based alternatives have inspired innovation, providing a new perspective and a change from traditional dietary preferences (Curtain & Grafenauer, 2019).

Canola oil has become an integral in basic cooking and in the global food industry with it being the third largest vegetable oil after palm and soybean oil. In 2024, the canola oil market was valued at approximately USD 36.6 billion with market studies indicating further growth (SMR, 2024). The widespread popularity of canola oil stems from it use in cooking, baking and food processing which is due to its ideal fatty acid profile, high concentration of monosaturated fats and Vitamin E (CCC). A number of clinical studies have successfully demonstrated that the use of canola oil instead of other oils rich in saturated fats reduce the amount of LDL cholesterol, which in turn contributes to cardiovascular health (Lin et al., 2013). The mild odour and flavour profile and its versatility is why canola oil is a constant in kitchens and in the food industry (CCC).

The extraction of Canola oil produces a substantial amount of canola meal, the solid seed residue. Internationally, the canola meal has exceeded 81million tonnes with producing at least 46 million tonnes of meal annually (Grain, 2023). The meal is used as a agricultural co-product, with it being primarily used as livestock feed for poultry, dairy or as agricultural fertilizer because of its high nutritional content (Bonnardeaux, 2007).

Looking at the nutritional profile of canola meal- it contains about 36-40% crude protein (Wickramasuriya et al., 2015). The major seed storage proteins factions are cruciferin (11S globulin) which is about 60-70% of total protein and napin (2S albumin) about 20-20% of total

protein. Cruciferin protein is known for having strong emulsifying and gel-forming properties and napin is known for its solubility across broad pH range making canola protein an asset in food applications (Zhu et al., 2024).

The aim of this master's thesis is to investigate the various characteristics and functional properties of a commercial canola protein isolate.

1.2 Research Objectives

The objective of this research is as follows:

- Objective 1: Examining physicochemical functional and composition properties

This objective seeks to investigate the proximate and functional properties of canola protein isolate using a broad variety of analytical and laboratory methods such as proximate analysis (protein, fat, ash and moisture), water holding capacity, foaming capacity and stability, amino acid profile, thermal stability and rheology. From the data available a benchmark is established from which the protein isolate performance and potential for the food industries was studied.

- Objective 2: Comparing CanolaPRO to literature data

The thesis aims to study CanolaPRO to existing scientific data on other types of canola and rapeseed proteins. Through the use of comparative analysis, the canola protein isolate characteristics were compared to the published results on other protein isolates e.g., protein purity, forming ability, gel formation, thermal stability, particle size, and overall nutritional profile.

- Objective 3: Achieving a stable gel or paste for rheological testing

The third objective focuses on improving and understanding gelation and rheological behavior of CanolaPRO under various conditions. This also includes the changes in rheological characteristics of CPI by blending with hydrocolloids like xanthan gum.

2. Literature Review

2.1 Canola

2.1.1 Brassica spp. History, Origin and Cultivation

The genus *Brassica* comes under the family Brassicaceae with the genus *Brassica* consisting of around 100 species which includes *Brassica napus*, *Brassica napa*, and *Brassica juncea* which are commonly called rape oilseed, rapeseed, or canola (Kirkegaard et al., 2021).

The historical traces of Brassica was found in the Neolithic village of Shanxi province of China dating back 7000 years with the earliest case of domestication of *Brassica juncea* being used in Indian agriculture dating back to 2300 BC (Regulator, 2017).

Brassica napus originated in southern parts of Europe with the species being introduced to Asia at the start of the 18th century. Evidence of rapeseed cultivation in Europe can be seen as early as the 13th century as it used in oil lamps and soap-making and was later introduced to Canada, Argentina and Australia as lubricants for ships in the years 1942, 1930s and 1960s respectively (Kirkegaard et al., 2021).

The theory of the “Triangle of U” describes the genetic connection between the different members of the *Brassica* genus. This theory was developed by a Korean Japanese botanist in 1935 by the name of Jang-choo Woo, whose name was changed to Japanese and became “Nagaharu U” (Shahidi, 1990). From the Figure 1 there are three basic species *B.nigra, B.oleracea* and *B.rapa*. Through years of artificial hybridization and chromosomal doubling the species *B.carinata, B.napus* and *B.juncea* were created (Eskin et al., 2020).

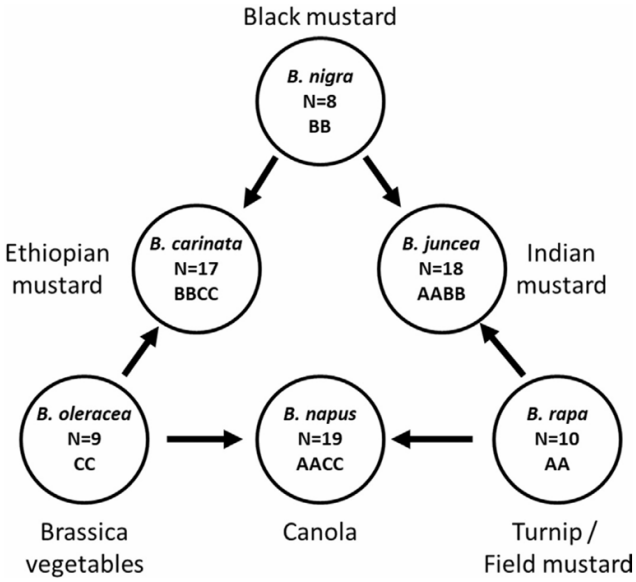

Figure 1: "Triangle of U" representing the evolution and genomic relationships of the *Brassica* plant genus (Kirkegaard et al., 2021)

The word 'Canola' was derived from **Can**adian **O**il, **L**ow **A**cid which was coined by the Western Canadian Oilseed Crushers Association in 1978. 'Canola' was used for seed oils with less than 2% erucic acid content in the oil and 30 μ mol g^{-1} aliphatic glucosinolate content in the meal. The trademark was later transferred to the Canola Council of Canada and now the term canola refers to three Brassica species: *Brassica napus* (Argentine canola), *Brassica rapa* (polish canola) and *Brassica juncea* (Indian mustard) (Kirkegaard et al., 2021).

Canola is cultivated as an oilseed and its yield averages around 40 - 45%. The byproduct of oil extracted rapeseed is a high-protein canola meal which is used animal feed. Due to the presence of a high oil content and high protein content meal canola is the third most crucial oilseed next to soybean and palm oil (Snowdon et al., 2007).

2.1.2 Transition of rapeseed to canola

Evidence of cultivation of rapeseed dates to 4000-year-old Asia where it had been used as lamp oil and in soap making. By the late thirteenth century Europe began cultivation as well to be used in the same way as it was in Asia (Eskin et al., 2020). By the start of the eighteenth century the Europeans and North Americans began using rapeseed oil as it showed potential as an excellent lubricant for high temperature steam engines and due to high demand it was exported from Asia and eastern Europe (Usha Thiyam-Holländer, 2013).

Later, *B. rapa* was introduced in western Canada by a polish farmer a Fred Solvoniuk and his wife in 1936. By WWII the production of rapeseed had grown to a substantial 80,000 acres in 1948 as in this period the need of industrial lubricants had spiked and an increase in cultivation was necessitated in Canada (Usha Thiyam-Holländer, 2013). During this period a Canadian plant breeder named William J. White with the University of Saskatchewan, and an oilseed chemist Hank Sallans conducted plant breeding research in rapeseed. By the early 1950s White and Sallans developed Canadas first rapeseed variety, Golden which had an increased oil content, early maturity, and yield (Eskin et al., 2020).

After WWII the steam engines were replaced with diesel engines and the need for rapeseed-based lubricants depleted. At this time there were talks of rapeseed as a new potential cooking oil in Canada as rapeseed grew very well in the Canadian weather. But the oil had a deep green colour, high acid content, and sharp taste also because of the high concentration of euric acid and glucosinolates which are classified anti-nutritional compounds. Due to the presence of these anti-nutritional compounds it rendered the oil and the resulting meal inedible or for use as an animal feed as euric acid caused animal heart lesions and glucosinolates inhibit thyroid function and impairs growth (Eskin et al., 2020).

Earlier research conducted on rapeseed was to combat the anti-nutrient content and to make the resulting oil edible. A vital factor to the success of breeding experiments was the development of gas- liquid chromatography (GLC) which was used to measure the euric acid content (Usha Thiyam-Holländer, 2013).

Later a important discovery by Keith Downey and Bryan Harvey of the Canada Agriculture Research Station found that the amount of euric acid depended on the seed and not on the maternal plant. That meant that seeds from the same plant could have different level of euric acid. This lead to the development of “Half-seed method” where a seed is cut in half, one half is tested for is euric acid content while the other half is left to grow and this method allowed for faster screening of rapeseed varieties (Usha Thiyam-Holländer, 2013).

Through the discovery of “Half-seed method” it led to Liho, a European rapeseed variety with 10% erucic acid. Through breeding experimentation, the low erucic acid features were transferred to *B. napus* variety which was named Low Euric Acid Rapeseed (LEAR). Keith Downey passed on the low euric acid trait to *B. campestris* using the “Half-seed method” making a low euric acid *B. campestris* is 1971(Eskin et al., 2020).

After the development of low euric acid varieties researchers turned their attention to glucosinolate. By 1973 through GLC and half seed method a low-glucosinolate *B. napus* variety was developed and through the end of 1974 the world first zero-erucic acid, low-glucosinolate *B. napus* variety was developed by Baldur Stefansson and not long after was followed by the world first zero-erucic acid, low-glucosinolate *B. campestris* being developed by Keith Downey (Shahidi, 1990).

The introduction of the double-low acid varieties was later renamed to 'Canola' by the Western Canadian Oilseed Crushers association in 1978. The seed classed as Canola had less than 2% erucic acid and less than 30 micromoles of glucosinolates and by 1985 Canola was given the Generally Considered as Safe (GRAS) notice in the United States of America opening up Canola to the world (Kirkegaard et al., 2021).

2.1.3 Global production and growth of canola

In the February of 1985 the U.S. Food and Drug Administration (FDA) designated Canola oil Generally Recognized as Safe (GRAS) - an oil having its beginning in rapeseed- which had a high amount of anti-nutritional compounds like erucic acid and glucosinolates, a distinct green colour and a sharp taste evolved into a leading oilseed globally. The GRAS designation assigned by the FDA allowed the entry of canola oil into the North American food market and further entry into the European markets ("GRAS status opens upnew market for canola oil ", 1985).

In the years after the GRAS status the canola production area grew exponentially in Canada and Europe with increased investment in crushing and refining infrastructure and quality control. By the late 1980s, Canada accounted for nearly 40% of global canola production, with only a few million tonnes in 1985, to almost 10 million tonnes 1995 (USDA, 2025).

Fast forward to the years 2023/2024 the canola oil production reached approximately 32.7 million metric tons and estimated to rise to 33.78 million tons in the year 2024/2025 (Shahbandeh, 2025). The global canola market was valued at USD 37.5 billion in 2024 (business, 2025) with analysts expecting canola to trade at USD 470 per tonne in 2025 with analysts expecting the value to go as high as USD 520 per tonne in 2026 which helps the farmers (Economics, 2025).

2.1.4 Canola seed protein

Canola seeds are rich in storage proteins, of which Cruciferin and Napin are by far the most abundant. These two proteins represent most of the seed's storage fraction (Chmielewska et al., 2021). Cruciferin is a large 11S/12S globulin (300–350 kDa) organized as a hexamer; each of its six subunits consists of an ~40 kDa acidic chain (254–296 amino acids) and a 20 kDa basic chain (189–191 amino acids) joined by a single disulfide bond (Wanasundara et al., 2016; Zhu et al., 2024). Napin is much smaller (a 2S albumin of ~12–15 kDa) and comprises two polypeptides (9.5 kDa and 4.5 kDa) held together by intermolecular disulfide bridges (Wanasundara et al., 2016). Together, Cruciferin and Napin make up roughly 80% of the total seed protein (Wanasundara et al., 2016). These storage proteins accumulate within membrane-bound protein bodies (protein storage vacuoles) in the cotyledons of the seed embryo (Zhu et al., 2024).

2.1.5 Canola seed byproduct management

Canola meal- the seed components remaining after oil extraction is commonly used as animal feed because of its high protein, fibre and residual oil content(Zhu et al., 2024).

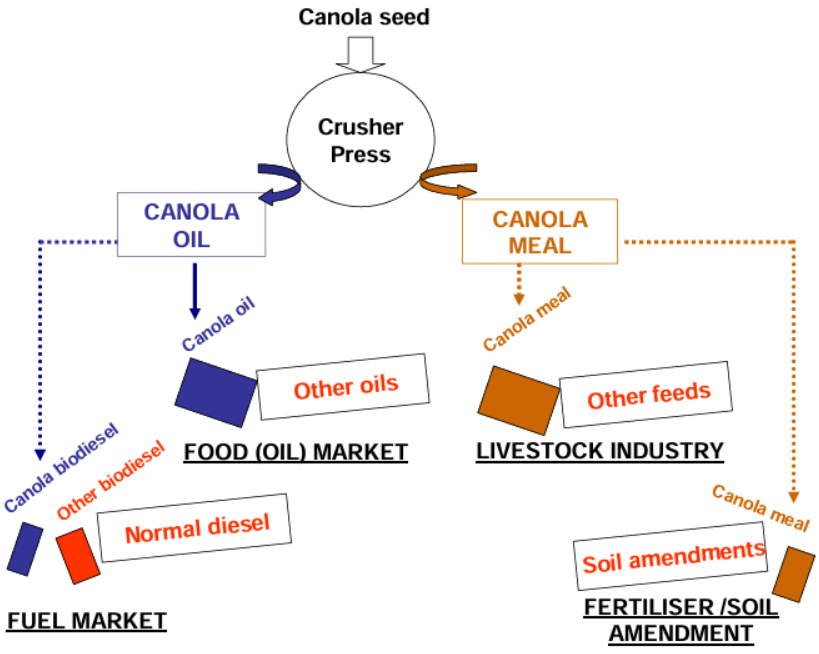

Figure 2: canola seed flowchart (Bonnardeaux, 2007)

There have been studies into alternate uses of canola meal other than as animal feed and on such study focused on the use of canola meal as a fertiliser/soil amendment. Farmers who crush their own canola for biodiesel can cut cost on transporting fertilizers as using the leftover meal

can be spread on the field thereby recycling the nutrients back to the soil and maintain the fertility of the soil (Bonnardeaux, 2007). There has also been research focused on the industrial application of canola meal with the focus on its high protein content to create plastics similar to the projects conducted on soy protein. The ENHANCE project in Europe derived a formulation called “protein II” from canola protein to be used in natural adhesives and researchers from INRA, France developed a biodegradable surfactant from canola meal which can eventually replace petrochemical detergents (Bonnardeaux, 2007).

2.2 Canola Protein Composition and Nutritional Profile

2.2.1 Major Protein Fractions: Cruciferin and Napin

Canola protein can be extracted through various methods that yield products containing different proportions of seed protein components. The two main storage proteins found in Brassica *napus* (canola) seed are cruciferin and napin, together accounting for approximately 85%–90% of the total protein content. In addition, small quantities of structural and metabolic protein are present. Cruciferin, a 11S globulin complex with a molecular weight ranging from 300–350 kDa, is the predominant seed storage protein. Napin, a smaller 2S albumin weighing between 14–16 kDa, occurs in lower concentrations relative to cruciferin (Tan et al., 2011; Wanasundara, 2011). Commercial canola or rapeseed protein products often comprise of these proteins in varying ratios depending on the processing technology. Since cruciferin and napin differ in physicochemical, functional and amino acid content their behaviour under conditions varies. Therefore, functional properties of canola protein ingredients are influenced by the abundance of cruciferin and napin in the product matrix (Campbell et al., 2016).

2.2.2 Amino Acid Profile and Nutritional Value:

Numerous studies have examined the amino acid profiles of canola protein isolate, revealing a well-balanced composition characterized by elevated levels of glutamine, glutamic acid, arginine, and leucine. Sulphur containing amino acids tend to be present in lower quantities which is most likely a result of industrial oil extraction (Aider & Barbana, 2011). The amino acid profile varies depending on the extraction technique applied to canola meal reside. Typically, alkaline extraction recovers up to 30% of total protein originally contained in rapeseed meal. Additionally, large scale

purification processes substantially influences the final amnio acid composition (Chabanon et al., 2007).

Several researchers analysed the amino acid content of canola meals, consistently nothing high glutamic acid concentrations (ranging from 16.77-18.63% w/w protein). However, they also reported reduced levels of tyrosine, methionine, cysteine. For instance, Shahidi et al. (1992) employed a two-phase solvent extraction and found it did not significantly alter the amino acid content of canola or rapeseed meals. Essential amino acids, cysteine, methionine, isoleucine and leucine also appeared at low concentrations. Protein Efficiency Ratios (PER) calculated from leucine, proline and tyrosine contents ranged between 2.19 and 2.64 (Shahidi et al., 1992).

Other studies on the amino acid composition of untreated canola meal have revealed similarities to proteins found in high erucic acid rapeseed, but with lower levels of cysteine and valine. An analysis of canola protein isolate (CMPI) showed higher amounts of leucine, phenylalanine, arginine, and asparagine compared to high erucic acid sources, while isoleucine levels were lower. In contrast to defatted canola meal, CMPI exhibited increased levels of isoleucine and arginine but decreased lysine, the only essential amino acid significantly lower than in raw commercial hexane-defatted canola meal. Despite the reduction in lysine, the quantities of serine and cysteine remained relatively constant (Shahidi, 1990).

The protein factions from rapeseed residue have been shown to have high digestibility and high amount of bioavailable amino acids. True ileal digestibility (TID) of these extracted proteins have been measured in in minipigs and Cruciferin has approximately 95% TID, whereas Napin has close to 87% TID. Interestingly, it was found that due to the branched chain amino acids of the Napin protein, DIAAS values, which indicates essential amino acid bioavailability were low at 82–89%, which is in contacts to the DIAAS values of cruciferin at 117% which allowed a balanced supply of amino acids with no limiting amino acid (Kapel et al., 2025). A technical article detailing the amino acid profile for isolated cruciferin and Napin fractions using a patented aqueous extraction process method shows that both fractions exceed essential amino acid needs for adults, with proteins like leucine, isoleucine, valine, methionine, and cysteine far above the FAO/WHO requirements. Notably, Napin particularly stands out in its high content of sulfur containing amino acids, which are critical for health at 86.5 mg/g vs the adult requirement of 24 mg/g**.** Therefore, both proteins present balanced amino acid profiles, highlighting their high nutritional value (Wanasundara & McIntosh, 2013).

2.2.3 Presence of Anti-Nutritional Factors

The presence of antinutritional factors is one of the biggest challenges faced by oil extracted canola meal. These include substances like glucosinolates, phenolics, phytates and high fiber content which contribute greatly to the undesirable qualities such as poor physicochemical properties reduced digestibility, bad taste and bad colour. Phenolic acid esters like sinapoylcholine (sinapine) and sinapoylglucose are the antinutritive components in canola seeds which are present in concentrations higher 30 higher than in soybeans. Sinapate ester can cause dark colour and bitterness to rapeseed meal and protein extracts, while also impacting digestion due to the formation of oxidised compounds with proteins during oil processing. Genetic factors also play a key role in sinapate ester content with reported content ranging from 5-17.7g seeds of *Brassica napus* (Aider & Barbana, 2011).

The level of glucosinolate present in canola meal (18-30 μ mol/g) is known to cause toxicity in animals, although lower levels like 0.61 μ mol/g, which is present in broccoli have been associated with have health benefits like a lower risk of cancer. Another known antinutritional compound in canola seeds is Phytic acid which often binds itself to micronutrients minerals like calcium, magnesium and potassium and found phytates. These phytates can significantly impact mineral bioavailability, lower digestibility and amino acid absorption. The presence of these types of antinutritional factors are the reasons of restricting major applications in animal feed and use as fertilizers (Aider & Barbana, 2011).

2.2.4 Comparative Analysis with Other Plant Proteins

When compared with other plant proteins canola proteins when processed with heat ranks among the highest quality plant proteins. This is supported by findings from two pivotal studies by (Fleddermann et al., 2013) and (Bailey et al., 2023), which analyses and compares the canola protein with other plant proteins like soy, rice, pea and even whey protein isolates and evaluates the nutritional quality, digestibility, and functional performance of canola protein isolate and its processed forms relative to soy and various plant proteins isolates.

(Fleddermann et al., 2013) conducted a randomised, double blind, crossover trial involving 28 adults, wherein the participants were given a specific amount of canola protein isolate/hydrolysate or soy protein isolate. Protein utilization and other important parameters were measured by measuring post-prandial amino acid response over a period of 8 hours and urinary nitrogen levels measured daily. However, (Bailey et al., 2023), used a swine model to analyse the standardized

ileal digestibility and DIAAS scores in heat treated and non-heat-treated canola protein isolate, soy protein isolate, whey isolates and pea and rice protein concentrates.

Their findings matched canola protein with soy protein in parameters like digestibility where CPI and CPH (Canola Protein Hydrolysate) triggered similar a post prandial amino response, with an early CPH peak than CPI and SPI suggesting a faster absorption due to it being a hydrolysed protein. Heat treated CPI matched and outperformed other plant proteins where it achieved DIAAS value ≥100 which meets the FAO criteria for excellent protein quality, equal to that of SPI and whey isolates and superior to pea or rice protein concentrates (<75). Moreover, heat treatment of the canola protein enhances its nutritional profile by improving digestibility and DIAAS scores, but hydrolysed canola protein shows an accelerated appearance of amino acid in circulation, making it by far the faster absorbed protein and makes canola derived protein a compelling candidate for nutritious plant-based alternative protein (Bailey et al., 2023; Fleddermann et al., 2013).

2.3 Conventional Extraction Methods:

2.3.1 Alkaline Extraction and Isoelectric Precipitation:

The use of alkaline extraction followed by isoelectric precipitation has become a popular method for the isolation of proteins due to it being an efficient, simple and cost-effective. The method involves suspending the plant material in a solution with the pH raised to a range of 8-12 which helps to dissolve the protein in the plant material. Solid residues that do not dissolve in the solution can be separated by processes like settling, centrifuging or filtering. Once residues are removed, the pH of the solution is lowered to its isoelectric point, which makes the protein charges ineffective and lower chances to repel each other which in turn allow the proteins to clump together and can be extracted from the liquid in the solution. To dry the extracted protein, it is either spray-dried or freeze-dried. But despite the method's popularity, there are several hurdles when it is changed into an industrial level. The hurdles include limited protein recovery rates, loss in purity, and altering of protein functionality (Cruz-Solis et al., 2023).

The use of high pH alkaline extraction can boost yield but can denature proteins thereby reducing solubility, and coextract unwanted antinutrients such as phenolics, glucosinolates, phytates which can alter the taste and quality of the final product (Zhang et al., 2020). Alkaline extraction can cause the resultant protein isolates to exhibit a strong water and lipid binding and emulsifying properties, which are both highly sensitive to the high pH used in alkaline extraction and

precipitation. Moreover, an elevated alkaline pH can increase the risk of protein denaturation, which is evident in the sample as can be seen in its protein profile and changes to its thermal stability (Manamperi et al., 2011).

Several technologies are being developed to increase the extraction yield, improve the purity, maintain protein functionality, and reduce the duration of the alkaline extraction process, including ultrasound, microwave, enzyme, and high-pressure homogenization methods. These technologies can be used to facilitate the disruption of the plant cell walls, the removal of the intracellular proteins, and the isolation of the proteins from polysaccharides, which thereby decreases process time, lowers pH requirements, and preserves functionality (Hadidi et al., 2023; Momen et al., 2021). This is incorporation of pre-treatment processes can improve yield and purity of the resultant isolate as evidenced by the incorporation of ultrafiltration after alkaline extraction significantly which is shown to enhance yield by 35%, with a purity >88% compared to standard AE–IEP which has a yield of about 20% (Hadidi et al., 2023).

2.3.2 Salt Extraction (Micellization)

Salt based extraction, also known as micellization is a technique which involves dissolving proteins in a mild neutral salt solution. After the proteins are fully solubilized, it can be recovered by lowering the ionic strength of the solution by dilution, dialysis or by membrane filtration. The use of a salt solution for extraction is a key factor of this process as this allows the protein to be fully dissolved which in turn helps to achieve a higher extraction rate. By relying on the salts, this This process allows for greater protein recovery rates of 40 percentage or higher when it is combined with processes like ultrafiltration. This can be seen in studies which involve pea and chickpea proteins, where the main emphasis of the extraction process is on isolating the proteins with fewer anti nutritional components such as phenolics and phytates. Due to these benefits of micellization, it stands as a highly effective option when it comes to separating proteins from various plant sources (Zhu et al., 2024).

2.3.3 Acid Precipitation:

Acid precipitation is commonly employed as a final step following alkaline or salt extraction of canola proteins from a defatted canola meal. This process involves reducing the pH of a protein-rich solution to precipitate proteins, matching its isoelectric point, whose adjustment renders the net charge of the protein nearly zero, minimizing its solubility and facilitating efficient protein separation, often using mineral or organic acids with or without additional salts like $CaCl_2$, to enhance yield and purity. The final precipitated protein is extracted by centrifugation (Zhu et al., 2024).

2.3.4 Impact of Extraction Methods on Protein Yield and Purity:

Scientists have researched various approaches to extracting proteins from canola, comparing methods like alkaline extraction combined with acid precipitation (Alk-Ac), alkaline extraction paired with ultrafiltration (Alk-Uf), as well as pre-treatment with ethanol followed by alkaline extraction and acid precipitation (Et-Alk-Ac) or ultrafiltration (Et-Alk-Uf). Among these protein extraction methods, the combination of alkaline extraction and ultrafiltration has proven most efficient for extraction and purification. The performance of alkaline extractions is primarily because of two reasons. Firstly, sodium hydroxide in the alkaline extraction helps break down plant fibers which helps in releasing more soluble compounds. Secondly, it helps to neutralize the side chain amines of basic amino acids like lysine and arginine, which increase the proteins negative surface charge and can help improve their solubility. The combination of ultrafiltration with alkaline extraction helps boost recovery rates, its pore size and molecular cut off helps for efficient separation which can result in a high purity and high yield for the protein isolates. The addition of ethanol pretreatment to the alkaline extraction did not necessarily improve the extraction results. This may be because ethanol has a low polarity which does not disrupt the cell walls and can limit how much protein can be extracted. Based on recovery rates and protein purity, Alk-Uf extraction method stands out as a suitable method for the isolation of canola proteins and also shows scope in scaling to industrial applications (Cháirez-Jiménez et al., 2023).

2.4 Structural and Physicochemical Characteristics

2.4.1 Molecular Structure and Conformation

Canola meal is dominated by two protein factions both of which are storage proteins; cruciferin, an 11S globular hexamer with a molecular weight in the range of 300–360 kDa and napin, an 2S albumin with a molecular weight in the range of 14–17 kDa (Zhu et al., 2024).

Cruciferin possesses a quaternary structure in a hexameric arrangement consisting of six individual subunits called protomers. These subunits display small variations in their tertiary structures which come from the multiple genes that are responsible for expressing cruciferin. Every protomer of cruciferin contains two polypeptide chains: an acidic α- chain and a basic β-chain. By using X-ray diffraction techniques, the structural analysis of rapeseed showed about 25-27 β-sheets, seven α-helices and 3-4 3_{10}-helices which showed a similarity to the A3B4 protomer structure of soybean glycinin. The combination of three crucifixion subunits is called a trimer and two of these trimers are stacked to form a hexamer. On the analysis of the protein storage vacuoles of Brassica napus the cruciferin subunits showed an octameric barrel-like configuration which weighed roughly around 420 kDa (Perera et al., 2016).

In comparison with cruciferin, napin consists of large and small polypeptide chains weighing about 9 kDa and 4 kDa respectively, connected by two interchain disulfide bonds. The large chain of the napin compound also contains 2 intra-chain disulfide linkages which create a compact helix-like structure stabilized by these bonds. Testing methods like spectroscopy confirm the very differing compositions of these proteins. Cruciferin, which is predominantly made up of β-sheets, which constitute about 45 – 46% of its secondary structure with 7-10% of α-helices. Whereas in comparison, napin is rich in α-helices, making up nearly 26-28% of its structure as seen by spectroscopy (Perera et al., 2016).

The primary structure of cruciferin in *Brassica napus* often ranges from 465 to 509 amino acids depending upon which gene is active, which gives rise to subunits designated CRU1, CRU2, CRU3, CRUA and CRU 5. Napin protein also behaves in a gene dependent manner with about 10 to 16 different genes being identified in *Brassica napus* for the coding of napin sequences. Some of these sequences give rise to subunits which include Napin-1, Napin-2, Napin-3, Napin-1A, Napin-B and Nap, with molecular masses spanning from 12.5 to 14 .5kDa (Perera et al., 2016).

2.4.2 Solubility and Isoelectric Point

Generally, plant proteins have their isoelectric points within an acidic range of pH 4.5 to 6, where rapeseed proteins stand out with a broad spectrum of isoelectric points. Studies have pointed out that roughly 1/5 to 2/5 of rapeseed protein molecules are almost neutral at alkaline conditions with isoelectric points in range of pH 11 while the rest fall into a common pH range of 4 to 8. The two main storage proteins found in rapeseed are cruciferin and napin with a minimum solubility of about pH 7.2 and pH 10.5 respectively. When sequential precipitation is applied to rapeseed meal extracts that are ethanol treated, the isolated protein mixtures show a variety of isoelectric points, and protein types. According to research, the protein fraction that first precipitates starts at the pH 10.5 is richer in napin and it is found in lower quantities in cruciferin, which shows that it more readily dissolved in acidic conditions (Georgiev et al., 2022).

Therefore, a process that utilizes an isoelectric precipitation at a range of pH 4.5–5.5 will have maximal extraction of the cruciferin and globulin, however further acidification to a range of pH 3.5–4 precipitates down any remaining albumins in the protein solution. Moreover, any pH employed that is above pH 7, will cause almost complete solubilization of the canola protein (Kalaydzhiev et al., 2020). Therefore, canola protein solubility is lowest at the isoelectric point, which is near neutral pH for cruciferin and very alkaline pH for napin and solubility is seen increase at pH values farther from these isoelectric points (Kalaydzhiev et al., 2020).

In addition to this, this precipitation can be accelerated by manipulating the ionic strength of the protein solution, where the addition of salts often improves the solubilization kinetics of the of canola proteins at acidic pH. Addition of 0.25 M NaCl was shown to increase the solubility of napin by more than 94% at a range of pH 4.5–6.5 to produce a napin-rich isolate. In contrast however, cruciferin-rich isolates are less sensitive to salt (Georgiev et al., 2022).

2.4.3 Thermal Stability and Denaturation Behaviour

The heat stability of proteins is shaped by a wide variety of structural and environmental factors. Factors like the specific amino acids present, interactions between protein molecules, the binding of metal ions as well as various linkages within the molecule. When purified, cruciferin and napin display higher temperatures for their main endothermic peaks than canola protein, probably because less pure samples contain a mix of proteins and other substances that can lower the thermal resistance. For cruciferin this peak occurs near 91°C, which is consistent with the general reported heat denaturation range for most globulin proteins. Reducing agents such as β-Mercaptoethanol

can break the disulfide bonds within proteins, transforming cysteine residues into sulfhydryl groups and lowering the protein stability as seen by drops in the denaturation temperature and energy required for this change. The addition of β-Mercaptoethanol weakens the cruciferin structure, which in turn lowers the denaturation temperature to around 76°C, which is similar to temperatures seen in soy and oat protein globulins. In the case of cruciferin however, the enthalpy of denaturation does not change with this temperature suggesting that its structure is protected by non-covalent interactions than by disulfide bridges (Wu & Muir, 2008).

Napin protein, on the other hand, is more heat stable with denaturation temperatures reported at 110°C with several research confirming this high thermal stability. When heat is applied, the protein structure becomes unstable, and bonds are broken. When in the presence of β-Mercaptoethanol the denaturation temperature and energy decrease sharply. Experiments show that napin subunits are connected by both interchain and intrachain disulfide bridges, which provides napin's exceptional resistance to heat. The disruption of the interchain and intrachain disulfide bridges can reduce both the denaturation temperature and the enthalpy of heating. Both cruciferin and napin react differently to the application of heat. Cruciferin remains completely folded up at 80°C and only begins to unravel at 90°C. Napin, on the other hand, retains its native shape until it is exposed temperatures higher than 100°C (Wu & Muir, 2008).These data mean that canola proteins require severe heating to denature and simple pasteurization temperatures of <80 °C will not fully unfold cruciferin, whereas sterilizing temperatures of >100 °C will cause extensive aggregation. When heated to above 85°C at neutral pH, cruciferin begins an irreversible thermal denaturation process, which often leads to gelation or precipitation upon cooling. Similarly, Napin's denaturation also leads to irreversible gelation but due to strong S–S bonds in the gel network it leads to bad water retaining gel structure, leading to extreme syneresis (Wanasundara et al., 2016).

2.4.4 Interaction with Other Biomolecules

Canola protein isolates generally struggle to produce strong gels in comparison with proteins of soybean or eggs, which are known to form strong gel networks. The ability of a protein to form gels strongly relies on the hydrophobic attractions and the hydrogen bonding of the molecules when heat is applied. Canola protein in its untreated form does not require a higher concentration for gelling, but introducing a polysaccharide or a preformed protein aggregates can help enhance the resulting gel strength. The foam stability of canola protein isolates is influenced by factors such

as the size of the protein molecule, the presence of polyphenols and phytic acid, and any prior heat exposure. Research has shown that smaller protein molecules are generally less effective at supporting stable foams. Polyphenols, however, may benefit the foam structure because they help proteins form more stable complexes at water to air interfaces. Phytic acid, in contrast, tends to hinder the foaming property by binding with proteins and lowering their solubility. In addition, heat treatment can also cause denaturation, which typically reduces both foaming capacity and foam stability (Tan et al., 2011).

The gelation and emulsification properties of canola proteins can be remarkably improved with the addition of certain polysaccharides. For example, when κ-carrageenan is mixed with canola proteins, even when other types of bonds like covalent and dye sulfide bonds are not present. The addition of κ-carrageenan or guar gum can boost the emulsifying function of canola protein isolates. In order to achieve ideal pH, CPI and κ-carrageenan have to be mixed together at pH 6, while canola protein isolate and guar gum will be most effective at pH 10. Another way to enhance the functional properties is protein hydrolysis, which increases protein solubility and in turn, their performance in emulsification and other applications. This modification pathway is particularly promising for CPIs, which otherwise tends to have low solubility under neutral pH conditions. Therefore, protein-polysaccharide stabilizing interactions are used to achieve better gelling properties, thickening, and stabilizing effects in canola protein-based products (Tan et al., 2011).

2.5 Functional Properties of Canola Protein Isolates

2.5.1 Foaming Capacity and Stability

Foam formation takes place at the boundary where air meets liquid and is largely carbon by the interfacial tension that is found in this interface. The physical stability of a foam depends upon ,the rate at which liquid drains out of the bubbles, the rate at which the bubbles merge together (coalescence) and the tendency of the bubbles to change size over time(coarsening). A crucial point is a proteins ability to reduce the interfacial tension and rapidly migrate to the air-water surface is highly critical for creating and maintaining foams. The foaming performance of proteins is highly increased when it is partially unfolded, where they are able to move to the air water interface. For a foam to be stable, it relies on a thin “mucilage” or a thin film that forms around the bubble. This film is both viscous and strong, and proteins must be reasonably water soluble and able to pack densely at the interface which can form cohesive and adhesive layers (Zhu et al., 2024).

Canola seed protein is known for its strong foaming capacity, which can sometimes even outperform soybean protein in some studies, and its foam is known to remain stable for a long period of time. This ability of canola protein is closely related to its unique structure, composition, and electrical characteristics. Pretreatments like, exposure to pulsed electric fields or ultrasound have been explored to further enhance the foaming properties of certain plant proteins. It has been found that adjusting the voltage or ultrasound intensity as well as pH during the treatment can induce a partial denaturation of the proteins. This can make the protein more flexible in a solution and can boost its ability to gather at the air water interface, which can lead to a larger and more improved foam volume and foam longevity (Zhu et al., 2024).

2.5.2 Water and Oil Absorption Capacities

Canola protein isolates show good water and oil absorption capacities, which are crucial for giving texture in food. Characterization studies of canola protein isolates by (Cháirez-Jiménez et al., 2023), reveals that CPI extracted using alkaline extraction and ultrafiltration, had a water absorption capacity (WAC) of 3.86 g water/g protein and an oil absorption capacity (OAC) of 2.77 g oil/g protein. All CPIs produced demonstrated a lower WAC value compared to the control soy protein isolate. The observed variation in WAC profiles among plant-based protein isolates has been attributed but not limited to differences in protein structure, conformation, and availability of polar amino acids, which mediate protein–water interactions (Bocarando-Guzmán et al., 2022). These values for oil and water retention are relatively high such that the aqueous WAC exceeds typical values for many legume proteins, highlighting the hydrophilic nature of canola, while the OAC shows a good capacity to absorb fats. The study also reports that Alkaline extraction plus ultrafiltration resulted in the highest WAC and OAC however acid precipitation of the proteins leads to isolates with slightly increased foam stability and density. In an earlier work, (Khattab & Arntfield, 2009), reports WAC values in the range of 3-5 g/g and OAC in the range of 2-3g/g for canola meal proteins, consistent with higher values seen in more purified isolates. Overall, CPI tend to retain water and oil at par with other vegetable proteins, although extraction and purification methods play a huge role in these capacities.

2.5.3 Influence of Processing Conditions on Functionalities

Treatment parameters like extraction pH, heat, enzymes and more during processing of the canola meal can affect canola isolate functionality. For example, (Cháirez-Jiménez et al., 2023) observed that isolates which have been extracted using alkaline extraction followed by ultrafiltration yielded CPI with higher WAC, OAC and foaming properties than CPI extracted using simple isoelectric precipitation. Salt extraction combined with UF, in general, retains better functionality than basic precipitates. Moreover, adjusting the extraction pH, can determine what protein factions are extracted, for example precipitating at pH 3 vs pH 5 can favour napin vs cruciferin and alters solubility of the extracted isolate.

Regarding the effects of pH on emulsification,(Wang et al., 2018), found that higher pH improves the solubility which led to a thicker, more stable interfacial layers and produced a better emulsion. (Khattab & Arntfield, 2009), found that boiling of the canola meal was found to reduce emulsification more than dry roasting, with the lower nitrogen solubility pinned a s a plausible reason. However, his findings concur with the findings of (Ghodsvali et al., 2005), where he concludes that who found that canola meals were superior to soybean meal in the emulsifying activity and foaming properties.

Regarding the effects of enzymatic hydrolysis, controlled proteolysis with limited protein cation can enhance protein functionality by yielding surface active peptides. Studies on CM by (Alashi et al., 2018), indicates that moderate hydrolysis can improve emulsification at acidic pH and improve foam stability, since the resultant peptides can diffuse faster at interfaces. Conversely, however, prolonged hydrolysis was found to create very small peptides that cannot stabilize emulsions or foams.

Regarding the effects of high intensity treatments like ultrasound and pulsed electric fields, its shown that pre-treatment of canola isolates with these techniques can increase emulsification capacity as these treatments can unfold proteins and improve solubility (Zhu et al., 2024).

2.5.4 Rheology and Gelation of Canola protein isolates

The rheological and gelation properties of canola protein isolate play an important role in use in food systems, especially in products where texture and thickness are of vital importance. The main two protein groups in canola are cruciferin and napin which show different behaviors when they are exposed to heat and changes in composition during processing. These two main protein groups make up most of the canola protein isolate and each contribute differently to the protein's performance. But in comparison to soy and other animal-based protein isolates, canola protein usually forms weaker gels, but the gel structure and strength can be improved by the addition of hydrocolloids like xanthan gum or k-carrageenan (Uruakpa & Arntfield, 2004).

During thermal processing cruciferin unfolds and form aggregates around 89°C to 91°C, which leads to the formation of elastic gels through interactions like hydrophobic bonding and disulfide bridges. Napin protein, on the other hand, has a higher tolerance to heat, with a denaturation point of above 100°C. The disulfide bonds with napin helps create gels that do not easily revert to liquid state, though such gels often release water and retain less moisture (Tan et al., 2014).

The addition of hydrocolloids like xanthan gum and k-carrageenan have demonstrated an increase in gel elasticity and storage modulus (G'), especially after heat treatment. However, the addition of too much hydrocolloids can often interfere with the binding of protein molecules and weaken the gel. The improvement in gel elasticity and its storage modulus (G') often occurs because the binding between proteins and hydrocolloids helps compensate for the proteins natural tendency to form weak gel (Rezaee et al., 2022).

2.6 Challenges and Limitations

2.6.1 Effects of Industrial Processing on Protein Quality

Industrial processing can exert a beneficial effect on the quality of canola protein isolates. Severe processing conditions, particularly those involving high temperatures or strong processing conditions, are frequently associated with protein denaturation and can affect essential amino acid concentration and digestibility of produced isolates. With these present challenges, there is research that has identified specific types of heat analysis that can enhance the digestibility of rapeseed protein isolates. In comparison, heat untreated isolates consistently demonstrated higher digestibility than the untreated isolates. This improvement is generally because of the result of irreversible protein denaturation, which can be because at temperatures above 70°C and it is believed that it would increase the accessibility of the peptide chains to digestive enzymes. The application of moderate heat processing within temperature ranges of 50°C to 90°C, can promote the partial unfolding of proteins (Akharume et al., 2021). This allows for the exposure of intramolecular bonds and active sites, which are critical for enzymatic hydrolysis. The extent of the effect, however, is influenced by various factors, including protein concentration, applied pressure, the specific denaturation profile, transition temperature, and the strength of the molecular bonds present within the protein matrix. Rapeseed protein isolates are known to contain multiple disulfide linkages and heat induced cleavage of these bonds will enhance the enzymatic access and improve digestibility. Experimental data indicates that heat processing can improve lysine availability in rapeseed protein isolates when compared to non-treated isolates. The absence of a decline in lysine digestibility after heat exposure suggests that the processing conditions were not severe enough to have induced Maillard reaction, which is a common result of lysine loss. Additionally, mild thermal treatment has been shown to increase the Digestible Indispensable Amino Acid (DIAAS) score of rapeseed protein to levels that exceed those of soy protein isolate and can near values that is observed for whey protein isolates (Rutherfurd et al., 2015). Similar improvements in its nutritional value and DIAAS have been observed for pea and rapeseed concentrates produced from proteins meals by thermal processing. Several studies also have reported a decline in DIAAS values for proteins that are derived from nuts or cereal grains by exposure to temperatures that are higher than 100°C. These research findings emphasize the necessity of carefully controlling industrial heat treatment protocols for canola and other plant protein isolates. High temperature treatments can often lead to irreversible degradation of not just lysine but also other indispensable amino acid and diminish its biological and nutritive value (Bailey et al., 2023).

In contrast however, some studies have found that standardised ileal digestibility of the amino acids in canola meal was affected by heat and found that it reduces both the concentration and the digestibility of amino acids in canola meal (Almeida et al., 2014). Moreover, its observed that heat treatment has ill effects on the foaming properties as it causes protein denaturation and that native proteins have a higher foaming stability and capacity (Khattab & Arntfield, 2009; Lin et al., 1974).

2.6.2 Sensory Attributes and Consumer Acceptance:

As some anti-nutritional factors persist after extraction and even after processing some remain, which gives canola protein isolates undesirable sensory properties. Bitterness and astringency are some of the important sensory defects that are reported in rapeseed protein isolates. Research has shown that unfavorable taste characteristics often come from presence of specific flavonoid glycosides. Among them, kaempferol 3-O-(2‴-O-sinapoyl-β-D-sophoroside) (K3OSS) has been identified as the principal compound in giving both astringency and bitterness. Research has also revealed eight other kaempferol glycosides, which can contribute to the overall bitter and astringent taste profile of rapeseed protein isolates (Hald et al., 2019).

The concentration of K3OSS tends to increase during industrial protein processing due to it giving off this compound from its precursors. Sensory threshold analysis shows that the perception of bitterness of these glycosides can occur within a concentration range of 3.3 to 531.7 μmol/L, while astringency can be detected at lower concentrations ranging from 0.3 to 66.4 μmol/L. These research results show the important role played by kaempferol glycosides in explaining the taste of rapeseed protein isolate, as a result show the need for a focused strategy both in both plant breeding and processing methodologies to navigate these taste related issues (Walser et al., 2024).

Moreover, anti-nutritional factors can also change the colour of the protein as the oxidation of phenolics during processing or enzymatic browning can turn protein isolate to change to yellow or brown, which consumers can find unappealing and unattractive (Wu & Muir, 2008). In addition to this, functionality can also be affected as heat denatured canola protein will have poor foaming and gelling characteristics, potentially affecting texture (Khattab & Arntfield, 2009; Lin et al., 1974)

To improve the taste properties of canola protein, several strategies can be considered. Selective breeding can help reduce the biosynthesis of kaempferol 3-O-(2‴-O-sinapoyl-β-D-sophoroside) (K3OSS) and its precursor compounds. Biotechnological modifications post extraction, including fermentation, enzymatic treatments and solvent extraction, have been reported as effective

approaches to decrease the concentration of the compound responsible for the off tastes. Research shows enzymatic intervention has demonstrated potential. For example, feruloyl esterase derived from *Schizophyllum commune* has been reported to lower K3OSS levels by approximately 65%. Enzymes such as β-glucosidases and laccases can help in conversion of K3OSS into less bitter compounds or influence its molecular stability through oxidative mechanisms, which in turn can modify its sensory effects. Recent studies combining untargeted metabolomics with sensory evaluation have revealed that laccase treatments result in a reduction of K3OSS and other phenolic compounds which is seen with a notable decrease in bitterness and astringency (Spaccasassi et al., 2025).

2.6.3 Regulatory and Commercialization Hurdles

In the US, specialized canola protein products have been recognized as GRAS (Generally Recognized as Safe), where examples like Supertein™ and Puratein® (BurconNutraScience) and Vertis™CanolaPRO® (DSM-Firmenich) have generally recognized as GRAS by the US Food and Drug Administration (FDA) for food use (GRAS, 2010, 2017). Cruciferin-rich and napin-rich protein preparations show significant functionality in a variety of food systems. These proteins are well suited for applications in bakery items, food and vegetable-based beverages, functional drink formulations, egg replacers and range of processed meat products. Canola protein ingredient called Isolexx produced by TeuTexx proteins has obtained authorization from the European Food Safety Authority (EFSA) as novel food ingredient which supports its suitability for integration into product development initiatives (EFSA Panel on Dietetic Products & Allergies, 2013).

However, concern of potential allergenicity have been highlighted and the EFSA explicitly reports that the 2S napin, known as a ‘mustard allergen’ is likely to trigger allergic reactions in individuals with a pre-existing mustard allergy (EFSA Panel on Dietetic Products & Allergies, 2013). Seed storage proteins belonging to the 2S albumin family within the Brassicaceae species have been identified for their potential in inducing immunogenic reactions in sensitive individuals. Both napin, which is considered to have a higher allergenic probability, and cruciferin, which is relatively less allergenic, are known allergens that are present in yellow mustard (*Sinapis alba*) which is closely related to canola. A particular allergen that has been identified is the napin isoform Bran1 from *Brassica napus* shows how this isoform can react with the pediatric population that is sensitive to mustard and can form allergies to canola derived protein ingredients. As a

consequence, canola proteins must carry an allergen label indicating allergen warning for mustard or possible brassica allergies (Puumalainen et al., 2015).

Commercially, canola-based plant protein products are behind other plant protein products. Despite successful safety assessments in non-allergic participants, market uptake and commercialization efforts and marketing is slow. A review by (Campbell et al., 2016), notes that there are no commercially available canola-based protein products and discussed the potential opportunity presented by canola proteins like a well-balanced amino acid profile, with a competitive PDCAAS score, a brand recognition as healthy oil used in food processing and home and valuable co-products from canola protein fractionation like hull fibre, higher value protein fractions, lignin, phytic acid, polyphenols and canolol. (Campbell et al., 2016).

2.7 Future Perspectives and Research Directions

2.7.1 Strategies for Enhancing Functional Properties

A wide range of physical, chemical, and enzymatic strategies are being explored for boosting canola protein functionality. Treatment strategies such as Ultrasonic or high-pressure processing can unfold protein structure, reduce aggregation and expose hydrophobic cores. Studies by (Ye et al., 2025), showed that ultrasonication of the of CPI combined with glycosylation modification was found to significantly improve both the stability and emulsification properties, highlighting its potential as a plant-based emulsifier in the food sector and beyond. Sonication was found to have improved structural flexibility due to increased surface hydrophobicity resulting from the unfolding structure of CPI. Maillard-type glycosylation was performed with polysaccharides like (e.g. dextran, gum Arabic, flaxseed gum), which greatly enhanced solubility and emulsifying power. (Ye et al., 2025), showed that the dual treated (sonicated and glycosylated) CPI with 27.69 % glycosylation showed a solubility of 90.26 %, and an emulsification capacity of 80.87 m^2/g. The emulsions stabilized by dual treated CPI exhibited higher degrees of protein adsorption (84.56 %) and a smaller particle size (4.22 μm) with a more homogeneous distribution at the oil-water interface. Additionally, it demonstrated enhanced thermal and salt stability resulting from its increased surface hydrophobicity and net charge, illustrating a good synergistic combination of physical and chemical modification to improve functionality. In another study (Qu et al., 2018), found that conjugating dextran via traditional wet heating and ultrasonication at pH 6 and pH 3.6 had markedly higher emulsification indices compared to original protein isolate and reported that Ultrasonic dextran grafting was more efficient than wet-heating grafting in protein

functionality. Similarly, conjugation of flaxseed gum to CPI also improves its emulsification indices (Ye et al., 2025).

Other physical methods like pulsed electric fields (PEF) with assisted alkaline extraction of canola protein from isopropanol-defatted canola seeds have been tested to disrupt protein structure in oilseeds and it reportedly increases solubility and emulsifying action of globular rapeseed globulin and albumin proteins (Zhang et al., 2017).

Chemical and enzymatic processing can also have significant effects on canola proteins functional characteristics. Controlled proteolysis on canola proteins is shown to have significant effects in reducing the protein size and the potential release of bioactive peptides. For example; controlled hydrolysis of canola protein with enzymes like Alcalase with DH 3.1 to 7.7% is shown to significantly improved emulsification (Vioque et al., 2000). Partial hydrolysis can also raise the PDCAAS/DIAAS scores to 1.00, which shows the potential for canola protein's use as a valuable nutritional source (Fleddermann et al., 2013). The selected hydrolysis of canola protein has also shown its impressive potential as a source of valuable bioactive peptides for human health (Aider & Barbana, 2011). However, prolonged hydrolysis can reduce emulsifying capacity by creating very small peptides that reduces functional properties, so optimized hydrolysing protocols are needed. Chemical modifications such as Acylation (succinylation or maleoylation) of the lysine residues has been used to increase surface activity, leading to improvements in functional characteristics. For example, (Das Purkayastha et al., 2016), reported improvements in emulsifying capacity when maleic anhydride is acylated to the rapeseed protein isolate, via sonication in the ultrasonic water bath. They observed increased EC (45–80%) and decrease in droplet size with an increase in degree of maleylation and ES reached maximum (85%) at 20% maleylation. In essence, they act as small-molecule surfactants at interfaces and shows an interesting approach to increase emulsifying capacity and stability.

Enzymatic modification with transglutaminase (TG) can be used to enhance the gelling characteristics of canola protein isolate. Factors like protein and enzyme concentration, treatment temperature significantly impact gel strength. Gelation can be improved by increasing the amounts of protein and TG and by keeping the treatment temperature close to 40˚C (Pinterits & Arntfield, 2008).

2.7.2 Expanding Applications Beyond Food Industry

Beyond the applications in the food industries, canola proteins have immense potential as a biomaterial to be used in a plethora of applications. As a bio-based packaging material, canola protein can emulate the properties of biodegradable plastics and edible films. Recent research has shown that canola protein is a promising raw material to produce biodegradable polymer films. When combined with plasticizers such as water, glycerol, polyethylene glycol, or sorbitol, canola protein can demonstrate thermoplastic behavior. Although canola proteins show superior processing properties, the films that are fabricated from canola protein can exhibit limited mechanical properties, with tensile strength observed in the range of. 1.19 to 4.31 MPa. To deal with the challenge of these mechanical limitations, several approaches like incorporation of synthetic polymers, modification of protein functional properties by the use of controlled denaturation, and the addition of cross-linking have been used. The use of these approaches has given rise to canola protein-based bioplastics processing mechanical and moisture barrier properties which are comparable to the bioplastics derived from other plant proteins. As a result, canola protein based bioplastics show a future in applications in flexible bio-packaging like films, wraps and pouches (Zhang et al., 2018).

Industrially CPI has found use as an excellent wood adhesive, with a market for renewable alternatives to synthetic resins. (Bandara et al., 2017), demonstrated a canola protein–Graphite Oxide hybrid adhesive, composed of exfoliated graphite oxide with different oxidation levels with GO-B (1.40 C/O ratio), in a canola protein precipitate matrix. They observed that the exfoliated graphite oxide nanoparticles significantly increased the adhesive's dry and wet bond strength, with 1% GO B doubling the dry shear strength from 6.4 to 11.7 MPa and the wet strength from 1.98 to 4.85 MPa relative to protein matrix alone. They attributed the adhesives properties to the increased interlayer spacing, improved exfoliation properties, and increased adhesive and cohesive interactions (protein-protein, protein-GO and adhesive-wood surface), hydrophobic interactions and thermal stability. This study highlights the promise of using low cost nanofillers to enhance canola protein adhesion for industrial usage (Bandara et al., 2017).

2.7.3 Sustainability and Economic Considerations

Numerous economic hurdles exist for the proper valorisation canola of by-products for human food applications. (Campbell et al., 2016), notes that canola has a lower protein content (36%) than soy (48%), so the economic challenge of achieving high protein yields is inherently more difficult. The principal factor for adopting canola protein as a food ingredient is achieving cost efficiency through competitive pricing. For this. An examination of the market factors such as market scale, product valuation and the cost of raw materials is required. For canola proteins to be able to get into the market to be in competition with premium ingredients in substantial volume like the staples like breakfast cereals, it is necessary to have a precise study of the market potential and set feasible price points. Identifying the functional and nutritional benefits of canola protein can help support positioning and strategic marketing in protein ingredient market. One of the key requirements for the successful development of canola protein as a food ingredient is to price the product competitively in the market. To do that, an initial in-depth assessment of the various market parameters like the market size, product value and the price of the raw materials have to be studied. To be able to access high end or large markets like the breakfast cereal market, it is necessary to evaluate the pricing structure and market volume for higher-value protein ingredients (Campbell et al., 2016).

As canola protein fit the circular economy model by utilizing an oilseed by product its business model is largely sustainable and eco-friendly. As (Đermanović et al., 2025), using the rapeseed meal ‘in line with industrial synergy’ can provide high quality plant-based food products with nutritional value comparable to animal proteins. This focus on green extraction methods are most appealing to sustainable practices because deep eutectic solvents and enzymatic methods have low toxicity and are exceedingly biodegradable. As is proven by (Đermanović et al., 2025), using solvent based extraction minimizes damage to the protein structure and avoids the generation of toxic alkaline by-products and runoff.

2.8 Conclusion

The purpose of this review is to highlight the potential canola protein portrays as a plant protein isolate. Through this evaluation it has become clear that CPI provides a combination of high protein content and digestibility with a balanced essential amino acid content. the water holding capacity shows favourably comparable to other plant protein isolates supporting its use in moisture rendition in food formulations. CPI behaviour in functional tests demonstrates that it has strong foaming properties, high thermal stability of its cruciferin and napin storage proteins, a fine particle size.

Although the literature highlights the promising attributes of CPI, functional performance can vary with processing methods, with studies on commercial isolates like CanolaPRO remain limited. Therefore, this thesis is carried out in providing a comprehensive characterization of CanolaPRO CPI.

3. Material and Methods

This thesis involves the characterization of a Vertis CanolaPRO I90+ Rapeseed (Canola) Protein (Isolate) 90% provided by DSM Food Specialties, Netherlands. The product details provided by the producer provided details on the molecular information of CanolaPRO with it being in a roughly 1:1 mixture by weight.

The protein isolate is tested for its proximate content such as moisture, ash, fat, protein and carbohydrates (calculated as difference of moisture, ash, fat and protein). Various functionality tests are also carried out like Water Holding Capacity (WAC), Differential Scanning Calorimetry (DSC), Foaming Capacity and Foam stability. Protein Solubility, Rheology and Amino Acid Profile tests were also done to determine the protein absorption, viscoelasticity and the Amino acid content to discuss the quality of the protein.

3.1 Proximate analysis

3.1.1 Moisture

The moisture analysis was done by the hot-air oven method. The reading was taken in triplicate so three moisture dishes were taken and weighed with their lids and then 2g of sample was weighed into each dish and weighed. The dishes were then placed into the hot air oven with their lids open (with the lids underneath the dish) at a temperature of 108°C overnight.

The following morning the dishes are taken out of the oven and lids are quickly refitted on the dishes and placed into a desiccator to cool off. Once cooled the dishes are weighed are the moisture percentages are calculated.

% of Total Moisture (T.M.)

$$\%(\text{T.M.}) = \frac{w_2 - w_3}{w_2 - w_1} \times 100$$

Where, w_1 = Dish + lid (g)

w_2 = Dish + lid + sample (g) (before drying)

w_3 = Dish + Lid + Dried sample (g) (after drying)

3.1.2 Ash

The crucibles (triplicate readings) were heated in the muffle furnace at 525 °C for 60 minutes and transferred to a desiccator to cool off. 2g of CPI is taken and weighed into the crucibles and the crucibles are placed on a hotplate to char the CPI. Once the CPI is charred the crucibles are transferred to the muffle furnace at a temperature of 525°C - 550 °C for 4-5 hours. Remove the crucibles from the muffle furnace and transfer to a desiccator to cool and weigh to determine the ash percentage.

$$\% \text{ ash} = \frac{w_2 - w_1}{w_3} \times 100$$

Where, w_1 = Crucible wt.(g)

w_2 = Crucible + Ash (g)

w_3 = Original Sample weight (g)

3.1.3 Crude Protein

The reading is done in triplicate (Kjeldahl) a blank sample is taken as well. Weigh 0.1g CPI into weighing boats and transfer the CPI samples from the weighing boat to the digestion tubes. Add two Kjeltabs (containing 3.5g K2SO4 and 0.0035g Se) and 17ml of concentrated H2SO4 and leave the digestion tubes in a rack in a fume hood for about 2 hours to digest the CPI sample.

The tubes are taken out of the fume hood and the block digestor is set up and the sample are left to digest at 420°C for 45 to 60 minutes until a transparent solution is observed in the tubes. Switch off the heating element and place the tube in the fume hood to cool off. Fill a bucket with cold water and place the tubes into the buckets when the tops of the tubes are cool to touch. Add 70ml of distilled water to each of the tubes and shake gently to mix in the water making sure there are no solids in the solution.

250ml conical flasks with 25ml of 4% boric acid is taken and placed with the digestion tube to be distilled into a kjeltec 2100 distillation system. Once the distillation is completed the steam generation is stopped. The distil after distillation is received into the 250ml conical flask (receiver flask) placed in with the 4% boric acid. The receiver flask is removed, and the sample is titrated with a 0.1M HCl until the sample turns a grey-mauve colour. Based on the titre value and molarity of each sample the %nitrogen can be determined.

% nitrogen= $\frac{(A \times B) \times 14 \times 100}{1000 \times C}$

A = ml of HCl used

B = Molarity of HCl

C = Sample wt.(g)

% crude protein= % nitrogen × 6.38

3.1.4 Crude Fat

The CPIs fat estimation readings (Mojonnier Method AACC 30-10) are taken in triplicate with a blank sample. 2g of CPI samples is taken in a 50ml beakers and 2ml of ethanol is added to wet the dry CPI powder. 10ml of HCl is added to the beakers and mixed in and placed in a boiling water bath while stirring the samples for 30-40 minutes and set it aside to cool. 10ml of ethanol is added and mixed and the mixture is transferred to a Mojonnier fat extraction tube. Rinse the beaker with 25ml diethyl ether in three portions to rinse out the beakers of any residue of the mixture. Place stoppers on the tubes and rock each tube gently for a minute.

25ml of petroleum ether is added to the tubes and rocked for 30 seconds and placed into a centrifuge for 2 minutes at 600rpm. Two distinct layers can be seen in the tubes. The organic solvent layer is decanted into a pre-weighed conical flask by gradually tilting the tube into a horizontal position careful not to decant the layer below. 5ml of ethanol is added into the tubes and mixed to prevent emulsions from forming. Then again 15ml of diethyl ether is added to the tubes and rocked for 1 minute and 15 ml of petroleum ether is added, rocked and centrifuged for 600rpm and any remaining organic solvent is decanted to the pre-weighed flask. The flasks are then placed on a steam bath to dry the flask and once the fat is seen in the flask and all the organic solvent is evaporated the flask is moved to a hot air oven at 100°C for 90 minutes to dry the flask of moisture. Once the flask is dry move to room temperature to allow the flask to cool and weigh the flask to find the fat in the flask.

% crude fat = $\frac{w_2 - w_1}{w_3} \times 100$

w_1 = Flask wt.(g)

w_2 = Wt. of flask and fat (g)

w_3 = Weight of sample(g)

3.1.5 Carbohydrate

Carbohydrate % is calculated using indirect subtraction method of protein%, Fat%, Ash% and Moisture%.

100% - [protein% + Fat% + Ash% + Moisture%]

3.2 Water Holding Capacity (WHC)

The water holding capacity was calculated in accordance with the method of (Jia et al., 2021). The readings were taken in triplicate with 3g CPI samples taken in 50ml centrifuge tubes with 25ml of RO water. The tubes with the CPI and water were placed on a vortex shaker for 500 rpm for 2 minutes each make sure to break all the lumps in the tube. If lumps are observed the tubes are vortexed for an additional 2 minutes. The samples are left in 2°C in a refrigerator for 24 hours for hydrating the protein isolate. The sample are left in room temperature for 30 minutes and the tubes are vortexed again for 2 minutes each for 500 rpm. The sample are then placed into the centrifuge (Multifuge X Pro series, Thermo scientific, NZ) for 3000g for 30 minutes.

After centrifugation the supernatant is decanted, and the tube is reweighed with the wet sediment. Moisture dishes are taken, weighed and the wet sediment from the tubes is transferred to the moisture dishes. The dishes are then transferred to a hot air oven for 108°C for 24 hours for drying. The dishes are then weighed to obtain the weight of the dry sediment.

$$WHC = \frac{M_w - M_d}{M_d}$$

M_w = Hydrated mass(g)

M_d = Dry mass(g)

3.3 Foaming Capacity and Stability

The foaming capacity is calculated in triplicate. This test is based on the foaming capacity and stability by (Vioque et al., 2000). 200ml of water is taken in a beaker and heated to 40ºC and stirred using a magnetic plate stirrer. 4g of CPI is added to the stirring RO water unit no lumps are observed, and the CPI is completely dissolved. Then the mixture is poured into a waring blender and blended for 2 minutes on low. Immediately after the 2 minutes the solution is poured into a 500ml measuring cylinder making sure to not get the solution on the walls of the cylinder. As soon as the mixture is poured into the cylinder record the volume of the liquid and volume of the foam. At regular intervals of 1-minute intervals for the first 5 minutes, and then at 3-minute intervals for the next 20 minutes. The foam and liquid volumes are also measured for time intervals of 30, 60, 90 and 120 minutes. By the data obtained for the gradual foam decrease and liquid increase the foam capacity and foam stability is calculated by using the formulas given below.

Foaming capacity is calculated as the foam volume decrease by the initial volume of the protein solution prior to blending (200ml). Foam stability is calculated as the foam volume percentages at time intervals of 0mins, 30mins, 60mins, 90mins, 120mins.

$$Foaming\ Capacity\ FC\ (\%) = \frac{V_{foam} - V_{liquid}}{V_{liquid}} \times 100$$

$$Foaming\ Stability\ FS\ (\%) = \frac{V_{foam\,t}}{V_{foam\,0min}} \times 100$$

3.4 Thermal Properties

The thermal property of CPI was investigated by using Differential Scanning Calorimetry (DSC) (Q2000 DSC, TA instruments, New Castle, DE, USA). The protein isolate prepared was a 50% thick paste and set aside for few hours to allow hydration of the CPI.

For the DSC measurements two aluminium pans are used- one pan for the sample and one reference pan. The weights of both pans and sample pan before sample is placed is noted.10-12mg of sample is weighed into the sample aluminium TA pan with a aluminium lid on top and sealed using a Tzero press. The heating range of 20°C - 105°C at rate of 5°C/min was set in the system. The lid is then opened, and both the reference and sample pans are placed into the machine and the resultant graph is obtained with specific peak. (Kim et al., 2016).

3.5 Amino Acid Profile

The Amino Acid Profile of CPI was done by Nutrition lab, Massey University Palmerston North. The acid stable amino acid was determined by AOAC 994.12, Cysteine and Methionine by AOAC 985.28, Tryptophan by Alkaline Hydrolysis.

3.6 Particle size

The particle size distribution of CanolaPRO was determined using a Mastersizer 2000 laser diffraction particle size analyser. A 10% CPI solution was used with the relative refractive index at 1.20 (Östbring et al., 2019).

3.7 Rheology

Rheological measurements of CPI were made using an Anton Parr MCR 301 rheometer equipped with a plate-on-plate geometry (PP40 40mm stainless steel plate) with a 1mm measuring gap. The rheometer is also equipped with a thermostat bath controller with help to control the temperature during measurements.

For achieving a thick consistency for the isolate, a 60% protein sample was required as working with sample with a lesser concentration produced a lot of bubbles which could tamper with rheological readings. Centrifugation of the samples proved insufficient as it would create sediments during the process. To minimize the presence of bubbles in samples all samples were prepared 14 hours prior and stored in 4°C to allow proper hydration and air release. Prior to placing the samples on the rheometer, samples were gently stirred with a spatula to ensure the absence of bubbles. To align with literature specific concentrations use of hydrocolloids were employed to incorporate thickness and gelling which would make it possible to perform rheological measurements. A 10% protein solution with 2% xanthan gum (2% XC) and a 10% protein solution with a 3% xanthan gum (3% XC) is used for rheological measurement along with the 60% protein paste. A comparative study of the strain, temperature and frequency of the three protein samples are conducted.

The initial strain sweep was performed where the storage modulus G’ and loss modulus G” was calculated as a function of strain (0.001 – 100%) at a constant temperature of 25°C and constant frequency of 1Hz for 51 measuring points. The strain sweep was done in triplicate and is used to

calculate the Linear Viscoelastic Range (LVE). It is acknowledged that the initial strain sweep used to estimate the LVE was conducted at 25 °C, where the CPI 60% behaved as a viscous or weakly structured system rather than as a fully developed gel. In thermo-gelling protein systems, the LVE of the final gel state is typically narrower than that of the initial liquid state, because gels fracture at much lower strains than liquids. Ideally, the LVE for subsequent temperature and frequency sweeps should therefore be confirmed at the most solid state of the system (i.e., after gel formation on heating/cooling) (Moakes et al., 2015). In this study, the strain used for the temperature ramps and frequency sweeps was selected conservatively based on the low-strain region of the 25 °C strain sweep; however, no additional post-heating strain sweep was performed on the fully gelled samples. As a result, it cannot be definitively confirmed that all data in the cooling curves (e.g., Figure 9) were collected strictly within the LVE of the final gel.

Equivalent strain sweep tests (0.001–100% strain, 1 Hz, 25 °C) were also performed on the 10% protein solutions containing 2% xanthan gum (2 XC) and 3% xanthan gum (3 XC) to identify their LVE at 25 °C. For all samples, the LVE was defined as the strain interval over which G′ and G″ remained essentially independent of strain (variation within ±3%). The strain amplitudes used in subsequent temperature and frequency sweeps for each formulation were chosen within these pre-heating LVE limits. No additional strain sweep tests were carried out after heating/cooling, so the post-gelation LVE of the xanthan-containing systems was not directly determined.

A thin layer of mineral oil is applied around the sample to avoid evaporation in the sample. Temperature sweep is conducted on the CPI sample by initiating with a heating cycle of ramping the temperature at a rate of 5°C/min for a temperature range of 25°C - 95°C at a constant frequency of 1Hz at a strain amplitude of 0.05% for the 60% protein paste and the 10% protein solution with 3% xanthan gum (3% XC). The 10% protein solution with 2% xanthan (2% XC) had a strain amplitude of 0.1%. Then held at 95°C for 30 seconds followed by the cooling cycle from 95°C - 25°C at a rate of 5°C/min at constant frequency of 1Hz and strain amplitude along with the sample being held at constant temperature of 25°C for 30 seconds. A frequency sweep was conducted on the sample that underwent temperature sweep at a frequency range of 0.1 – 10Hz for a constant strain amplitude and constant temperature of 25°C.

Frequency sweeps are later tested on the CPI sample, tested within the LVE range. It is measured from a frequency range of 0.1 – 10Hz for a constant strain amplitude and constant temperature of 25°C.

In addition to CPI 60%, rheological measurements were conducted on CPI–xanthan mixtures containing 10% protein with 2% xanthan gum (2 XC) and 10% protein with 3% xanthan gum (3 XC). No measurements were carried out on xanthan gum solutions without canola protein. This is a limitation, as xanthan gum is known to form gels and viscoelastic networks on its own, and a pure xanthan control would have enabled a direct assessment of whether CanolaPRO reinforces or weakens the xanthan gel structure.

3.8 Statistical analysis

All quantitative analysis was performed using Microsoft Excel. Means and standard deviations for each experiment were calculated using Excel functions. All graphs and tables shown in this work was generated using Microsoft Excel and all experiments were done in triplicate.

4.Results and Discussion

4.1 Proximate Analysis

Table 1: Proximate analysis of Canola Protein Isolate

Moisture	7.37±.033%
Ash	0.89±0.02%
Crude Fat	1.47±0.06%
Crude protein	**89.03±0.33%**
Carbohydrates	1.54%

Data was obtained as triplicates, and the data was calculated to obtain standard deviations.

The CanolaPRO protein isolate was used to conduct proximate analysis which yielded results of 7.37±.033% moisture, 0.89±0.02% ash ,1.47±0.06% fat, and a high protein content of 89.03±0.33% (Nx6.25). The level of purity of the canola protein makes it a superior plant protein which can be comparable to those produced through alkaline extraction combined with ultrafiltration (Alk-Uf) or ethanol pretreatment followed by Alk-Uf which yielded high protein contents in the range of 88-90%(Cháirez-Jiménez et al., 2023).

The low fat and ash content of CanolaPRO protein isolate is consistent with that of commercial canola protein isolates and are a characteristic of high purity extraction which is extracted with a combination of salt extraction and membrane based purification (GRAS, 2017). Although the presence of the moisture content (7.37%) is slightly higher than other ultra filtrated isolates, but these finds are aligned with other commercial spray-dried CPI powder(GRAS, 2010, 2023).

Canola protein isolates with protein range from 89-90% protein have high nutritional and functional properties. The CPIs produced via Et-Alk-Uf method attain a crude protein content of 89.7% and Protein Digestibility Corrected Amino Acid Scores (PDCAAS) of 1.0(Cháirez-Jiménez et al., 2023).

4.2 Water Holding Capacity (WHC)

Table 2: Water Holding Capacity of Canola protein isolate

Hydrated mass (g) Mw	**Dry mass (g) Md**	**WHC=Mw-Md/Md**
1.3947±0.02	0.2875±0.1	**3.85±0.15**

Data was obtained as triplicates, and the data was calculated to obtain standard deviations.

The Water Holding Capacity (WHC) of CanolaPRO protein isolate was tested to be 3.85±0.15 g water/ g dry pellet protein performed by the method described by (Jia et al., 2021). This range of Water Holding Capacity reported by Jia et al. (2021) comes close to the WHC obtained in CanolaPRO which was conducted in triplicates and shows very low standard deviation (Jia et al., 2021).

The WHC values obtained for CanolaPRO is very similar to the values reported in literature. Jia et al. (2021) reported a WHC of approximately 3.50g/g for Rapeseed Protein Isolate (RPI), with a higher WHC of 6.7g/g for Rapeseed Protein Concentrate (RPC). The lower WHC for RPI compared to the concentrate is attributed to the presence of hydrophilic materials which is lost during the purification process(Jia et al., 2021). Similar values of 3.86 g/g were reported with samples that are extracted using alkaline extraction followed by ultrafiltration (Alk-Uf) and ethanol pretreatment followed by Alk-Uf (Cháirez-Jiménez et al., 2023).

When comparing CanolaPRO with other plant protein isolates like Soy Protein Isolate (SPI) a value of 7.57g/g is reported which is higher than other pulse protein isolates and this high WHC is attributed to its protein structure and high presence of hydrophilic amino acids. In Pea Protein Isolate (PPI) WHC reported value is 5.14g/g. In the cases of Faba Bean Protein Isolate (FPI) and Lentil Protein Isolate (LPI) the values are 3.20g/g and 2.20g/g (Kai Kai Ma et al., 2022).

The WHC value of 3.85g/g obtained for CanolaPRO shows that although it does not compare with values reported for proteins like and SPI and PPI it can show comparative and in some cases higher WHC with proteins like FPI and LPI(Kai Kai Ma et al., 2022).

4.3 Foaming Capacity (FC) and Foaming Stability (FS)

Table 3:Foaming Capacity (FC) and Foaming Stability (FS) of CanolaPRO

time(mins)	CPI foam volume	Foam capacity	Foam Stability
0	483.3 ±2.36	141.7%	100.0%
30	455.7±1.55	127.9%	94.3%
60	414.7±2.05	107.4%	85.8%
90	389±3.74	94.5%	80.5%
120	371.3±4.11	85.7%	76.8%

Data was obtained as triplicates, and the data was calculated to obtain standard deviations.

The foam stability and capacity of the commercial CanolaPRO protein isolate was performed based on the experimental method of Vioque et al. (2000)(Vioque et al., 2000). From the Table 3 a progressive decline is observed on the foam capacity and foam stability over a period of 120 minutes. Initially at 0 mins a foam capacity of 141.7% and foam stability of 100% is observed.

Foaming capacity of canola and rapeseed protein isolates may vary depending on the extraction and processing conditions. For example, the work by Cháirez-Jiménez et al. (2023) on canola protein isolates extracted by alkali-acid extraction excellent foaming capacity (505%) and stability of (93.75%), which are values better than reported for unmodified rapeseed/canola isolates(Cháirez-Jiménez et al., 2023). A study by Ivanova et al. (2018) shows that acid-soluble protein-rich ingredients (ASP) can achieve great amount of foaming 90% and foam stability stays at a higher percentage if salts are avoided along with extreme pH ranges.

Foam capacity and stability depend not only on processing conditions but also on innate protein characteristics like solubility, molecular weight, surface hydrophobicity which can influence foam formation(Zhang et al., 2025). When compared against other plant protein isolates, canola protein's foaming is better than that of chickpea (30%-44%) and pulse isolates (30%-70%) often varying depending on the experimental method (K. K. Ma et al., 2022). Soy protein isolate is popular in the food industry for its strong foaming properties in specific pH and processing conditions is slightly better or match canola protein isolate although outcomes may vary depending on the post-extraction product(J. Li et al., 2021).

4.4 Thermal Properties (DSC)

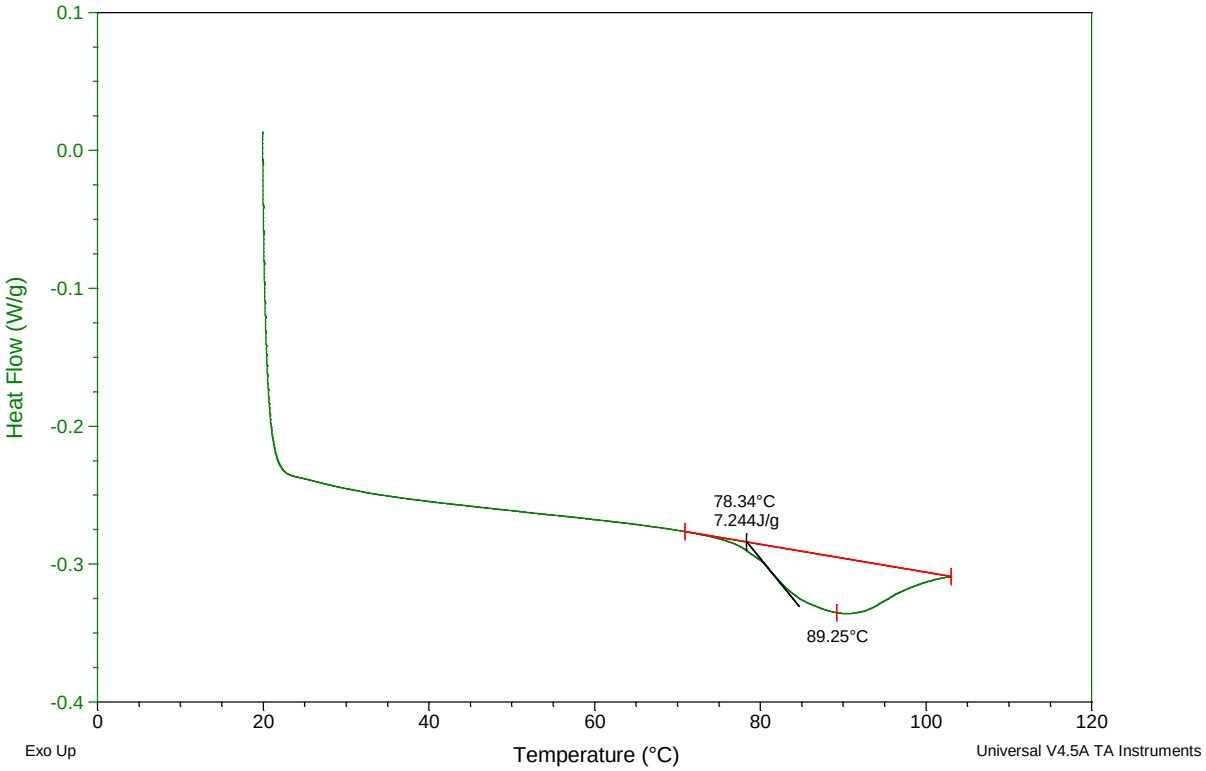

Figure 3: DSC graph of CanolaPRO

The differential scanning calorimetry (DSC) conducted on CanolaPRO gave an endothermic peak (Figure 3) with an onset temperature (T_m) of 78.0±0.3°C, denaturation temperature (T_d) of 89.2±0.005°C and denaturation enthalpy (ΔH) of 7.0±0.2 J/g (Table 4). These values for a DSC analysis of CanolaPRO closely correspond with other CPI cited in literature like Kim et al., 2016 whose CPI was of 98% protein purity reported the onset temperature of 78.6 ± 0.4 °C and denaturation temperature of 87.1 ± 0.8 °C(Kim et al., 2016).

Table 4:The thermal denaturation of CanolaPRO

Sample	**T_m (°C)**	**T_d (°C)**	**ΔH (J/g)**
CanolaPRO Protein Isolate	78.0±0.3	**89.2±0.005**	7.0±0.2

T_m (°C) - onset temperature

T_d (°C) - denaturation temperature

ΔH (J/g) – enthalpy of denaturation

Data was obtained as triplicates, and the data was calculated to obtain standard deviations.

Wu et al. (2008) reported similar finding in their study on Cruciferin and Napin proteins, (which are the two main storage proteins present in canola protein) which have the denaturation temperature 90.7°C and 109.9°C respectively. The thermal denaturation tests conducted on canola protein isolates provided the onset temperature of 77.9°C and denaturation temperature of 83.9°C which are very similar values to CanolaPRO(Wu & Muir, 2008).

When comparing the thermal properties of canola protein with other plant protein isolates it provides additional perspective. Soybean protein isolate is one of the most popular plant protein isolates and one of the most studied plant proteins shows denaturation temperature for β-conglycinin and glycinin, which are the two main storage proteins of soybean protein as 76.7°C and 94.1°C (Tang et al., 2007). Pea Protein Isolate (PPI) shows denaturation temperature for its globular protein 11S legumin and 7S vicilin as 84°C and 71°C respectively (Kuang et al., 2023).

The thermal analysis of CanolaPRO shows excellent thermal stability which places it among other plant protein isolates. The denaturation temperature of 89.2°C provides CanolaPRO with flexibility in various food applications which ensures it can undergo controlled thermal modification while maintaining structural integrity. This high thermal stability ensures that CanolaPRO is particularly suitable for applications in baked goods, extruded products and thermally processed beverages.

The denaturation temperature of CPI, observed at approximately 89 °C in the DSC thermogram, is expected to correspond to major structural transitions that can alter gelation behaviour and viscoelastic properties. This temperature therefore provides a useful reference point for interpreting the rheological response of CPI and CPI–xanthan systems during heating and cooling in the rheology experiments.

4.5 Amino Acid Profile

Table 5:The amino acid composition of CanolaPRO (CPI)

Non- essential amino acids	
Alanine	3.51
Arginine	5.89
Aspartic acid	5.91
Cysteine	2.74
Glutamic acid	20.76
Glycine	4.00
Proline	5.77
Serine	2.90
Tyrosine	2.01
Essential amino acids	
Histidine	1.88
Isoleucine	3.41
Leucine	6.26
Lysine	4.99
Methionine	2.37
Phenylalanine	3.53
Threonine	3.09
Tryptophan	1.40
Valine	4.60

The amino acid analysis of the CanolaPRO is reported as mg/100mg of protein. The findings on Table 5 confirms the published findings in recent years on the animo acid composition of canola protein isolate. Arginine content of CanolaPRO is seen to be at 5.89mg/100mg, aspartic acid is measured at 5.91mg/100mg and proline at 5.77mg/100mg. The glutamic acid content (20.76mg/100mg) of CanolaPRO is the highest reported amino acid value in the Table4 and it is a repeatedly reported value for glutamic acid in canola protein isolate from the range of 17 to 23 mg/100mg(Chmielewska et al., 2021; X. Li et al., 2021; Zhu et al., 2024).

The essential amino acids like Leucine (6.26mg/100mg), Isoleucine (3.41mg/100mg) and Valine (4.60mg/100mg) are comparable to the values reported for canola, soy and other plant proteins (Cháirez-Jiménez et al., 2023; Fleddermann et al., 2013). These amino acids are called the BCAAs (Branched-Chain Amino Acids) and these amino are not synthesised by the human body and is required for the synthesis of muscle proteins and recovery (Erdman J, 2011).

Cysteine (2.74mg/100mg) and Methionine (2.37mg/100mg) are the sulfur-containing amino acid which is comparable or sometimes greater than the values reported for soy, pea and other plant proteins(Fleddermann et al., 2013). Histidine (1.88mg/100mg), Threonine (3.09mg/100mg), Phenylalanine (3.53mg/100mg), Tryptophan (1.40mg/100mg) and Tyrosine (2.01mg/100mg) are the other amino acids which closely align with the other documented values for canola protein isolates and other high-quality isolates which were prepared by a number of extraction methods (Cháirez-Jiménez et al., 2023; Karabulut et al., 2025).

Difference in extraction methods and extraction source (seed meal) can lead to minor changes in amino acids values but not cause a significant change in the nutritive profile of the protein isolate (Cháirez-Jiménez et al., 2023; X. Li et al., 2021). The amino acid analysis of CanolaPRO suggested that this is an ideal source of high-quality protein and meet the requirements for an adult (Chmielewska et al., 2021).

4.6 Particle size

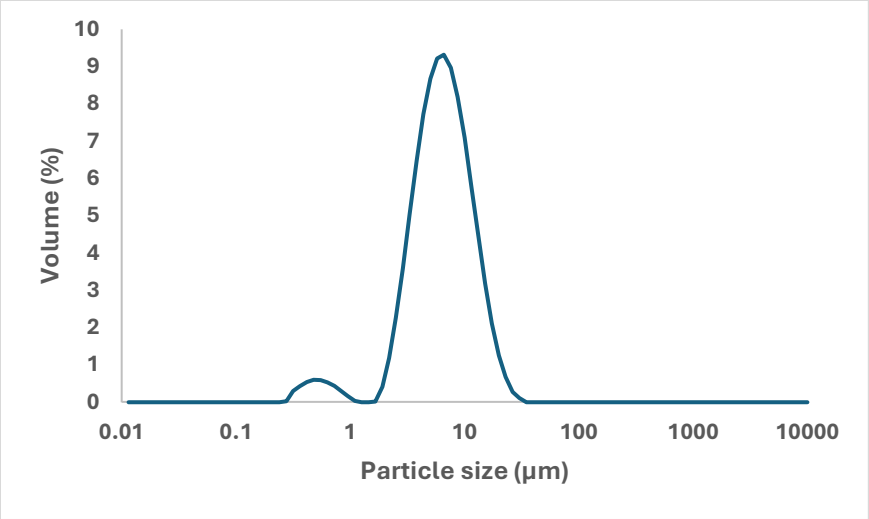

Figure 4: A graphical representation of the particle size distribution of CanolaPRO

Table 6:Particle size distribution of CanolaPRO

D [3,2]	**3.783**
D [4,3]	6.778
d (0,1)	2.745
d (0,5)	5.876
d (0,9)	12.179

In this study a 10% CPI solution was used for the estimation of particle size distribution and mean particle size. The Mastersizer 2000 data for CanolaPRO shows a unimodal graph with the volume distribution seen around 6µm (D [4,3] = 6.78 µm, d (0,5) = 5.876 µm) indicating that most of the particles fall between 3 and 12 µm. The mean particle D [3,2] of CanolaPRO is 4 µm and the D [4,3] is 7µm. The d (0,9) value 12µm shows the largest particle size with 90% of CanolaPRO being below that level. (Table 6). These values indicate that CanolaPRO is a fine particle with even smaller particle size that other commercial plant protein isolates like soy and pea protein isolates (Dadi et al., 2025; Schmid et al., 2024).

When CanolaPRO is compared with other plant protein isolates, it shows a significantly smaller and a more uniform particle size. Studies on Soy Protein Isolate (SPI) using a Mastersizer showed a significantly larger size and broad distribution of particles. Schmid et al.,2024 worked on investigating the physicochemical properties of different commercial soy protein isolate she investigated the particle size of the SPIs. The D [3,2] and D [4,3] values in this study for six SPI commercial brands are at least five times larger than the CanolaPRO. The D values ranges

from 22µm to 72µm in dry powder analysis and in a hydrated sample the d values range from 45µm to 202µm(Schmid et al., 2024).

The comparison of particle size distribution of protein powders of mung bean, pumpkin, pea, fava bean, and soy bean also shows that the D values greatly differ in with the difference in particle size with the greatest in particle size being pea>fava bean>soy bean>pumpkin>mung bean (Scott, 2025). This comparative data analysis of CanolaPRO with other plant protein isolate indicates that CanolaPRO has a more finer particle size than other plant protein sources.

4.7 Rheology

The rheological analysis of CanolaPRO protein isolate gives and detailed insights into the viscoelastic behaviour and gel-forming capabilities under various conditions. The rheological study of CanolaPRO includes a strain sweep analysis which can help determine the Linear Viscoelastic range (LVE), temperature sweep followed by a frequency sweep and an independent frequency sweep without heat treatment. The study is conducted on three samples: a 60% CanolaPRO paste (CPI 60%), a 10% CanolaPRO solution with 2% Xanthan Gum (2% XC) and a 10% CanolaPRO solution 3% xanthan gum (3% XC) hydrocolloid.

4.7.1 Strain sweep

The strain sweep for the CPI samples, provided an LVE range of 0.05-1% for CPI 60% (Figure 5) and 3% XC (Figure 6) whereas for 2% XC, it was 0.1-1%. Based on these LVE values the temperature and frequency sweeps were conducted. These LVE ranges are consistent with the ranges reported for other plant protein isolates, where low strain is associated with a brittle gel network (Tang & Ghosh, 2021b). For both 2% XC and 3% XC, the LVE at 25 °C extended up to 0.05-1% before G′ started to decrease, indicating the onset of non-linear behaviour. These pre-heating LVE limits were used to set the strain amplitudes for the temperature and frequency sweeps of the xanthan-containing samples. However, because no strain sweeps were carried out after gelation, potential changes in the LVE range (and thus brittleness) of the gels upon heating/cooling could not be quantified directly, and any discussion of brittleness changes is therefore qualitative rather than quantitative.

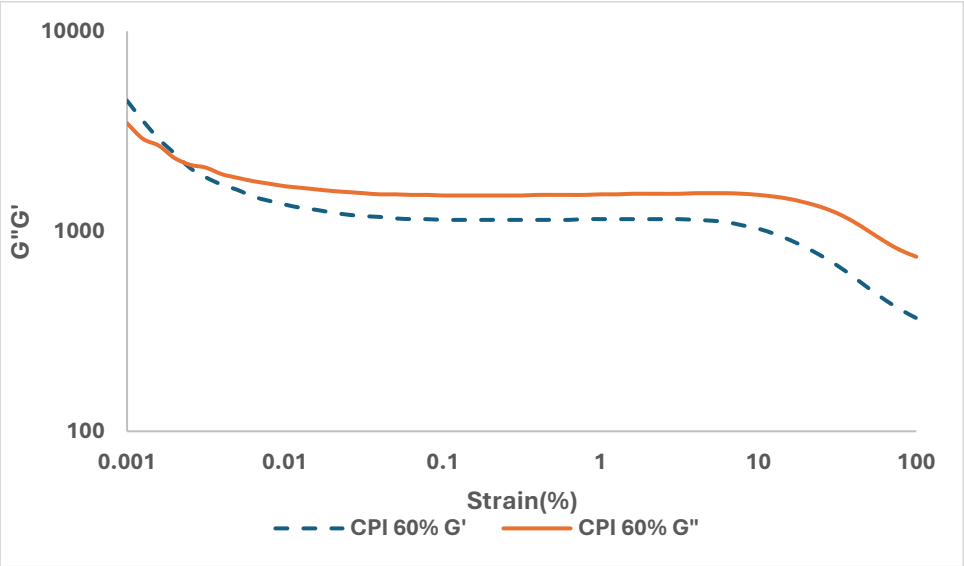

Figure 5: Strain sweep (0.001-100%) of CPI 60%

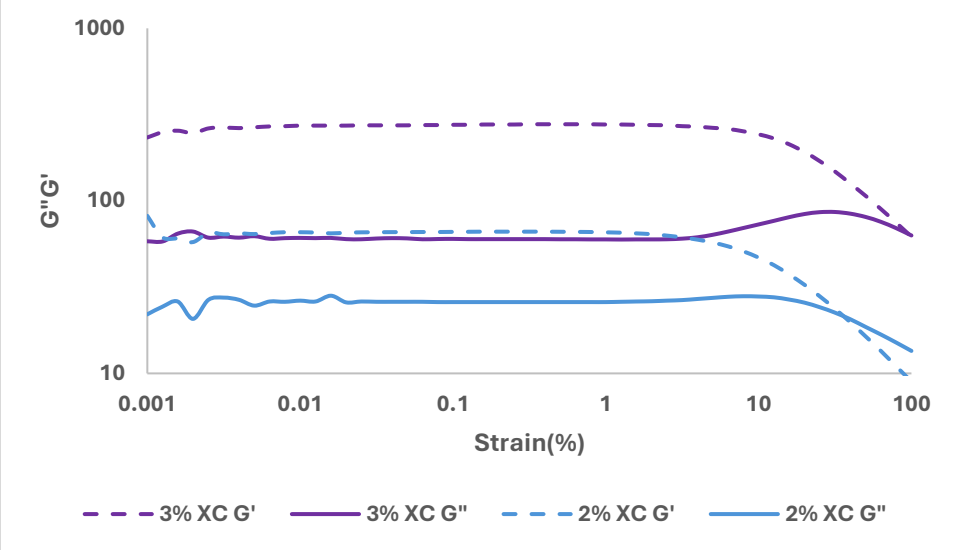

Figure 6: Strain sweep (0.01-100%) of 2% XC and 3% XC

4.7.2 Temperature ramp combined with frequency sweep

In Figure 7 it is seen that the G" is greater than G' and it is seen that G' value rises at a range of 75-80°C. Before the rise of G' the sample is seen as a thick yet viscous liquid but the rise in G' is correlated with the denaturation of CPI where there is an unravelling of protein structure with protein aggregation by hydrophobic interaction and the formation of disulfide bonds.

The heating cycle of the CPI xanthan samples in Figure 8 it is seen that for the 2% XC samples the G' and G" values decline in the temperature range of 60-70°C whereas for the 3% XC there is a temperature range of 80-85°C. The 3% XC has a higher modulus than the 2% XC during the heating cycle.

During the heating cycle (Figure 7, Figure 8), G′ began to increase markedly as the temperature approached and passed the DSC denaturation temperature (~89 °C), consistent with protein unfolding and aggregation leading to network formation.

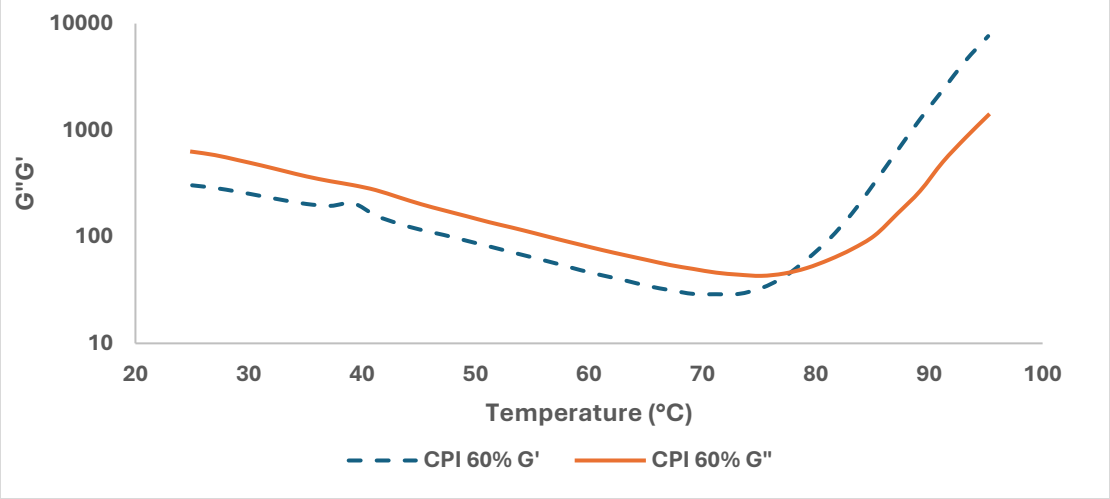

Figure 7: Heating cycle (25-95°C) of CPI 60%

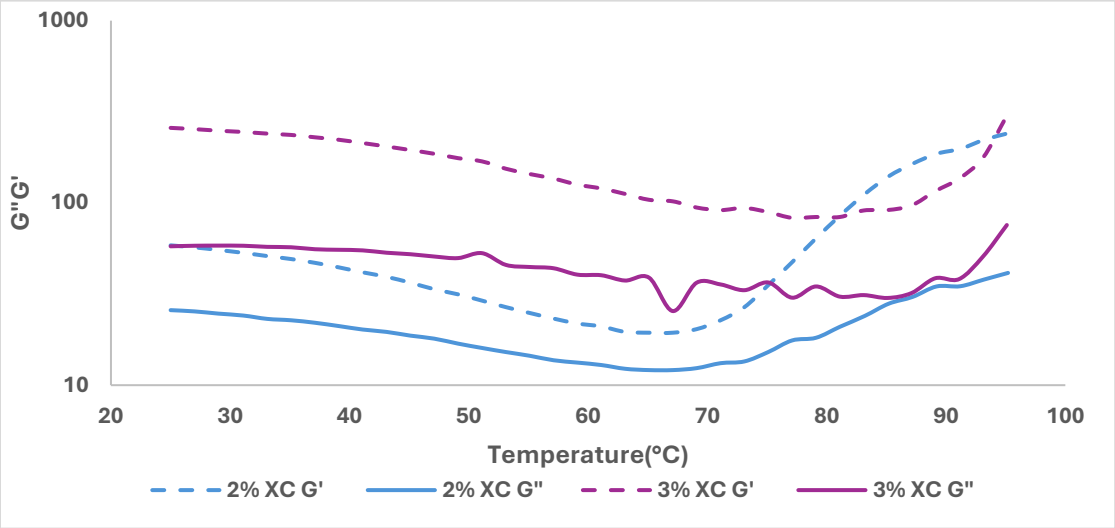

Figure 8: Heating cycle (25-95°C) of 2% XC and 3% XC

The strain amplitude applied during the temperature sweeps was chosen in the low-strain region determined at 25 °C; however, because no additional strain sweep was carried out after gelation, the LVE of the fully gelled CPI 60% during cooling (Figure 9) can only be assumed and not rigorously verified. In Figure 9 the cooling cycle is seen for the CPI 60%, the G' rises above the G" and it experiences a drop a then gradually increasing as the temperature decreases. The gradual increase in G' and G" values indicate a strong elastic gel after heat set. This is seen as the protein-protein aggregate and the hydrogen bonding is becoming more prevalent an gel structure is becoming stronger (Léger & Arntfield, 1993). Although G′ remained higher than G″ during cooling, indicating gel-like behaviour, it should be noted that the absence of a post-heating strain sweep means these measurements may partially extend beyond the strict LVE of the final gel structure, particularly at lower temperatures where the gel is most rigid. Figure 10

shows the cooling cycle of the XC samples. It is seen that the 2% XC finishes higher than the 3% XC. Excess xanthan may partially disrupt protein-protein connectivity after the thermal cycle causing a less elastic protein system (Rezaee et al., 2022).

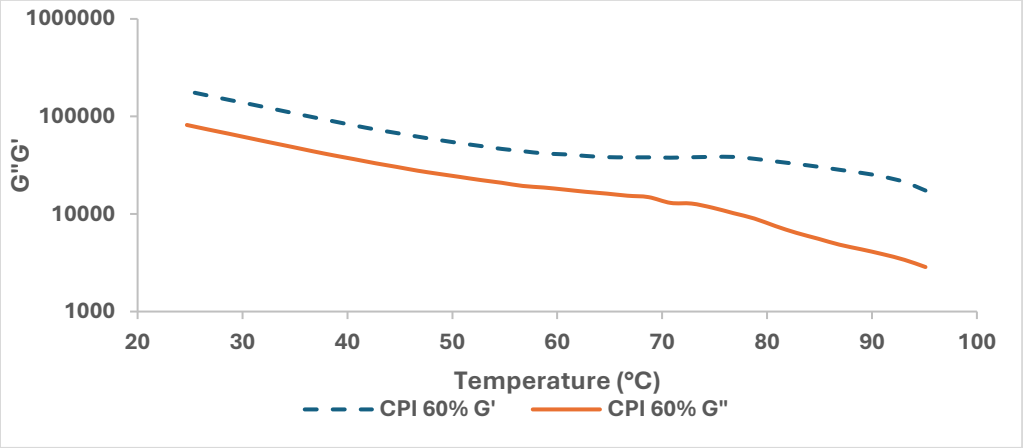

Figure 9: Cooling cycle (95-25°C) of CPI 60%

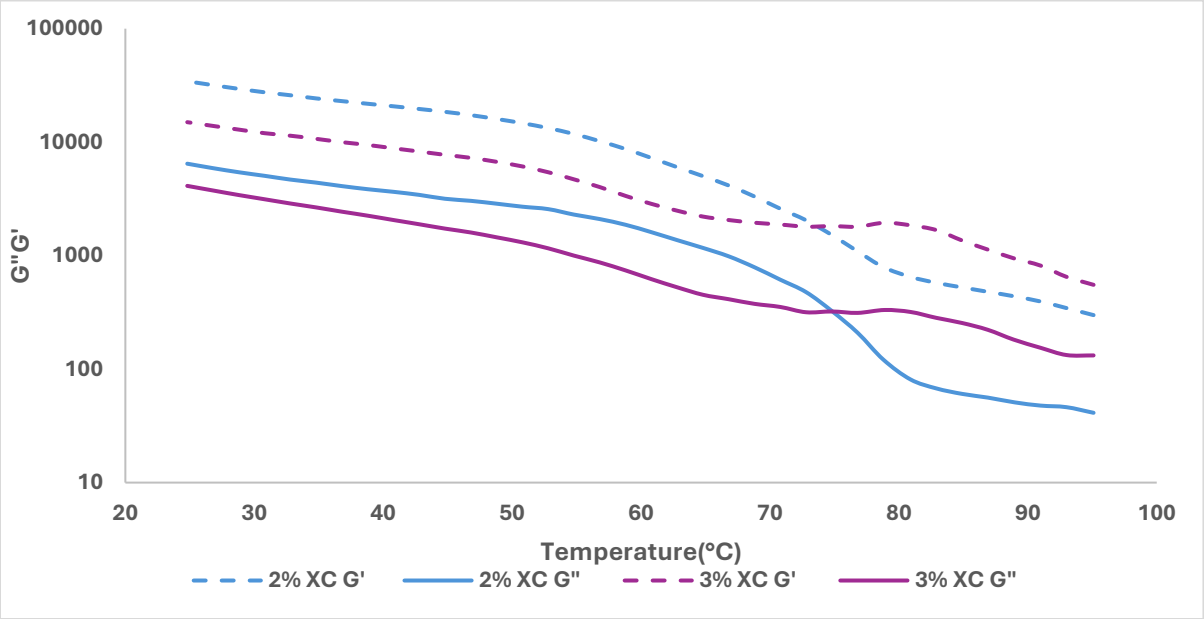

Figure 10: Cooling cycle (95-25°C) of 2% XC and 3% XC

In Figure 11 it is observed that in frequency sweep after temperature sweep the G’ > G” and both G’ and G” increased in a steady pace as a function of frequency. The G’ represents the number of cross-links which exists within the protein system. A greater G’ value indicates greater inter-molecular network and enhanced protein-protein interaction. It is also seen that the G’ and G” are parallel to each other which show the typical behaviour of weak gels (Tan et al., 2014).

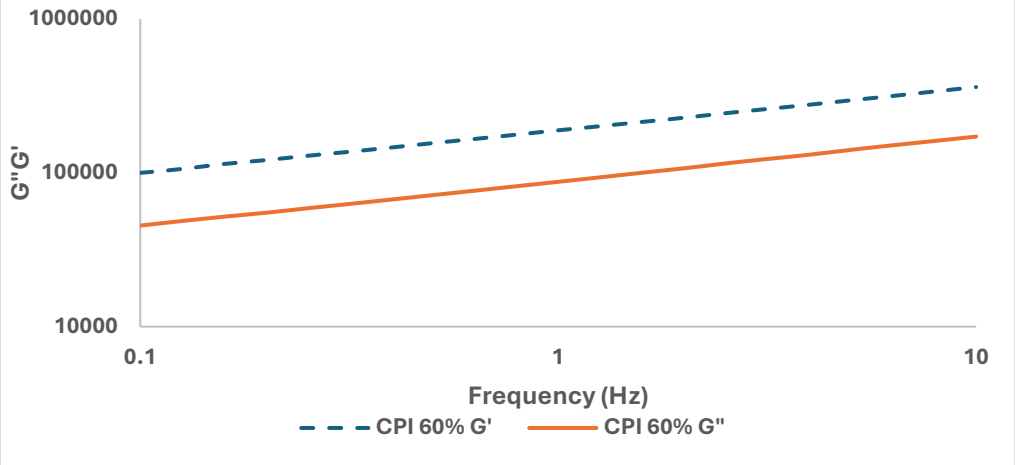

Figure 11: Frequency sweep of CPI 60% after temperature sweep

The thermal history of the 2% XC and 3% XC (Figure 12) gives the xanthan canola samples and advantage as it reflects a better protein-protein linkage with 2% XC seen with a better moduli than the 3% XC. This is correlated to the cooling cycle of the xanthan canola samples which shows that a moderate amount of xanthan can produce better linkage and gels.

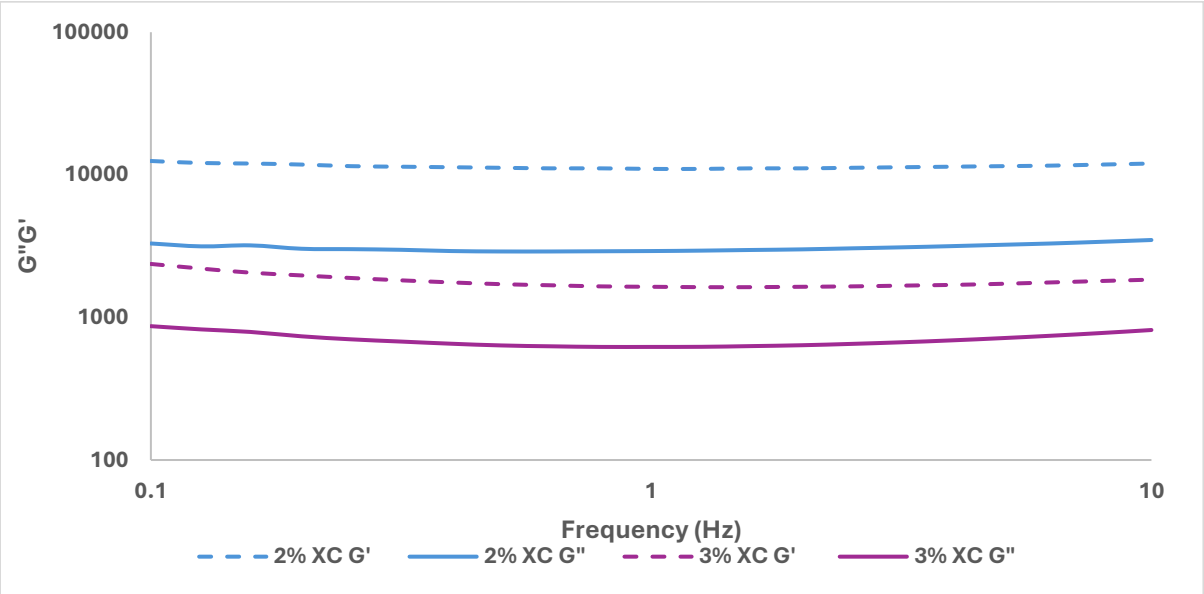

Figure 12: Frequency sweep of 2% XC and 3% XC after temperature sweep

4.7.3 Frequency sweep

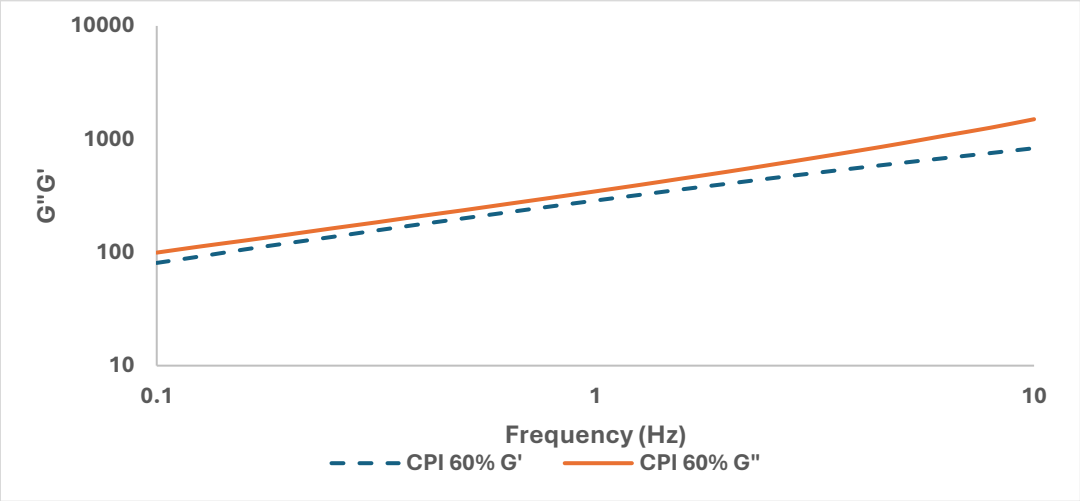

Figure 13:Frequency sweep of CPI 60%

In the Figure 13 on which the CPI 60% is tested for frequency sweep and is seen that G" > G' buy a small margin. A comparison of the frequency sweeps in Figure 11 and Figure 13 shows a big gap. This shows that thermal denaturation is required to build strong elastic networks in CPI gels. In the research by Tang et al. 2021 it is seen that the heat treated CPI sample shows comparatively strong gel network than the untreated CPI emulsion (Tang & Ghosh, 2021a).

In the frequency sweep of XC samples (Figure 14), a reversal is observed to the temperature sweeped frequency samples. The more the amount of xanthan the greater is the G' which indicates that xanthan alone can create a more viscoelastic network without heating.

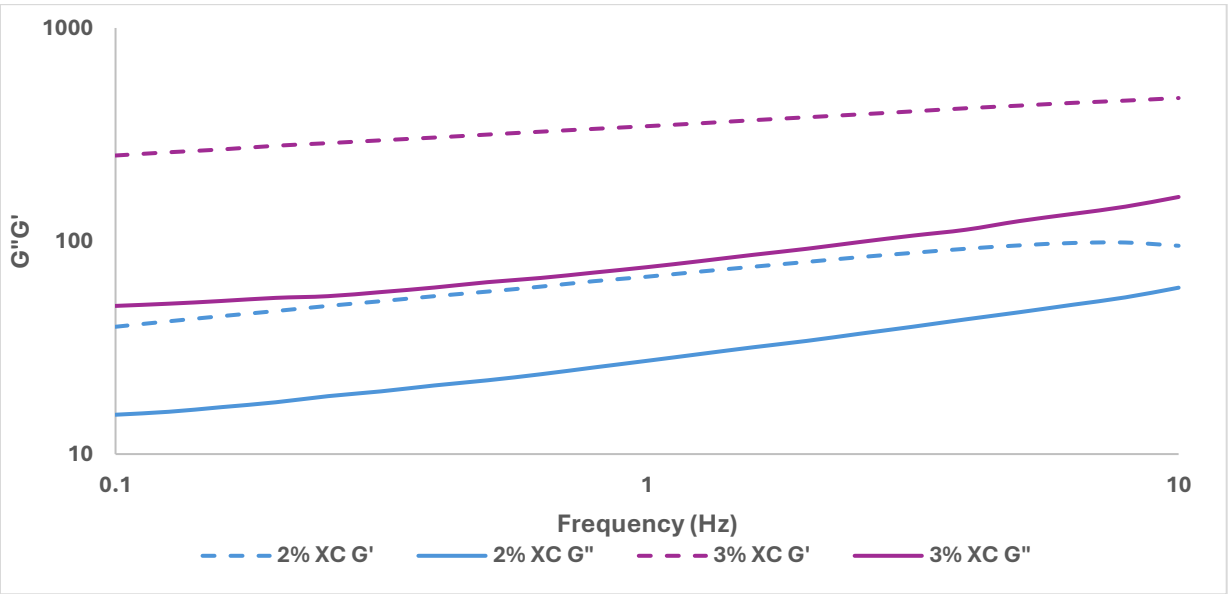

Figure 14: Frequency sweep of 2% XC and 3% XC

Addition of xanthan gum to Cpi showed a definite change in rheological characteristics in the protein system. Adding 2–3% xanthan gum to CanolaPRO at the same protein level boosted storage modulus (G′) and kept G′ > G″ across frequencies, forming a stronger elastic network than CPI alone. Xanthan blends also showed wider LVE at 25°C and firmer gels after heating-cooling, confirming xanthan reinforces protein structure rather than just thickening.

5. Conclusion

This research has achieved its primary objective of investigating the various functional properties and characterization attributes of a commercial canola protein isolate (CanolaPRO I90+). This study provides valuable insights into the properties that make canola protein isolate a innovate and sustainable alternative to traditional animal and plant-based protein in food applications.

The proximate analysis of CanolaPRO showed a high protein content of 89.03±0.33%, low fat content of 1.47±0.06% fat, a minimal ash content 0.89±0.02% ash and a moisture content of 7.37±.033% which is in acceptable levels for protein isolates which ensure a longer shelf-life and stability.

Ther water holding capacity (WHC) of CanolaPRO is 3.85±0.09 g water/ g protein which is in a range of most other commercial plant protein isolates, which shows its potential for retaining moisture in food formulations.

The foaming property demonstrated by CanolaPRO show excellent surface-active properties with an initial foaming capacity of 141.7% and a good stability of 76.8% after 120 minutes. These properties can vary by extraction method and show a higher foaming capacity and stability than most other plant protein isolates which makes it a suitable in food applications involving foam formation and stability such as in bakery and beverages.

Thermal analysis through Differential Scanning Calorimetry (DSC) revealed the onset temperature as 78.0±0.3°C and denaturation temperature as 89.2±0.005°C. These values closely align with literature on canola protein isolates and can be an important parameter in food applications. The high denaturation temperature indicates good thermal stability, which allows various heat treatments while maintaining protein integrity.

Amino acid profile analysis confirms the excellent nutritional availability of CanolaPRO with glutamic acid being in the highest amount of 20.76mg /100mg protein which is a value consistent with literature for canola proteins. The presence all essential amino acids, branched-chain amino acid (leucine 6.26mg /100mg, isoleucine 3.41mg /100mg, valine 4.60mg /100mg) and sulfur- containing amnio acid (cysteine 2.74mg /100mg, methionine 2.37mg /100mg), demonstrates Casolaro’s ability to meet an adult’s nutritional requirements and compete with other high-quality plant protein isolates.

Particle size analysis of CanolaPRO revealed that the particle size to be approximately 6µm which is significantly smaller than other commercial soy and pea protein isolates. This fine particle size enhances mouthfeel, solubility and incorporation into food formulations, where a smoother texture is desirable.

Rheological analysis also provided insights into the gelling properties of CanolaPRO. The study showed that thermal denaturation is essential for the development of strong gels. It is demonstrated that incorporation of a moderate amount of hydrocolloids produced better gel properties than at higher amount, showing that excess hydrocolloids can cause a bad protein-protein interaction.

The comparative analysis of CanolaPRO throughout this study highlights the properties and matching or exceeding the functional properties of other plant protein isolates offering an advantage against soy protein in particle size and thermal stability making canola protein a sustainable and high- quality protein source.

However, this study also highlights areas for future research, in areas of working with different processing methods to provide the maximum functional properties and work on ingredients which can work with canola proteins to improve areas where canola protein is lacking.

In summary, this investigation of the characteristics of CanolaPRO demonstrates that it possesses the necessary functional and nutritional attributes that make it an effective alternative protein source for food applications.

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Appendix A

Figure 15:Consistency of CPI 10% - 30%

Figure 16: Consistency of CPI 40% - 60%

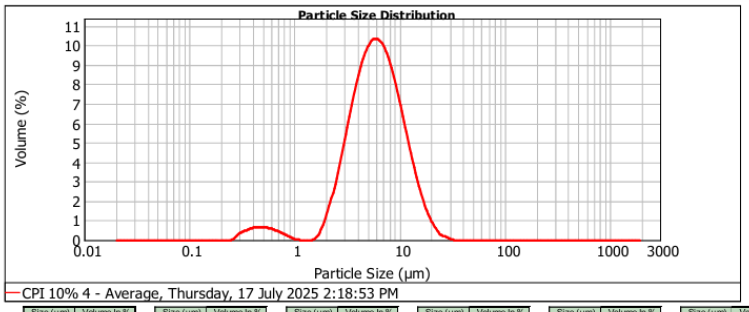
Appendix B

MASTERSIZER 2000

Result Analysis Report

Sample Name: CPI 10% 4 - Average	**SOP Name:**	**Measured:** Thursday, 17 July 2025 2:18:53 PM	
Sample Source & type:	**Measured by:** 24006113	**Analysed:** Thursday, 17 July 2025 2:18:54 PM	
Sample bulk lot ref:	**Result Source:** Averaged		
Particle Name: canola	**Accessory Name:** Hydro 2000SM (A)	**Analysis model:** General purpose	**Sensitivity:** Normal
Particle RI: 1.200	**Absorption:** 0.01	**Size range:** 0.020 to 2000.000 um	**Obscuration:** 18.94 %
Dispersant Name: Water	**Dispersant RI:** 1.330	**Weighted Residual:** 0.356 %	**Result Emulation:** Off
Concentration: 0.0152 %Vol	**Span :** 1.605	**Uniformity:** 0.508	**Result units:** Volume
Specific Surface Area: 1.59 m²/g	**Surface Weighted Mean D[3,2]:** 3.783 um	**Vol. Weighted Mean D[4,3]:** 6.778 um	

d(0.1): 2.745 um **d(0.5):** 5.876 um **d(0.9):** 12.179 um



Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %	Size (µm)	Volume In %
0.010	0.00	0.105	0.00	1.096	0.00	11.482	4.45	120.226	0.00	1258.925	0.00
0.011	0.00	0.120	0.00	1.259	0.00	13.183	3.18	138.038	0.00	1445.440	0.00
0.013	0.00	0.138	0.00	1.445	0.00	15.136	2.11	158.489	0.00	1659.587	0.00
0.015	0.00	0.158	0.00	1.660	0.02	17.378	1.26	181.970	0.00	1905.461	0.00
0.017	0.00	0.182	0.00	1.905	0.41	19.953	0.68	208.930	0.00	2187.762	0.00
0.020	0.00	0.209	0.00	2.188	1.19	22.909	0.27	239.883	0.00	2511.886	0.00
0.023	0.00	0.240	0.00	2.512	2.27	26.303	0.11	275.423	0.00	2884.032	0.00
0.026	0.00	0.275	0.03	2.884	3.59	30.200	0.00	316.228	0.00	3311.311	0.00
0.030	0.00	0.316	0.30	3.311	5.04	34.674	0.00	363.078	0.00	3801.894	0.00
0.035	0.00	0.363	0.43	3.802	6.47	39.811	0.00	416.869	0.00	4365.158	0.00
0.040	0.00	0.417	0.55	4.365	7.73	45.709	0.00	478.630	0.00	5011.872	0.00
0.046	0.00	0.479	0.60	5.012	8.68	52.481	0.00	549.541	0.00	5754.399	0.00
0.052	0.00	0.550	0.59	5.754	9.22	60.256	0.00	630.957	0.00	6606.934	0.00
0.060	0.00	0.631	0.54	6.607	9.32	69.183	0.00	724.436	0.00	7585.776	0.00
0.069	0.00	0.724	0.43	7.586	8.97	79.433	0.00	831.764	0.00	8709.636	0.00
0.079	0.00	0.832	0.30	8.710	8.19	91.201	0.00	954.993	0.00	10000.000	
0.091	0.00	0.955	0.16	10.000	7.09	104.713	0.00	1096.478	0.00		
0.105	0.00	1.096	0.03	11.482	5.79	120.226	0.00	1258.925	0.00		

Operator notes: *10% canola PI*

Malvern Instruments Ltd.
Malvern, UK
Tel := +[44] (0) 1684-892456 Fax +[44] (0) 1684-892789

Mastersizer 2000 Ver. 6.01
Serial Number : MAL1057136

File name: 10% CPI
Record Number: 38
17/07/2025 2:26:59 PM

Figure 17: Mastersizer Data for CPI 10%

Appendix C

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Figure 18: License for the use of Figure 1

Appendix D

AI declaration

AI tools like ChatGPT, Perplexity and Quill Bot were used during the preparation of this thesis. These tools were used only to polish the language, help with complex concepts and help with writer block. No AI system contributed to the original research, analysis or with experimental data. All scientific findings and conclusions are the author’s own.