Effects of Atmospheric Cold Plasma Treatment on Canola Protein Structural and Functional Properties

by

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ABSTRACT

Canola meal contains a considerable concentration of protein after oil extraction, and it is a potential alternative to other plant proteins because of its functional properties and well-balanced amino acid composition. However, canola meal contains undesirable compounds such as phenolic compounds including sinapine, the principal anti-nutritional compound among all phenolic compounds. Atmospheric cold plasma (ACP) is a nonthermal technology, which can potentially improve the functional properties of proteins. ACP is capable to degrading the antinutritional compounds in canola protein concentrate (CPC), making it more suitable for various applications in the food industry. In this research, the application of ACP treatment resulted in 74 and 43.6% reductions in total phenolic compounds and sinapine concentration of CPC, respectively. Moreover, the ACP treatment led to enhanced functional properties of canola protein including improved solubility, emulsification, and foaming properties, accompanied by notable structural modification. The solubility of CPC was dependent on the pH and the ACP treatment time. The ACP treatment improved the emulsifying properties. The exposure to ACP unfolded the CPC structure as demonstrated by the Fourier transform infrared spectroscopy analysis. After ACP treatment, the β-sheets and random coil structures of CPC increased, but the β-turn and α-helix decreased. The structural changes induced by ACP treatment, with increased random coil, β-sheets, and decreased β-turn, and α-helix can affect different canola protein functionalities by potentially improving solubility and enhancing emulsification properties.

The second part of this research aimed to determine the combined effect of ACP treatment with pH shifting to improve the quality of canola protein and degrade the antinutritional compounds. The CPC underwent ACP treatment for a duration of 10 min at pH levels of 6, 8, and 10, and subsequently the pH was adjusted to 7. The combined ACP treatment and pH shifting led to a

substantial reduction in the sinapine from 94.6 to 12.6 μg/mL after 10 min. Also, the solubility of CPC was increased after the pH shifting with ACP treatment, especially in alkaline conditions. The CPC treated by ACP for 10 min at pH 10, followed by pH shifting to 7 resulted in a significant increase in emulsification activity. Additionally, the emulsification stability of the treated samples was observed to be 54.5%, showing a substantial improvement compared to the control. A similar trend was observed for foaming capacity and foaming stability, wherein their values increased significantly at pH 10 following the combined treatment of ACP and pH shifting. These findings highlighted the efficacy of the ACP treatment and pH shifting combination in enhancing the emulsification and foaming properties of CPC, particularly at a pH level of 10.

The results from this study indicate the potential of the ACP treatment on improving the properties of canola protein, and the degradation of antinutritional compounds from canola protein concentrate.

PREFACE

This thesis is an original work done by Muhammad Faisal Arif at the Food Safety and Sustainability Engineering Lab at the University of Alberta under the supervision of Dr. Roopesh Mohandas Syamaladevi.

A manuscript based on the study in Chapter 3 will be prepared for publication. A technical research abstract based on the results in Chapter 3 was peer-reviewed, and an oral presentation was delivered at the American chemical society (ACS) Conference, held virtually from Aug 22 – 28, 2021.

The acquisition of the spectra and the data analysis in Chapter 3 was conducted with the help of graduate student Sitian Zhang. Several other collaborators helped in this study, and their contributions were acknowledged accordingly.

Dedicated

To my beloved

Mom and Dad

Sahiba and Arif

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CP	Canola protein
CPC	Conola protein concentrate
ACP	Atmospheric Cold Plasma
UV	Ultraviolet
SH	Sulfhydryl
pI	Isoelectric point
FTIR	Fourier transform infrared
EA	Emulsification activity
ES	Emulsification stability
FC	Foaming capacity
FS	Foaming stability
PDA	photodiode array
GPPI	Grass pea protein isolate
RONS	Reactive oxygen and nitrogen species
ROS	Reactive oxygen species
TPC	Total phenolics content
FAO	Food and agricultural organisation
CPI	Canola protein isolate
UV	Ultraviolet
WHO	World health organization
YE	Yeast extract

Chapter 1: Chapter 1. Introduction and Objectives

1.1 Introduction

Over the past few years, there has been a substantial market demand and growing enthusiasm for plant proteins. Plant proteins are vital components of the human diet. There is an increase in the utilization of plant proteins in the food industry due to their availability and high nutritional value. This trend has been increasing because of a suggested lower environmental impact and health benefits of plant proteins compared to animal protein sources. However, further research is needed to fully understand and evaluate these claims. The production of animal protein can impose an adverse impact on soil, groundwater, and tropical forests (Aiking, 2011). Global sales of plant protein products reached $35 billion in 2018 and are projected to increase to 45 billion by 2023, with a compound annual growth rate of 4.93% from 2019 to 2023 (Aschemann-Witzel et al., 2021).

Canola (*Brassica napus* L.) possesses proteins with high nutritional value and, well-balanced essential and non-essential amino acid profile. Canola meal is generally a leftover material after oil extraction. It contains proteins with high nutritional value and technological functional properties, however, it is mostly used as livestock feed or fertilizer (Chmielewska et al., 2021). There is potential for utilizing canola meal as a protein source for human consumption (Wanasundara et al., 2017). Due to its high protein content, excellent nutritional value, and balanced amino acid profile, canola protein can serve as a viable substitute for other plant proteins. (Wanasundara et al., 2016b; Wanasundara et al., 2017).

Canola meal contains certain undesirable antinutritional compounds such as glucosinolates, phenolics, and phytates (Mailer et al., 2008; Wu & Muir, 2008). The presence of anti-nutritional compounds remains a significant challenge in the utilization of canola meal by the food industry

(Wu & Muir, 2008). Phenolic acids are considered the main phenolic components in canola meal. The most prominent phenolic compound is the choline ester of sinapic acid known as sinapine, which is 80% of all the phenolic compounds present in the canola meal (Wanasundara et al., 2016). Sinapine is responsible for undesirable properties of canola meal, such as poor functional properties and low solubility in canola protein (Zum Felde et al., 2007). Additionally, sinapine imposes a negative impact on the digestibility of canola protein.

Previous research reported the effect of conventional processing methods on the antinutritional components of canola meal or protein. For instance, (Mahajan & Dua, 1998) reported a significant reduction in glucosinolates, phenols, and phytic acid after enzymatic modification of canola meal using pepsin, papain, trypsin, ficin and hemicellulose. However, limited information is available on the application of novel processing technologies on the reduction of antinutritional compounds and improvement in canola protein functional properties.

Recent studies reported the application of non-thermal atmospheric cold plasma (ACP) as a novel technology to improve the functional properties of plant proteins. Plasma is produced by ionizing a gas by supplying energy, resulting in the dissociation of molecular bonds and production of high energy reactive species including reactive oxygen species (ROS) (e.g., O_3, O_2, O^-, and H_2O_2), reactive nitrogen species (RNS) (e.g., N_2^+, N^+, NO), neutral particles, electrons, and ultraviolet (UV) radiations (Venkataratnam et al., 2019). ACP treatment of plant proteins potentially can change the protein structures. Zhang et al. (2021) reported improvement in pea protein gelling properties after ACP treatment. The high energy reactive plasma species can possibly degrade the antinutritional phenolic compounds in canola protein due to their interaction with these molecules, hence improve the functional properties of CP. However, no previous studies reported the use of ACP treatment on

the degradation of antinutritional compounds in canola protein and the resulting functionality improvement of canola protein.

1.1 Hypotheses

The overall hypothesis of this research is that ACP technology can potentially degrade the anti-nutritional phenolic compounds present in canola protein. Furthermore, this technology will enhance the functional properties of canola protein, such as solubility, emulsification, and foaming properties. The efficacy of ACP treatment to degrade phenolic compounds in canola protein and improve its functionality depends on different process and product parameters, such as ACP treatment time, pH of the protein solution etc.

Through ACP treatment, the solution pH of canola protein can shift from neutral to acidic due to the generation of nitrates, nitrites, and nitric acid. Consequently, pH reduction during ACP treatment depending on the process condition can diminish the protein functionality. By combining ACP treatment with pH shifting, it is possible to address the limitations associated with pH reduction during protein treatment using ACP. Further research is required to investigate the effects of combining ACP treatment with pH shifting on the structural and functional properties of canola protein.

1.2 Objectives

The primary aim of this thesis was to determine the degradation of antinutritional compounds and enhance specific functional properties of canola protein by ACP technology. The specific objectives were to:

1) determine the reduction of overall anti-nutritional phenolic compounds and quantify the

degradation of sinapine after ACP treatment (chapter 3).

2) evaluate the structural changes and improvement in selected functional properties of canola protein including solubility, surface charge, emulsification capacity and stability, foaming capacity, and stability after ACP treatment (chapter 3),

3) investigate the effect of ACP treatment followed by pH shifting on the degradation of sinapine and the selected functional properties of canola proteins (chapter 4).

1.3 References

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity–A practical and critical review. *Trends in food science & technology*, **22**(1), 21-39.

Aiking, H. 2011. Future protein supply. *Trends in Food Science & Technology*, **22**(2-3), 112-120.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Aschemann-Witzel, J., Gantriis, R.F., Fraga, P., Perez-Cueto, F.J. 2021. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**(18), 3119-3128.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944

Mahajan, A., Dua, S. 1998. Improvement of functional properties of rapeseed (Brassica campestris var toria) meal by reducing antinutritional factors employing enzymatic modification. *Food Hydrocolloids*, **12**(3), 349-355.

Venkataratnam, H., Sarangapani, C., Cahill, O., Ryan, C.B. 2019. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, **52**, 368-375.

Wanasundara, J., Tan, S., Alashi, A., Pudel, F., Blanchard, C. 2017. Proteins from canola/rapeseed: Current status. in: *Sustainable protein sources*, Elsevier, pp. 285-304.

Wanasundara, J.P., McIntosh, T.C., Perera, S.P., Withana-Gamage, T.S., Mitra, P. 2016b. Canola/rapeseed protein-functionality and nutrition. *OCl*, **23**(4), D407.

Wu, J., Aluko, R.E., Muir, A.D. 2008. Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food chemistry*, **111**(4), 942-950.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

Zum Felde, T., Baumert, A., Strack, D., Becker, H., Möllers, C. 2007. Genetic variation for sinapate ester content in winter rapeseed (Brassica napus L.) and development of NIRS calibration equations. *Plant Breeding*, **126**(3), 291-296.

Chapter 2: Literature Review

2.1 Plant proteins

The planet's ecosystem is affected by an increasing population, resulting in the need for larger quantities of food production, especially animal proteins in the coming decades. To address this challenge, production and utilization of plant proteins for partial or full substitution of animal-derived proteins is necessary (Aiking, 2011) Plant protein diet is reported to fulfil the nutritional needs of growing human populations while reducing the harmful effects of food production on the climate. Among plant-based proteins, rapeseed or canola protein can be useful for the development of plant-based foods, because of its nutritional value and functional properties.

Canola is suited to different farming environments with high production volumes (Carré & Pouzet, 2014; Von Der Haar et al., 2014; Wanasundara et al., 2016b). Canola belongs to the family Brassicaceae. *Brassica napus* is grown worldwide and thus studied extensively. Over 95% of the seeds produced in Canada are *Brassica napus*. Canola is a term commonly used in Canada and Australia, wherein Europe, the name rapeseed or double zero rapeseed is preferred (Wanasundara et al., 2016b). The protein-rich canola meal, remaining after extracting oil from the seeds, is typically utilized as a valuable protein source in the livestock industry. Canola meal can also be used for human consumption. However, its use as a commercial food source has not been established (Wanasundara et al., 2016b). The quality of canola meal or protein can be improved by decreasing the anti-nutritional compounds present in canola meal (e.g., glucosinolates and erucic acid). The glucosinolates level in canola meal usually accounts for less than 30 μmol/g and the erucic acid content in the oil is below 2%. Many industries are interested in developing and marketing canola

proteins with the establishment of the canola protein production technology (Mupondwa et al., 2018). The proteins in canola meal can be extracted and its functionality can be possibly improved by using novel technologies. This literature review provides information about the amino acid composition, antinutritional compounds, and different functionalities (for example solubility, gelation, hydrophobicity etc.) of canola protein.

Canola protein (CP) is low in histidine, cysteine, and methionine but high in aspartic acid and glutamic acid (Shi & Dumont, 2014). CP also has a variety of applications in industrial products, biofuels, and cosmetics (Wanasundara et al., 2016a). There's also some evidence that CP has strong functional and rheological properties (Tan et al., 2011a). All of these indicate that CP is a potential replacement to soybean proteins and other plant and animal proteins for use in the food industry. However, Canola meal has antinutritional compounds such as fiber, phytates, phenolics, glucosinolates, and these anti-nutritional factors are the main barrier to using oil-free canola meal for human food production (Tan et al., 2011a). These antinutritional factors can cause unsuitable properties, for example, poor structural properties, bad taste, objectionable color, and low digestibility (Wu & Muir, 2008) of canola meal. Sinapate ester with sinapoylcholine (Sinapine) is considered the most important phenolic compound in CP. Sinapine is responsible for undesirable properties in canola meal and the extracted CP, such as dark color and bitter taste (Chmielewska et al., 2020). Furthermore, sinapine imposes a negative impact on CP digestibility. This might be due to the formation of complexes by sinapine with protein through oxidation during rapeseed oil production, which decreases the digestibility of CP (Shahidi & Naczk, 1992).

2.2 Components and properties of canola meal protein.

2.2.1 Amino acid composition of canola protein

The quality of protein is determined by the essential amino acid content as they are the building subunits of proteins. Canola proteins have high nutritional value due to a well-balanced essential amino acid profile, including histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and sulfur-containing amino acids such as methionine and cysteine. Canola meal contains 24% proteins, 40% oil and 20% carbohydrates (Bhatty et al., 1968). The major components of the canola protein isolates are 12S cruciferin and 2S napin, which contribute to 20% of the total canola proteins (Tan et al., 2011a). Cruciferin represents 60% of the total seed proteins in canola (Burgess, 1991). The studies showed that cruciferin contains arabinose, glucosamine, mannose, galactose, and inositol. Cruciferin has the ability to dissociate into smaller components under extreme pH or in the presence of urea similar to other 12S seed globulin. Different factors such as ionic strength and pH can dissociate the 12S globulin ascribed to the breakage of covalent disulfide bonds present in the polypeptide chains (Burgess, 1991). Prakash & Narasinga Rao (1986) established that the native structure of globulin is formed through hydrophobic interactions.

2.3 Antinutritional compounds in canola protein

The main hurdle to using canola protein for human food production lies in the presence of antinutritional factors within the oil-free canola meal. These include glucosinolates, phenolic compounds, and phytates (Mailer et al., 2008; Tan et al., 2011b; Wu et al., 2008). Antinutritional compounds are produced as side products during various metabolic processes and belong to the secondary metabolites of plants. The presence of antinutritional compounds results in undesirable canola meal properties including, weaker physical and chemical properties, low digestibility, bad taste, and unpleasant color (Wu et al., 2008). In canola seed, phenolic acid esters are known to be

the main antinutrient compounds (Ismail et al. 1981) Phenolic acid esters concentration is around 30 times higher than that of soybean (Kozlowska et al. 1990; Shahidi et al. 1992). Canola meal contains 32.4% dietary fibre, 4.2 μm/g glucosinolates, 2.3% phytic acid, and 1% sinapine (Tan et al., 2011a). The presence of these antinutritional factors is also recognized as the primary drawback in employing canola protein for food applications, despite having potential health effects, such as reducing cancer risk (Tan et al., 2011a; Wanasundara et al., 2017). During the production of canola proteins, 100% of tannic acid, 92% of phytic acid and 95% of glucosinolates could be removed. The sinapate esters can have a detrimental effect on canola meal digestibility. Sinapine can form protein complexes during the processing of canola oil by oxidation, which can reduce the digestibility of the canola meal (Kozlowska et al., 1990; Shahidi & Naczk, 1992). Velasco & Möllers (1998) recorded a range of sinapate ester content of 5 to 17.7 g/kg Brassica napus seed in their analysis of 1361 canola meal samples.

The amount of glucosinolate in canola meal is relatively high with concentration in the range of 18 to 30 μmol/g. Glucosinolates are glycoside containing sulfur and nitrogen and their appearance in canola meal is a main problem for their use as feed. Different animal studies have demonstrated that these compounds exhibit anti-nutritional or toxic characteristics. Surprisingly, it has been stated that a lower amount of glucosinolate content has good health effects. (Song & Thornalley, 2007) found that a decreased risk of cancer can be associated with a glucosinolate level of 0.61 μmol/g in broccoli. Reducing the quantity of glucosinolates in canola meal protein to make it suitable for human consumption is a challenging task.

Another antinutritional compound in canola meal is phytic acid, usually contained as a mixed salt of calcium (Ca), magnesium (Mg), and potassium (K) (Mills & Chong, 1977; Yiu et al., 1982). At normal pH, the molecule is negatively charged; thus, with cations such as minerals, it is very reactive

(Thompson & Serraino, 1986). Depending on the method of protein isolation, phytate levels for protein isolates and concentrates are almost 10% and 2% to 5% for defatted meal (Uppström & Svensson, 1980). Phytic acid also binds with protein, in addition to binding with minerals, decreasing the digestibility of proteins and making available amino acids (Thompson & Serraino, 1986) . Moreover, phytic acid decreases the digestion and absorption of starch by reducing the amylose activity (Yoon et al., 1983). Therefore, these antinutritional compounds impact canola protein applications for human food. As a result, the current utilization of canola meal remains restricted to animal feed and fertilization purposes.

2.4 Degradation of antinutritional compounds.

Several studies have shown the effect of processing on the degradation of the antinutritional compounds from canola. The degradation up to 94% of glucosinolates, 67% of tannic acid, and 43% phytic acid in the canola meal was tested when subjected to heat treatment (Mansour et al., 1993). In a study by Jensen et al., (1990), it was observed that high temperatures led to the degradation of glucosinalotes in canola meal, simultaneously enhancing its flavor and palatability. The addition of organic solvents, such as ethanol, methanol, and acetone during the extraction process has been identified as an alternative and efficient method to extract glucosinolates from canola meal. It is important to process canola meal in a manner that minimizes the presence of anti-nutritional factors. Hence the development of a method, which can degrade the antinutritional compounds in canola without impacting the sensory and nutritional characteristics of canola protein is required for the food industry.

2.5 Functional properties of canola protein

Incorporation of proteins influences the functional properties of food; stabilizes the structure and adds nutritional value to foods. Plant protein digestibility is lower than animal proteins (Tomé,

2013). Application conditions, intrinsic factors, and type of treatment are some of the main factors that can affect the functional properties of protein. The functional properties of canola protein, including foaming and emulsification properties, and solubility, make it highly promising for various human food applications.

2.6 Solubility of canola protein

Solubility is an important requirement for the utilization of proteins in food matrices and it is closely linked to other functional properties for example gelation, emulsifying, and foaming capacity (Ostrowska et al., 2018). Different studies have shown that cruciferin and napin exhibit distinct solubility behaviors that depend on salt level, pH, and temperature of canola protein isolate (Wanasundara et al., 2016b).

The solubility of a protein is greatly influenced by its molecular structure. The research demonstrated that the solubility of rapeseed protein isolate was directly impacted by changes in its secondary and tertiary structure resulting from ultrasonic treatment (Li et al., 2020). Rapeseed protein isolate to ultrasound conditions can disrupt the spatial and molecular structure by breaking electrostatic and hydrogen bonds, leading to an increased unfolding of the protein chains. Furthermore, analysis of the spatial structure using FTIR indicated an increase in β-turn and β sheets, while there was a decrease in random coils and α-helix. In general, implemented modifications resulted in an enhancement of solubility. Noncovalent linkages in canola protein, including hydrophobic interactions, hydrogen bonding, and van der Waals interactions have also been reported by Wu et al. (2008). They suggested that such interactions played a significant role in stabilizing the native conformation and affects of solubility of canola protein.

The solubility of canola protein or canola meal is pH dependent. Previously the solubility of canola protein and its meal was examined at various pH levels (Pedroche et al., 2004). The solubility of canola protein increased at alkaline pH, while at acidic pH levels, the solubility of the canola meal was higher. The lower solubility observed in the canola meal at alkaline pH, as compared to the extracted canola protein, may be attributed to the presence of other components such as antinutritional glucosinolates and phenolic compounds within the meal that exhibit reduced solubility. Furthermore, the canola meal's heterogeneous nature allows for other components and proteins, potentially altering the net charge and hydrophobicity of the protein, thereby influencing its solubility. The increased solubility of the canola meal at the acidic pH, in contrast to canola protein, can be attributed to the loss of proteins soluble at low pH during the preparation of canola protein. The common method for preparing canola protein involves solubilizing proteins from defatted meals in alkaline solutions, followed by precipitation at the acidic isoelectric point (pI). The pH at which proteins exhibit their lowest solubility is known as the isoelectric point (pI). This information holds significant importance when determining the applicability of a protein, particularly in the field of food processing.

Different functional properties and solubility are also highly dependent on the protein's molecular structure (Li et al., 2020). The solubility of canola protein is directly influenced by the modifications of its secondary and tertiary structure during food processing. In comparison to cruciferin, Wanasundara et al. (2012) reported that napin is soluble in strong acid conditions, while both proteins remain in solution at strong alkaline conditions. Solubility is also strongly dependent on other conditions such as ionic strength, temperature, and processing conditions.

2.7 Emulsifying properties

Proteins are highly sought after for their exceptional emulsifying properties, and their hydrophilic and hydrophobic characteristics (Hristov et al., 2011). Proteins, in contrast to low molecular weight emulsifiers, exhibit a large size and slower diffusion, resulting in excellent emulsion-forming abilities as they efficiently diffuse to the interface (Chang et al., 2015). To bring hydrophobic amino acids that are hidden within the protein to the surface, some degree of potential denaturation is frequently required when proteins are situated at the interface. The proteins then re-align themselves at the interface, extending hydrophobic groups into the hydrophobic (oil) phase and hydrophilic groups into aqueous phase. Thus, the emulsifying ability of proteins tends to be lower than that of small molecular weight emulsifiers, considering the complexity and partial denaturation at the interface.

The emulsifying properties of proteins are significantly influenced by their physiochemical characteristics (Bueno et al., 2009). The protein's capacity to adsorb at the interface is influenced by the degree of surface hydrophobicity (Cumby et al., 2008). Conversely, the solubility of proteins in the aqueous phase is influenced by the surface charge of the proteins. It is necessary to have a high solubility to achieve a faster diffusion rate at the interface (Karaca et al., 2011). After the formation of the viscoelastic film, the charge of droplets is determined by whether the pH of the emulsion is above or below the protein's isoelectric point, resulting in the droplets assuming either a negative or positive charge, respectively. Greater emulsion stability is typically observed when there is a high level of electrostatic repulsion between oil droplets. However, droplet aggregation dominates under pH conditions close to the isoelectric point of the protein leading to instability (Chang et al., 2015). The inclusion of protein in the continuous phase of an emulsion results in an increase in viscosity, which in turn decreases the mobility within the emulsion.

(Sibt-e-Abbas et al., 2020a) investigated emulsifying properties of oilseed proteins. According to their findings, the emulsifying stability of canola protein isolate was measured at 72%, emulsifying capacity was 65%. The low emulsifying capacity of canola protein isolate was due to the reduction in the hydrophobic residue in protein, influencing the diffusion of the oil droplets. (Tan et al., 2014) investigated the emulsifying properties of canola meal isolate components such as globulin and albumin. The globulin fraction outperformed the albumin fraction in terms of emulsifying capabilities and exhibited the smallest droplet size. In canola protein isolate (CPI), the presence of high molecular weight polypeptides can cause poor emulsifying activity (Aider & Barbana, 2011). The emulsifying capacity was highly dependent on the pH (Aider & Barbana, 2011).

2.8 Foaming properties

The amphiphilic structure of proteins enables them to form foams by reducing surface tension at the interface between air and water. The stability of foams is also attributed to the presence of a viscoelastic film of proteins that surrounds air bubbles (Malabat et al., 2001). Foams have the capacity to counter the gravitational and mechanical stress (Gerzhova et al., 2015). In contrast to cruciferin proteins, napin proteins (almost 93%) had an outstanding ability to produce stable foams (Wanasundara et al., 2016). A study by (Sibt-e-Abbas et al., 2020) reported that the foaming capacity of the canola protein isolate was relatively low, attributed to the cleavage of disulfide bonds, resulting in reduced flexibility of protein structures. At various pH (4.0, 7.0, 9.0) conditions, the foaming properties of CPI were examined by (Gerzhova et al., 2015) . This study showed that at pH 9, CPI has excellent foaming ability. At pH 3, foaming capacity was marginally lower and at pH 7, a small reduction was observed. A steady decrease was observed after assessing the stability of the foam within 1 hour, indicating that the foam was not stable. However, at pH 4 lowest foaming stability was observed compared to pH 7, but the highest foaming stability was obtained at pH 9.

The foaming capability was influenced by parameters such as solubility and hydrophobicity, which were identified as important factors. Also, the capacity to foam is linked to the nice balance of hydrophilic and hydrophobic groups that promote the stability of bubbles. The findings suggested that hydrophobicity may have a stronger correlation with foaming capacity compared to solubility (Chmielewska et al., 2021). The proteins with low foam capabilities demonstrated low hydrophobicity, independent of their solubility (Chmielewska et al., 2021). It should be emphasized that a high foam capacity is not necessarily associated with high foam stability, as the molecular characteristics required for these properties are different.

(Flores-Jiménez et al., 2019) investigated the foaming capacity that was observed to be relatively low within the pH range of 4-6, with the lowest value recorded at pH 4. The protein isolates exhibited an increase in foam capacity when exposed to basic pH conditions, reaching its highest value at pH 10. The higher values seem to be a result of improved solubility and greater net charges, leading to weaker hydrophobic interactions and increased flexibility of proteins. As a result, protein diffusion towards the air-water interface increased, promoting the encapsulation of air particles, and ultimately improving the formulation of foam.

2.9 Food applications of canola protein

In food products, proteins perform many distinct roles, including binding of water and fat, emulsification, foaming and gelation. Protein gels add strength and texture to several foods including jelly and products made from fish paste. With a composition rich in oil, protein, and fiber, canola seed is globally recognized as the second-largest oilseed crop in terms of production. Numerous food applications utilizing canola proteins, including bakery, dairy and meat analogue products have been reported. Over the past few decades, research has been conducted to explore the use of canola protein as a viable alternative to animal-derived proteins in food products. In food, canola protein may play

different roles such as texturizer, emulsifier, gelling agent or just a source of protein. Canola protein has bioactive characteristics partially due to the embedded sequence of bioactive peptide. These peptides can be released through regulated hydrolysis, which can contribute to its benefits. In bakery products including bread, rolls, croissants, cakes, brioches, bagels, croutons, muffins, biscuits, crumpets, scones, tortillas, cookies, crepes, donuts, bars, waffles, pancakes, canola protein isolates can be added as an ingredient. In high-protein nutrition bars, canola protein can be added as a protein source. Canola protein concentrate can be used to replace eggs in pound cakes. Canola protein isolate and concentrate can be used up to 18% in food applications including bakery, dairy, and meat products to replace wheat flour, without affecting the dough properties and causing any adverse effects (Tan et al., 2011; Wanasundara et al., 2016) . Canola protein can be added as egg substitutes to improve the gel-forming capacity and emulsification capacity in mayonnaises with similar firmness (Von Der Haar et al., 2014). The utilization of protein ingredients in food products is primarily determined by their functional properties, which are considered crucial.

2.10 Atmospheric cold plasma

Plasma, the fourth state of matter is generated when various gases (for instance air, nitrogen, helium, argon) and a combination of gases are electrically energized. This results in the formation of a partially ionized gas (Niemira, 2012). Plasma consists of free electrons and fully ionized atoms (ions) or partially ionized atoms/molecules. The energy used to disrupt the chemical bonds in a gaseous environment produces plasma, which is fully or partially ionized gas, resulting in the generation of cocktail of reactive oxygen species (ROS) (O_3, O_2, O^-, and H_2O_2,), and reactive nitrogen species (RNS) (N_2^+, N^+, NO) and ultraviolet (UV) radiations (Venkataratnam et al., 2019).

Plasma can be divided into hot thermal plasma and cold plasma (CP). When the nucleus (protons and neutrons) is in thermal equilibrium with the electrons, and the plasma temperature in such

conditions can be in the order of 10^6 to 10^8 K, the plasma is characterized as thermal. On the other hand, in cold plasma, the partially ionized atoms/molecules are at nearly ambient temperature, although the free electrons are at much higher temperatures, the heavier partially ionized atoms/molecules are at nearly room temperature in such cases (Zainal, Redzuan, & Misnal, 2015). The plasma discharge occurs between two electrodes, with at least one electrode containing a dielectric barrier to enhance plasma formation and stability. When the high voltage is applied, it creates an electric field that ionizes the gas, forming a plasma. The energetic electrons in the plasma collide with gas molecules, transferring energy and creating further ionization. This process generates a cascade of reactions, leading to the production of ROS and RNS, including hydroxyl radicals, ozone, atomic oxygen, and nitrogen oxides (Deng et al., 2020). Non-thermal atmospheric cold plasma (ACP) represents a promising and innovative technology with different applications in surface modification, sterilization, and cleaning but also in modifying the protein functionality (Zhang et al., 2021b). As the agri-food industry seeks sustainable alternatives to minimize energy and resource consumption, ACP emerges as a viable and environmentally friendly option to achieve those goals. In addition to reducing the need for solvents and chemicals, ACP technology leaves no residue, providing food and food ingredients with a "clean label" appearance (Misra & Roopesh, 2019).

ACP treatment changes protein functionality in several ways, one of which is the reaction between reactive species and protein molecules. ACP treatment has the potential to induce cleavage of covalent bonds within protein molecules and promote the oxidation of sulfur amino acids, potentially resulting in the formation of disulfide bonds (Dong et al., 2017b). The formation of S-S bonds can result in the formation of the sulfhydryl (SH) group, which can further alter the protein's shape and improve the dough strength of wheat gluten and enhance the foam stability (Dong et al., 2017b).

ACP treatment offers the possibility of modifying proteins in terms of their functional, chemical, and physical properties (Akharume et al., 2021).

ACP technology can possibly reduce the antinutritional phenolic compounds and enhance functional properties such as solubility, emulsification and foaming capacity, and stability of canola protein. Until now, there has been no study investigating the effect of ACP treatment on the degradation of antinutritional phenolic compounds in CP or the improvement in the functional properties of canola protein concentrate.

Numerous reports have provided supporting evidence for the impact of plasma on dairy proteins (Coutinho et al., 2018) and animal proteins (Pérez-Andrés et al., 2019). The clear mechanism of enhancing the plant protein with ACP is not clear yet and it needs to be investigated. However, the general mechanism by which ACP improves the functionality of plant proteins is through various physical and chemical interactions. Atmospheric cold plasma generates reactive oxygen and nitrogen species, such as ozone (O_3), hydrogen peroxide (H_2O_2), and nitric oxide (HNO_3), which can induce modifications in the protein structure. These modifications may include oxidation of certain amino acids, crosslinking of protein chains, and disruption of disulfide bonds. These alterations can lead to changes in protein conformation, solubility, emulsifying properties, and enzymatic activity, ultimately improving the functionality of plant proteins. Additionally, ACP treatment can also influence the microbial load and decontamination of protein-based food products, further enhancing their functionality and safety.

2.11 Summary

Given the prevailing trends in the global food markets, it is crucial to expand the availability of plant-based protein sources. Canola, currently ranking as the world's second-largest oilseed crop

after soy, shows great promise as a viable alternative. Dark color, negative taste, the occurrence of antinutritional factors are the key issues related to the processing of canola. Significant research is needed to understand the potential of novel technologies such as atmospheric cold plasma to degrade the antinutritional factors of canola protein and improve its functional properties. This gap in the literature is addressed in Chapter 3, where the effect of ACP on sinapine (antinutritional compound) was investigated. In chapter 4, ACP was combined with pH shifting to understand their synergistic effect on selected functional properties of canola protein.

2. 12 References

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity–A practical and critical review. *Trends in food science & technology*, **22**(1), 21-39.

Aiking, H. 2011. Future protein supply. *Trends in Food Science & Technology*, **22**(2-3), 112-120.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Aschemann-Witzel, J., Gantriis, R.F., Fraga, P., Perez-Cueto, F.J. 2021. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**(18), 3119-3128.

Bhatty, R., McKenzie, S., Finlayson, A. 1968. The proteins of rapeseed (Brassica napus L.) soluble in salt solutions. *Canadian journal of biochemistry*, **46**(10), 1191-1197.

Burgess, D.A. 1991. Purification, characterization, and the micelle response of the 12S canola globulin.

Carré, P., Pouzet, A. 2014. Rapeseed market, worldwide and in Europe. *Ocl*, **21**(1), D102.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Dong, S., Gao, A., Zhao, Y., Li, Y.-t., Chen, Y. 2017. Characterization of physicochemical and structural properties of atmospheric cold plasma (ACP) modified zein. *Food and Bioproducts Processing*, **106**, 65-74.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food Bioscience*, **12**, 128-138.

Hüsken, A., Baumert, A., Milkowski, C., Becker, H.C., Strack, D., Möllers, C. 2005. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (Brassica napus L.). *Theoretical and Applied Genetics*, **111**, 1553-1562.

Jensen, S., Olsen, H., Sørensen, H. 1990. Aqueous enzymatic processing of rapeseed for production of high quality products. *Canola and rapeseed: production, chemistry, nutrition and processing technology*, 331-343.

Kozlowska, H., Naczk, M., Shahidi, F., Zadernowski, R. 1990. Phenolic acids and tannins in rapeseed and canola. *Canola and rapeseed*, 193-210.

Karaca, A.C., Low, N., Nickerson, M. 2011. Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International*, **44**(9), 2991-2998.

Li, Y., Cheng, Y., Zhang, Z., Wang, Y., Mintah, B.K., Dabbour, M., Jiang, H., He, R., Ma, H. 2020. Modification of rapeseed protein by ultrasound-assisted pH shift treatment: Ultrasonic mode

and frequency screening, changes in protein solubility and structural characteristics. *Ultrasonics Sonochemistry*, **69**, 105240.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Mansour, E., Dworschák, E., Lugasi, A., Gaál, Ö., Barna, É., Gergely, A. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food chemistry*, **47**(3), 247-252.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Malabat, C., nchez-Vioque, R.I., Rabiller, C., Gu guen, J. 2001. Emulsifying and foaming properties of native and chemically modified peptides from the 2S and 12S proteins of rapeseed (Brassica napus L.). *Journal of the American Oil Chemists' Society*, **78**(3), 235-242.

Mills, J., Chong, J. 1977. Ultrastructure and mineral distribution in heat-damaged rapeseed. *Canadian Journal of Plant Science*, **57**(1), 21-30.

Mupondwa, E., Li, X., Wanasundara, J.P. 2018. Technoeconomic prospects for commercialization of Brassica (cruciferous) plant proteins. *Journal of the American Oil Chemists' Society*, **95**(8), 903-922.

Misra, N., Roopesh, M. 2019. Cold plasma for sustainable food production and processing. in: *Green food processing techniques*, Elsevier, pp. 431-453.

Ostrowska, A., Kozlowska, M., Rachwal, D., Wnukowski, P., Nebesny, E., Rosicka-Kaczmarek, J. 2018. Rapeseed protein-fibre concentrate: Chemical composition and functional properties. *Żywność Nauka Technologia Jakość*, **25**(4).

Prakash, V., Narasinga Rao, M. 1986. Physicochemical properties of oilseed protein. *Critical Reviews in Biochemistry*, **20**(3), 265-363.

Pedroche, J., Yust, M., Lqari, H., Girón-Calle, J., Alaiz, M., Vioque, J., Millán, F. 2004. Brassica carinata protein isolates: chemical composition, protein characterization and improvement of functional properties by protein hydrolysis. *Food chemistry*, **88**(3), 337-346.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Shi, W., Dumont, M.-J. 2014. Processing and physical properties of canola protein isolate-based films. *Industrial Crops and Products*, **52**, 269-277.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Sultan, M.T., Saddique, M.S., Shahid, M. 2020. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Song, L., Thornalley, P.J. 2007. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and chemical toxicology*, **45**(2), 216-224.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011a. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011b. Extraction and characterization of protein fractions from Australian canola meals. *Food Research International*, **44**(4), 1075-1082.

Thompson, L.U., Serraino, M.R. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. *Journal of agricultural and food chemistry*, **34**(3), 468-469.

Tomé, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements—functional aspects. *Food and nutrition bulletin*, **34**(2), 272-274.

Uppström, B., Svensson, R. 1980. Determination of phytic acid in rapeseed meal. *Journal of the Science of Food and Agriculture*, **31**(7), 651-656.

Velasco, L., Möllers, C. 1998. Nondestructive assessment of sinapic acid esters in Brassica species: II. Evaluation of germplasm and identification of phenotypes with reduced levels. *Crop Science*, **38**(6), 1650-1654.

Venkataratnam, H., Sarangapani, C., Cahill, O., Ryan, C.B. 2019. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, **52**, 368-375.

Von Der Haar, D., Müller, K., Bader-Mittermaier, S., Eisner, P. 2014. Rapeseed proteins–Production methods and possible application ranges. *Ocl*, **21**(1), D104.

Wanasundara, J., McIntosh, T., Perera, S., Withana-Gamage, T., Mitra, P. 2016a. Canola/rapeseed protein-functionality and nutrition. OCL 23. *D407*.

Wanasundara, J., Tan, S., Alashi, A., Pudel, F., Blanchard, C. 2017. Proteins from canola/rapeseed: Current status. in: *Sustainable protein sources*, Elsevier, pp. 285-304.

Wanasundara, J.P. 2011. Proteins of Brassicaceae oilseeds and their potential as a plant protein source. *Critical reviews in food science and nutrition*, **51**(7), 635-677.

Wanasundara, J.P., Abeysekara, S.J., McIntosh, T.C., Falk, K.C. 2012. Solubility differences of major storage proteins of Brassicaceae oilseeds. *Journal of the American Oil Chemists' Society*, **89**, 869-881.

Wanasundara, J.P., McIntosh, T.C., Perera, S.P., Withana-Gamage, T.S., Mitra, P. 2016b. Canola/rapeseed protein-functionality and nutrition. *OCl*, **23**(4), D407.

Wu, J., Aluko, R.E., Muir, A.D. 2008. Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food chemistry*, **111**(4), 942-950.

Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.

Yiu, S., Poon, H., Fulcher, R., Altosaar, I. 1982. The microscopic structure and chemistry of rapeseed and its products. *Food Structure*, **1**(2), 4.

Yoon, J.H., Thompson, L.U., Jenkins, D. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *The American journal of clinical nutrition*, **38**(6), 835-842.

Yoshie-Stark, Y., Wada, Y., Schott, M., Wäsche, A. 2006. Functional and bioactive properties of rapeseed protein concentrates and sensory analysis of food application with rapeseed protein concentrates. *LWT-Food Science and Technology*, **39**(5), 503-512.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

Zum Felde, T., Baumert, A., Strack, D., Becker, H., Möllers, C. 2007. Genetic variation for sinapate ester content in winter rapeseed (Brassica napus L.) and development of NIRS calibration equations. *Plant Breeding*, **126**(3), 291-296.

Chapter 3: The Influence of Atmospheric Cold Plasma Treatment on Canola Protein Structural and Functional Properties

3.1 Introduction

The demand for renewable and sustainable protein sources is increasing. Plant-based diets are becoming more popular for a variety of reasons, including negative environmental implications of animal protein production (Capper, 2020), the risk of some chronic diseases because of excessive animal protein consumption (Itkonen et al., 2021), and the increasing trend of plant-based food consumption (Pojić et al., 2018). The Food and Agricultural Organization has estimated that by 2050, the demand for animal meat proteins is expected to double (Chmielewska et al., 2020). One of the promising alternatives to address these issues is the partial substitution of animal-derived proteins with plant-origin proteins, which may have a beneficial impact on human health.

Canola (Brassica napus L.) is the world's second most produced oilseed after soybean. Canola meal, a leftover from canola seed after oil extraction, contains 35-40% protein and is mostly used as a livestock feed or fertilizer (Chang et al., 2016). In addition to its application as an animal feed, canola meal could also be used for human nutrition (Wanapat et al., 2020). Canola protein (CP) has an excellent amino acid profile including cysteine and tyrosine, with a high protein efficiency ratio exceeding the established requirement of World Health Organization (WHO) and the Food and Agricultural Organization (FAO) (Chmielewska et al., 2021). Hence CP could be used as a potential replacement for soybean derivatives and other cereal and pulse proteins used in the food industry.

Canola meal is associated with certain antinutritional compounds such as phytates, phenolics, and glucosinolates. These antinutritional factors are the main barriers to the use of canola meal in the food industry (Tan et al., 2011a). These antinutritional factors in canola meal can lead to certain

undesirable properties; for instance, poor structural properties, bad taste, objectionable color, and low digestibility (Wu & Muir, 2008). In canola meal, certain phenolic compounds are considered as the main anti-nutritional factors (Ismail et al., 1981). The concentration of phenolics in CP is 30 times higher in comparison of soybean (Shahidi & Naczk, 1992). Sinapate esters (Sinapine) are considered the important phenolic compounds in canola meal, responsible for the undesirable properties, such as dark color and unpleasant taste in canola protein (Chmielewska et al., 2020). Furthermore, sinapine can have a negative impact on CP digestibility. This might be due to formed complexes between sinapine and protein through oxidation during the canola oil production, which decreases the digestibility of CP (Shahidi & Naczk, 1992).

Non-thermal treatment such as atmospheric cold plasma (ACP) is a revolutionary technique with numerous uses in food processing such as decontamination and protein functionality improvement (Zhang et al., 2021b). ACP is a partially ionized gas, consisting of a cocktail of reactive oxygen species (ROS) (O_3, O_2, O^-, H_2O_2, etc.), and reactive nitrogen species (RNS) (N_2^+, N^+, etc.), charged and neutral particles, electrons, and ultraviolet (UV) radiations (Venkataratnam et al., 2019). The functional, chemical and physical characteristics of protein can also be altered by ACP treatment (Akharume et al., 2021). ACP treatment can change protein functionality in several ways, possibly by the interaction between plasma reactive species and protein molecules. ACP with high energy can trigger the breakage of disulfide (S-S) bond by causing sulfur amino acid oxidation and the breaking of covalent bonds inside the protein molecules (Dong et al., 2017b). Sulfhydryl (SH) and sulfoxide (SO) groups may develop after the breakage of disulfide bond, that can further alter the protein's shape (Dong et al., 2017b). ACP can also interact with phenolic acid and change their structure and functionalities, for instance, (de Castro et al., 2020) reported TPC reduction after 15 min ACP treatment from camu-camu juice.

It was hypothesized that using ACP technology would improve the functional qualities of canola protein by removing undesired phenolic compounds. There is no study reported the effect of ACP treatment on the degradation of antinutritional phenolic compounds and improvement in the functional properties of CP. The objectives of this study were to 1) removal of antinutritional phenolic compounds from CP by the ACP treatment; and quantify the degradation of sinapine, and 2) determine the effect of ACP treatment on functional properties of CP including solubility, surface charge, emulsification capacity and stability, foaming stability, and capacity.

3.2. Materials and methods

3.2.1. Canola meal and chemicals

Canola meal (40.7% protein, 37.8% carbohydrates, 14% fat, 2% ash, 5.5% moisture) was commercially sourced in western Canada, from the 2019 harvest and processed in Alberta, Canada. Sinapine standard (2 mg) was purchased from Selleckchem (Ontario, Canada) and sodium dodecyl sulfate (SDS), acetonitrile (ACN), methanol, trifluoracetic acid (TFA), Folin-Ciocalteu (FC) reagent, trinitrobenzene sulfonic acid (TNBS), sodium phosphate buffer, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), glycine, and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma-Aldrich (Mississauga, Canada), and sodium carbonate was obtained from Fisher Scientific (St. Louis, USA).

3.2.2 Extraction of canola protein concentrate (CPC)

The extraction of canola protein concentrate is presented in Fig 3.1. Canola protein concentrate **(**CPC) was prepared according to (Zhang et al., 2021b) with some modifications. First, canola meal was combined with distilled water in a ratio (1:7), and pH of mixture was raised to 12 by adding 1M NaOH, followed by constant stirring for one hour at room temperature (25 ºC), then centrifugation at 10,000 ×g for 15 min. After centrifugation, the resulting mixture was collected, and pH was

adjusted to 4.5 by using 1M HCL or 1M NaOH. After that, the suspension was subjected to centrifugation at 10,000 ×g for 15 min and the collected pellet was mixed with distilled water and pH was raised to 7 by using 1M NaOH. Finally, the resulting pellet was freeze-dried to collect the CPC powder. The protein content of CPC (85.11%) was determined using a conversion factor of 5.96 by the Leco nitrogen analyzer (Leco®, USA) (Fujihara et al., 2001). Finally, CPC (0.4g) was dissolved in 10 mL of distilled water with 1% SDS to make 4% (w/v) solution and used for further analyses. Canola protein samples were subjected to ACP treatment using 4% (w/v) protein solution. Initial trials demonstrated that ACP treatment alone did not result in significant changes to functional properties. To address this, SDS was added to the canola protein samples, followed by ACP treatment. Also, SDS interacts with proteins, leading to the formation of a complex between SDS and the protein. This complex carries a negative charge and serves to disrupt the non-covalent interactions, such as hydrophobic interactions, hydrogen bonds and ionic bonds within the protein chains. Due to these properties, SDS finds extensive application in the study of protein structure

3.2.3 Atmospheric cold plasma (ACP) treatment

Canola protein concentrate suspension (4% w/v) was prepared by dispersing CPC (0.4 g) in distilled water (10 mL) and SDS (1%) with magnetic stirring at 700 rpm for 10 min at room temperature and then stirring overnight in the refrigerator (4 °C) to ensure the complete hydration. The CPC was taken out of the fridge and transferred to room temperature before any further analysis. CPC suspension was treated using a dielectric barrier discharge (DBD) ACP system (Advanced Plasma Solution, Malvern, USA). A plastic container with a diameter of 4cm and a height of 0.5cm was filled with three milliliter of 4% (w/v) CPC suspension, and it was placed between the ground electrode and high-voltage electrode. The standard output frequency, duty cycle, and output pulse width were 3500 Hz, 70%, and 10 µs respectively. The voltage output ranged from 0-30 kV, and the

protein sample was maintained a distance 2 mm from the high-voltage electrode. The CPC was treated for 5 and 10 min.

3.2.4 Total phenolic content determination

The total phenolic compound (TPC) was determined using the procedure outlined by (Li et al., 2019). Approximately 3 mL of treated CPC solution (4% w/v) was mixed with 5 mL of methanol and the resulting homogenate was centrifuged at 10000 ×g for 15 min. The mixture containing Folin-Ciocalteu 1 mL, distilled water 180 µL, extraction of mixture 20 µL, and sodium carbonate 0.8 mL was incubated at room temperature for 2 h. A spectrophotometer (Molecular Devices, USA) was used to determine the absorbance at 725 nm, and TPC was represented as milligrams per gram (mg/g), based on the gallic acid equivalent.

3.2.5 Quantification of sinapine by HPLC

High performance liquid chromatography (HPLC) was used to measure the sinapine concentration in CPC samples according to the methodology outlined by (Mailer et al., 2008). The CPC samples (4%) with 1% SDS were dissolved into 0.1% trifluoracetic acid (TFA) to make a 10 mL solution. ACP treatments of 3 mL of samples were performed for 5 and 10 min. After the ACP treatments, 448 µL acetonitrile (ACN) was added into the solution with 87% TFA and 13% ACN. Afterwards, the supernatant was extracted with a 1 mL syringe, followed by filtration through a 0.45 nylon syringe filter. These filtered samples were then transferred into HPLC vials for further analysis. The sinapine concentration was calculated by HPLC (Shimadzu Scientific Instruments, Inc. MD. USA). A reverse phase column Agilent Zorbax SB- C18 250 mm × 3 mm, 5 µm, was used. The solvents A, consisting of 0.1% TFA in H_2O, and solvent B, containing 0.1% TFA in ACN were employed. Throughout the experiment, the injection volume was 25 µL with a continuous flow rate of 1 mL/min

and column oven temperature was 40℃. Photodiode array (PDA) was used to determine sinapine concentration at 330 nm wavelength.

3.2.6 Canola protein concentrate (CPC) solubility

The CPC solubility was calculated using a method mentioned by (Yang et al., 2018) with some modification. First, CPC (0.4g) with 1 % SDS was dissolved in 10 mL of distilled water to make 4% (w/v) solution and six different pH (2, 4, 6, 8, 10 and 12) were used to analyze the solubility of CPC. The samples were treated with ACP for 5 and 10 min. After that, all the treated and untreated samples were centrifuged at 3000 ×g for 10 min. Then, the supernatant was freeze-dried to prepare the protein powder. The protein solubility was determined using the equation below.

$$\text{Protein solubility (\%)} = \frac{\text{Protein content in freeze-dried sample}}{\text{Initial protein content in the sample}} \times 100 \qquad (1)$$

3.2.7 Emulsification activity and stability

The emulsification activity (EA) and emulsion stability (ES) was calculated according to Flores-Jiménez et al. (2019). The ACP treated and untreated samples (3 mL) were dissolved in 15 mL of distilled water and mixed well. After that, 10 mL of canola oil was added. The resulting mixture was homogenized for 5 min at 10,000 rpm and then centrifuged for 15 min at 3000 ×g. Then, the sample was descended into 50 mL of measuring cylinder and left for 15 minutes to stabilize the emulsified layer.

$$\text{Emulsification activity (\%)} = \frac{\text{Volume of emulsified layer}}{\text{volume of total volume in tube}} \times 100 \qquad (2)$$

The emulsion descended into 50 mL measuring cylinder and waited for 15 min to stabilize the emulsification layer. The control and treated samples were heated at 80 ºC for 30 min and then

cooled at 15 ºC and centrifuged as described above in EA for determining the ES. The ES was determined according to the equation (3).

$$\text{Emulsification stability (\%)} = \frac{\text{Volume of the emulsified layer after heating}}{\text{total volume in tube}} \times 100 \quad (3)$$

3.2.8 Foaming capacity and foaming stability

The evaluation of foaming properties was conducted with some modification based on the methodology employed by Flores-Jiménez et al. (2019). To prepare the samples, 3 mL of the treated or untreated samples were dispersed in 100 mL of distilled H_2O. The resulting suspension was then stirred with a homogenizer at 10,000 rpm for a duration of 1 min. Further, the sample was carefully descended into a 150 mL measuring cylinder, and the total volume of foam was measured and recorded after a 30 second interval. The foaming capacity (FC) was assessed using the equation (4).

$$\text{Foaming capacity (\%)} = \frac{\text{Volume of foaming layer}}{\text{total volume in tube}} \times 100 \quad (4)$$

The foam volume in the measuring cylinder was recorded for 30 min and the foaming stability (FS) was determined according to the equation (5).

$$\text{Foaming stability (\%)} = \frac{\text{Foam volume after 30 min}}{\text{Initial foam volume}} \times 100 \quad (5)$$

3.2.9 Zeta potential of CPC

The zeta potential of CPC was measured using laser Doppler velocimetry (Malvern Instruments Ltd., UK). The refractive index of the protein was tuned to 1.45, whereas the refractive index (RI) of the dispersion medium was set to 1.33. The ACP treated and untreated samples were suitably diluted before measuring the surface charge.

3.2.10 Canola protein secondary structure analysis

Fourier transform infrared (FTIR) spectrogram of the CPC was recorded from 4000 to 400 cm^{-1} using a Nicolet 6700 FTIR spectrophotometer to determine the structural changes after ACP treatment. All the data was acquired across 128 scans with a spectral resolution of 4 cm^{-1}. To prepare the CPC suspension, D_2O was used as the solvent. During the testing process, the samples were placed between two CaF_2 windows with a 25 μm polyethylene terephthalate film spacer to create a sandwich-like configuration. A lab gas generator (Parker Hannifin Corp., USA) was used to continuously purge the spectrophotometer with dry air. The ohmic 8.1 software (Thermo Fisher scientific, MA, USA) was utilized to analyze the amide 1 (1700-1600 cm^{1}) band region and determine the secondary structure of each spectrum of CPC.

To assess the fluorescence spectra of the CPC suspension, a spectraMax M3 microplate reader was employed for samples with or without ACP treatment. Prior to measurement, appropriate dilutions were made for all samples. Using an excitation wavelength of 295 nm, the emission spectra were recorded in the range of 300-400 nm at a scanning speed of 10 nm/s. Both slits (excitation and emission) were configured to a width of 3 nm.

3.2.11 Content of free sulfhydryl (total and exposed)

The analysis of the free sulfhydryl (-SH) content in CPC samples followed the methodology described by (Zhang et al., 2021). The SH content was determined according to the equation (6).

$$SH\ (\frac{\mu mol}{g}) = \frac{73.53 \times A \times D}{C} \quad (6)$$

Where A is the absorbance at 412 nm, C is the sample concentration (mg/mL), and D is the dilution multiple.

3.2.12 Statistical analysis

One-way analysis of variance (ANOVA) was used to analyse the data in R version (Cary, NC, USA). The significance ($P < 0.05$) level was assessed using the Tukey's multiple comparison test. For all the analyses, results were presented as the mean value ± standard deviation of triplicate samples.

3.3 Results and discussion

3.3.1 Degradation of total phenolic compounds (TPC) and sinapine

As one of the advanced oxidation methods, ACP has the potential for degrading phenolic compounds. Due to the production of oxidative species (H_2O_2, O_3, $OH^{\bullet}$, $O^{\bullet}$, etc.) as well as initiating a variety of physical and chemical processes such as high electric field, and ultraviolet light (UV) radiation, ACP can possibly decompose chemical compounds (Zhang et al., 2009). When compared to the control treatment, the ACP 5 and 10 min treatment showed a notable reduction of TPC from CPC. After 5 min ACP treatment, the TPC concentration significantly decreased from 66.9 µg/mL to 35.1 µg/mL and after 10 min ACP treatment, the phenolics concentration further decreased to 17.6 µg/mL. Phenolic compounds are antioxidants, and these antioxidant compounds are degraded by the reactive oxygen and nitrogen species (RONS) in ACP. These phenolic compounds are particularly important as they may influence the interactions between proteins and plasma generated species, and the resulting intermediates.

Sinapine (Figure 3.2) is the choline ester of sinapic acid (Cao et al., 2019) and it is also considered a phenolic compound (Chmielewska et al., 2020). Sinapine is the principal antinutritional phenolic compound among all TPC and is responsible for many undesirable properties. Reduction of sinapine could improve the quality of CPC as an animal feed and in the food sector. The result of this study showed that ACP treatment decreased the sinapine concentration in CPC (Figure 3.3). The 5 min ACP treatment reduced the concentration of sinapine from 60.6 to 44.7 µg/mL and 10 min ACP

treatment reduced it to 34.2 µg/mL. The OH radical and ozone are suggested to be the most important species for degrading phenols via oxidation (Lukes & Locke, 2005). Hydroxylation has been suggested as the main oxidation pathway for phenol degradation during pulsed electrical discharges under an oxygen atmosphere. The OH radical and ozone can have anelecterophilic attack on the aromatic ring of a phenol compound and form dihydroxybenzenes such as catechol and hydroquinone (Lukes & Locke, 2005). Ozone may also cleave the aromatic ring through 1,3-dipolar cycloaddition mechanism (Lukes & Locke, 2005) and form intermediate low molecular weight compounds, mainly organic acids. These intermediates could further be oxidized during hydration and hydroxylation and form CO_2 (Cheng et al., 2012; Zhang et al., 2009). In another study by Torres et al. (2011), ozone decreased the phenolic content of apple juice which further suggests the importance of ozone in ACP for phenol degradation. The other reactive species formed during ACP treatment such as superoxide (O_2^-), singlet oxygen ($^1O_2$), and H_2O_2 participate in chemical reactions, leading to the formation of ozone and OH radicals (Zhang et al., 2009).

The reactivity of H_2O_2 with phenols is low at ambient temperature; however, ultraviolet radiation from ACP can result in photolysis of the H_2O_2 and form OH radicals (Sugiarto & Sato, 2001). Also, H_2O_2 can oxidize the phenol compound in a slow reaction and produce catechol and hydroquinone. These two compounds could be further oxidized to benzoquinone, which will finally lead to the formation of organic acids such as maleic and oxalic acids via subsequent oxidation reactions (Yi et al., 2008). Also, UV radiation during ACP can result in the photolysis of ozone into OH radicals (Gurol & Vatistas, 1987), which can affect the degradation pathway of phenols.

Another factor that plays a role in determining the pathway and rate of degradation is pH of the solution. During the ozonation of phenols at high pH, OH radicals are the dominant oxidants of phenols compare to low pH, in which ozone is the dominant oxidant (Gurol & Vatistas, 1987).

Overall, as ACP consists of several chemicals and physical processes, a variety of mechanisms could be involved in the degradation of TPC and sinapine during treatment. Ozone and OH radicals could be the main species responsible for the degradation of phenolic compounds, which could be affected by the pH, UV radiation intensity in ACP, and the concentration of other reactive species in the media. Further research is needed to clarify the exact degradation pathway of TPC and sinapine via ACP treatment.

Sarangapani et al. (2017) reported the reduction of TPC after 5 min plasma (80 kV) treatment from 210 mg/100g to 150 mg/100g in blueberries. Also,(Garofulić et al., 2015) reported the phenolic acid degradation from 232 mg/100g to 163 mg/100g in sour cherry Marasca (*Prunus cerasus* var. Marasca) juice after a 30 min of plasma treatment.

3.3.2 Protein solubility

Th influence of pH on the solubility of ACP treated and untreated CPC solution was presented (Figure 3.4). All samples of CPC showed the minimum protein solubilities of 14.8, 22.7 and 26.5%, for untreated (control) treatments at pH 4 after ACP treatments for 0, 5, and 10 min, respectively. The maximum protein solubility of CPC at pH 12 was 71.8% after 10 min ACP treatment. The protein solubility of the CPC after 10 min ACP treatment increased up to 20%, when the pH range was 2 to 8 as compared to the untreated samples. The ACP generated reactive species possibly disrupted the hydrogen bonding, and disulfide linkages (S-S). The disruption of the disulfide bond increased the SH and SO groups, which probably unfolded the protein molecules and introduced new hydrophilic groups, resulting in the increased solubility and interaction between the protein and water molecules (Dong et al., 2017). Another potential process impacting the solubility of proteins is surface etching. On the surface of the protein, active sites are produced by the bombardment of plasma reactive species. The pH of the protein solution was decreased by the electrolysis of water

vapors, which introduced new acidic groups to the surface. This process improved the surface polarity, which may have a significant impact on improving the solubility (Ji et al., 2019).

The minimum solubility of CPC was observed at pH 4, which could be the isoelectric point (pI) of CPC. Previous studies showed that proteins have minimum protein solubility at pI, because the electrostatic forces of attraction are very low at pI and the interaction of protein molecule with water is very low (Fox, 1989; Mann & Malik, 1996; Wong et al., 1996). Protein molecules are more likely to approach each other, precipitate and possibly aggregate under these specific conditions. At pH level above or below the pI, where a protein has a net negative or net positive charge, more water interacts with the protein molecules. Previous studies also reported similar results that ACP treatment has the ability to improve the solubility with peanut protein (Ji et al., 2018). This study showed that 3 min cold plasma (CP) treatment increased the solubility by enhancing the exposure of active substances on the protein surface increasing the interaction with water molecules activated by CP treatment. (Dong et al., 2017a) demonstrated the concentration of soluble zein protein increased in an acidic solution after 3 min cold plasma treatment. Also, grass pea protein isolate (GPPI) solubility increased after the CP treatment (Mehr & Koocheki, 2020). This study reported that the increase in GPPI solubility after 60 s cold plasma treatment was due to an increase in surface charge and a reduction in protein particle size. Also, (Flores-Jiménez et al., 2019) reported canola protein isolate solubility increased to 78% at 12 pH after 30 min ultrasound treatment. ACP resulted in lower solubility change compared to the ultrasound treatment, but ACP is more energy efficient and could have reduced treatment time compared to the other methods.

3.3.3 Emulsification activity and stability

The ability of proteins to produce emulsion is an important quality for food applications. This property is based on the protein's ability to absorb the oil-water interface, decrease the interfacial

area, and then generate a strong enough repulsive force to prevent the droplet from aggregating with its neighboring molecules. (Noshad et al., 2016). The ability to emulsify is often well associated with the increased protein solubility. For a protein to exhibit effective emulsifying capability, it is crucial to rapidly absorb at the water-air and water-oil interface and establish a strong cohesive film by interacting with nearby molecules (Sharafodin & Soltanizadeh, 2022). The emulsification activity (EA) and emulsion stability (ES) of CPC were both significantly increased by 5 and 10 min ACP treatment (Figure 3.5). This increase in EA after ACP treatment is attributed to the protein's partial denaturation and exposure of its hydrophobic groups of protein molecules, which improved the protein's ability to absorb the oil-water interface (Sun et al., 2013). Proteins consist of both hydrophobic (Glycine, Alanine, Leucine, Isoleucine) and hydrophilic (Serine, Threonine, Cysteine, Tyrosine) amino acids; hydrophobic amino acids usually contained within the protein's internal regions and on the other hand, hydrophilic amino acids present on the surface of protein structure. ACP can improve emulsifying ability and interfacial activity by partially unfolding and degradation of protein aggregates by plasma reactive species (Mehr & Koocheki, 2020). Protein oxidation occurs due to the active free radicals produced by ACP that can alter the initial structure of the protein, which increase their activity that is responsible in the exposure of hydrophobic groups of CPC, thereby an increase in emulsification (Sharifian et al., 2019). The ES of CPC increased (40.83% increase) after 10 min ACP treatment. Electrostatic repulsion within the protein molecules is caused by the charge particles that the plasma introduce, and the repulsion improves the stability of the emulsion (Mehr & Koocheki, 2020). This is because ACP treatment can introduce charges onto the surface of the CPC particles, that results in electrostatic repulsion between them. Like charges repel each other, causing the particles to distribute more evenly throughout the emulsion, leading to

improve emulsion stability (Mehr & Koocheki, 2020). Also, the structural alterations in CPC as well as their unfolding appear to increase the ES (Sharifian et al., 2019).

3.3.4 Foaming capacity and stability

The foaming capacity (FC) values of the CPC treated by ACP for 5 and 10 min indicate no significant difference observed compared to the control (Figure 3.6). The FC is determined by the protein's ability to diffuse at the air-water interface due to the protein's structure unfolding. On the other hand, the development of a substantial cohesive layer around the air bubble determines foaming stability (FS). ACP treatments for 10 min had no significant effect on FS, while FS was significantly decreased after 5 min ACP treatment. The general unfolding of the proteins during the initial stages of ACP treatment may have resulted in the formation of a more flexible structure capable of organizing itself at the air-water interface. Protein structure altered during unfolding and partial surface denaturation, and the molecule in the film then rearranged to achieve the conformation with the lowest free energy. However, the value of foaming stability was significantly reduced after 5 min of ACP treatment. This reduction might be due to the development of aggregates among proteins, which seems to have a detrimental role in the foam formation. (Croguennec et al., 2006) found a direct correlation between foam stability and surface elasticity. Furthermore, the decrease in foam stability can be attributed to the reduced surface elasticity, which causes the interfacial films to become more resistant. Moreover, the presence of the protein network acts as a mechanical barrier, preventing the bubbles from rupturing and coalescing. Our findings regarding stability are explained by the existence of the aggregates to the extent that the protein network and structure produced rigid film because of the high packaging density and effective intermolecular interactions.

The findings of this study were in agreement with the (Segat et al., 2015) where foaming stability also reduced after 30 and 60 min plasma treatment. Even though there were very small variations

among the sample, a similar trend was also found in the emulsion capacity. The ACP treated protein solutions likely behaved differently and showed greater affinity in a water-air system as opposed to a water-oil system. The FC and FS values of canola protein may be affected by the pH of the solution. For instance, (Gerzhova et al., 2015) studied the foaming capacity and foaming stability of canola protein isolate at pH levels of 4.0, 7.0 and 9.0, and reported that the FC and FS were improved at alkaline pH (9.0) and the lowest FC and FS were observed at acidic pH (4.0). (Sibt-e-Abbas et al., 2020b) reported that the low FC in canola protein isolate was due to the cleavage of the disulfide bond, resulting in low flexibility. (Zhang et al., 2021a) reported that short plasma treatment can improve the foaming properties while plasma treatment with 5 and 10 min imposed a negative impact on these properties. The ability of the proteins to realign themselves at the oil–water/air–water interfaces following short-term plasma treatment may explain the creation of a moderately unfolded structure with increased flexibility. However, insoluble protein aggregates produced due to the extensive oxidation by longer ACP treatment had an adverse effect on their interfacial properties.

3.3.5 Protein secondary structure analysis

The functional properties of a protein heavily rely on its secondary structure. The successful alteration of the secondary structure of CPC appears to be a result of the interaction with reactive species during ACP treatment. The amide 1 band (1700−1600 cm^{-1}) of CPC was selected for analysis (Figure 3.6) because it is connected to the secondary structure of proteins, which constituted the most significant and prominent band of the protein's backbone. The bands 1650-1660 cm^{-1} represent α helix, β sheet (1618-1640 and 1670-1690 cm^{-1}), β-turn (1660-1670 and 1690-1700 cm^{-1}) and random coil (1645 cm^{-1}) (Haris & Severcan, 1999; Law et al., 2008; Wang et al., 2014). In comparison to the untreated sample, the CPC secondary structure was changed after ACP treatment. ACP treatment increased the random coil, and β-turn. After 5 min ACP treatment, the random coil

increased to a maximum of 14.7% compared to the control treatment (Table 3.1). As a result, the increased random coil content of CPC samples helped to improve the emulsifying functional properties significantly. This is because emulsification properties rely on the ability of proteins to form and stabilize oil-in water interface. An increase in β sheets can enhance the ability of proteins to form a stable interfacial film, improving the emulsification properties. Improving the β sheets can provide a more ordered and rigid structure at the oil-water interface, reducing droplet coalescence and increasing the stability of the emulsion (Basak & Annapure, 2022). This finding matched with emulsifying capacity and stability after ACP treatment (Basak & Annapure, 2022). The findings of this study were consistent with (Misra et al., 2015), who found that wheat flour treated with ACP exhibited an increase in β-sheets, β-turns and a decrease in α-helices. A decrease in α-helices and an increase in β-sheets and random coils have been observed (He et al., 2021). The stability of protein structure is primarily dictated by various types of hydrogen bonds, predominantly associated with the secondary structure of proteins for instance, greater hydrogen bonding associated with the higher β-sheets value that can improve the water-holding capacity and extend the protein structure. The protein structure became more disordered, as the number of β-turns increased because it showed more distortion and asymmetry. The α-helix content decreased compared to the control by 4.68% after 10 min ACP treatment, suggesting the orderly structure of CPC was destroyed (Basak & Annapure, 2022).

3.3.6 Zeta potential and free sulfhydryl (SH) group (total and exposed)

The pH is the most important factor that affects the zeta potential. The ACP treatment was carried out at neutral pH and the ACP treatment led to pH reduction. The zeta potential of the samples was negative. This might be due to the change in pH induced by the ACP treatment, leading to the

negative zeta potential. Protein colloids are regarded as electrically stabilized if their absolute zeta potential is high (~30 mV). The zeta potential values of untreated and ACP treated (5 and 10 min) CPC samples were negative (Table 3.2). The electrostatic attraction between the particles and the suspension's pH or ionic strength has a significant impact on zeta potential. The electrical double layer decreases as the ionic strength of the suspension increases, or when the pH is low, which in turn lowers the zeta potential (Salgın et al., 2012). The zeta potential decrease of the plasma treated canola protein was compelling because cold plasma interactions are frequently accompanied by pH reduction as a result of the production of acidic groups.

Different modifications in the amino acid side chains may occur due to the interaction of ACP generated ROS, for example, $^{\bullet}OH$ (Eazhumalai et al., 2023) and H_2O_2 (Zhang et al., 2021). The total -SH groups were reduced significantly ($P<0.05$) (Table 3.3). A similar pattern was noted in the peanut and squid mantle protein following ACP treatment (Ji et al., 2018). After ACP treatment, the amount of exposed -SH groups slightly increased, but the increase was not significantly different. An increase in exposed SH groups suggests a higher accessibility of these reactive sites on proteins. The SH groups can interact with water molecules and form hydrogen bonds, enhancing the protein's solubility in aqueous solutions (Chen et al., 2019). The decrease in the total sulfhydryl group is well associated with the increase in carbonyl content (Zhang et al., 2021). The S-S group and sulfonic acid group were made after the reduction of free -SH group. The degradation of free -SH group is associated with two possible mechanisms. The -SH radicals are created when the •OH groups react with free sulfhydryl groups, and they lead to the creation of disulfide bond because of their instability. Additionally, these radicals have the capacity to generate SOO• (thiol peroxyl radical), which can then be changed back into the S-S bond (Mehr & Koocheki, 2021). The oxidation of thiol group to form disulfide bonds is one way in which ozone can potentially degrade sulfur-containing

amino acids. The total -SH group was decreased after 5 and 10 min of ACP treatment. The result of this study is in agreement with (Zhang et al., 2021) where 10 min ACP also reduced the free -SH content from pea protein concentrate.

3.4 Conclusions

Atmospheric cold plasma (ACP) promising non-thermal approach for changing the functional characteristics of canola protein concentrate (CPC). One important mechanism by which ACP treatment affects protein structure and its functionality is the reaction between protein molecules and reactive species. High energy ACP could be able to break down the covalent bond of protein molecules and cause cleavage of disulfide bonds because of reactive species generated by plasma. Break down of disulfide bonds can develop the sulfhydryl (SH) or sulfoxide (SO) group which can further alter the protein structure. After 5 min ACP treatment, the TPC concentration significantly decreased from 66.9 µg/mL to 35.1 µg/mL and after 10 min ACP treatment, the phenolics concentration further decreased to 17.6 µg/mL and the concentration of sinapine from 60.6 to 44.7 µg/mL and 10 min ACP treatment reduced it to 34.2 µg/mL. The improvement in different functional properties of CPC such as solubility, emulsification, foaming properties, surface charge, and changes in the secondary structure after ACP treatments were analyzed.

The ACP technology could degrade the phenolic compounds and sinapine significantly in CPC and improve the functional properties after 10 min treatment, hence enhancing the potential of canola protein to be used in the food industry. However, more research is required to determine the actual mechanisms of total phenolics and sinapine degradation during ACP treatment.

3.5 References

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Alashi, A.M., Blanchard, C.L., Mailer, R.J., Agboola, S.O. 2013. Technological and bioactive functionalities of canola meal proteins and hydrolysates. *Food Reviews International*, **29**(3), 231-260.

Brandenburg, R., Ehlbeck, J., Stieber, M., v. Woedtke, T., Zeymer, J., Schlüter, O., Weltmann, K.D. 2007. Antimicrobial treatment of heat sensitive materials by means of atmospheric pressure Rf-driven plasma jet. *Contributions to Plasma Physics*, **47**(1-2), 72-79.

Caessens, P.W., De Jongh, H.H., Norde, W., Gruppen, H. 1999. The adsorption-induced secondary structure of β-casein and of distinct parts of its sequence in relation to foam and emulsion properties. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, **1430**(1), 73-83.

Capper, J.L. 2020. Opportunities and Challenges in Animal Protein Industry Sustainability: The Battle Between Science and Consumer Perception. *Animal Frontiers*, **10**(4), 7-13.

Chang, C., Tu, S., Ghosh, S., Nickerson, M. 2015. Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, **77**, 360-367.

Chen, C., Liu, C., Jiang, A., Guan, Q., Sun, X., Liu, S., Hao, K., Hu, W. 2019. The effects of cold plasma-activated water treatment on the microbial growth and antioxidant properties of fresh-cut pears. *Food and Bioprocess Technology*, **12**(11), 1842-1851

Coutinho, N.M., Silveira, M.R., Fernandes, L.M., Moraes, J., Pimentel, T.C., Freitas, M.Q., Silva, M.C., Raices, R.S., Ranadheera, C.S., Borges, F.O. 2019. Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry*, **278**, 276-283

Chang, P.G., Gupta, R., Timilsena, Y.P., Adhikari, B. 2016. Optimisation of the complex coacervation between canola protein isolate and chitosan. *Journal of Food Engineering*, **191**, 58-66.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Coffmann, C., Garciaj, V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, **12**(5), 473-484.

Dong, S., Gao, A., Xu, H., Chen, Y. 2017a. Effects of dielectric barrier discharges (DBD) cold plasma treatment on physicochemical and structural properties of zein powders. *Food and Bioprocess Technology*, **10**(3), 434-444.

de Castro, D.R.G., Mar, J.M., da Silva, L.S., da Silva, K.A., Sanches, E.A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F.A., Campelo, P.H. 2020. Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, **131**, 109044.

Dong, S., Gao, A., Zhao, Y., Li, Y.-t., Chen, Y. 2017b. Characterization of physicochemical and structural properties of atmospheric cold plasma (ACP) modified zein. *Food and Bioproducts Processing*, **106**, 65-74.

de Castro, D.R.G., Mar, J.M., da Silva, L.S., da Silva, K.A., Sanches, E.A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F.A., Campelo, P.H. 2020. Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, **131**, 109044.

Eazhumalai, G., Kalaivendan, R.G.T., Annapure, U.S. 2023. Effect of atmospheric pin-to-plate cold plasma on oat protein: Structural, chemical, and foaming characteristics. *International Journal of Biological Macromolecules*, 125103.

He, T., Wang, J., Hu, X. 2021. Effect of heat treatment on the structure and digestion properties of oat globulin. *Cereal Chemistry*, **98**(3), 740-748.

Feizollahi, E., N.N. Misra., & M. S. Roopesh (2021) Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications, Critical Reviews in Food Science and Nutrition, 61:4, 666-689, DOI: 10.1080/10408398.2020.1743967

Flores-Jiménez, N.T., Ulloa, J.A., Silvas, J.E.U., Ramírez, J.C.R., Ulloa, P.R., Rosales, P.U.B., Carrillo, Y.S., Leyva, R.G. 2019. Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (Brassica napus L.) protein isolate. *Food Research International*, **121**, 947-956.

Fox, P. 1989. The milk protein system. *Developments in dairy chemistry. 4. Functional milk proteins.*, 1-53.

Fujihara, S., Kasuga, A., Aoyagi, Y. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science*, **66**(3), 412-415.

Garofulić, I.E., Jambrak, A.R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., Herceg, Z. 2015. The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (Prunus cerasus var. Marasca) juice. *LWT-Food Science and Technology*, **62**(1), 894-900.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food bioscience*, **12**, 128-138.

Grzegorzewski, F., Ehlbeck, J., Schlüter, O., Kroh, L.W., Rohn, S. 2011. Treating lamb's lettuce with a cold plasma–Influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta. *LWT-Food Science and Technology*, **44**(10), 2285-2289.

Haris, P.I., Severcan, F. 1999. FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. *Journal of Molecular Catalysis B: Enzymatic*, **7**(1-4), 207-221.

Hou, H., He, H., Wang, Y. 2020. Effects of SDS on the activity and conformation of protein tyrosine phosphatase from thermus thermophilus HB27. *Scientific Reports*, **10**(1), 3195.

Ismail, F., Vaisey-Genser, M., Fyfe, B. 1981. Bitterness and astringency of sinapine and its components. *Journal of Food Science*, **46**(4), 1241-1244.

Itkonen, S.T., Päivärinta, E., Pellinen, T., Viitakangas, H., Risteli, J., Erkkola, M., Lamberg-Allardt, C., Pajari, A.-M. 2021. Partial replacement of animal proteins with plant proteins for 12 weeks accelerates bone turnover among healthy adults: A randomized clinical trial. The Journal of Nutrition, **151**(1), 11-19.

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., Chen, Y. 2018. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, **11**(2), 344-354.

Jiang, J., Wang, Q., Xiong, Y.L. 2018. A pH shift approach to the improvement of interfacial properties of plant seed proteins. *Current opinion in food science*, **19**, 50-56.

Law, H.-Y., Choi, S.-M., Ma, C.-Y. 2008. Study of conformation of vicilin from Dolichos lablab and Phaseolus calcaratus by Fourier-transform infrared spectroscopy and differential scanning calorimetry. *Food research international*, **41**(7), 720-729.

Li, M., Li, X., Han, C., Ji, N., Jin, P., Zheng, Y. 2019. Physiological and metabolomic analysis of cold plasma treated fresh-cut strawberries. *Journal of agricultural and food chemistry*, **67**(14), 4043-4053.

Lukes, P., Locke, B.R. 2005. Degradation of substituted phenols in a hybrid gas− liquid electrical discharge reactor. Industrial & engineering chemistry research, **44**(9), 2921-2930.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Makris, D.P., Rossiter, J.T. 2002. Hydroxyl free radical-mediated oxidative degradation of quercetin and morin: a preliminary investigation. *Journal of Food Composition and Analysis*, **15**(1), 103-113.

Mann, B., Malik, R. 1996. Studies on some functional characteristics of whey protein-polysaccharide complex. *Journal of food science and technology (Mysore)*, **33**(3), 202-206.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Mu, L., Zhao, M., Yang, B., Zhao, H., Cui, C., Zhao, Q. 2010. Effect of ultrasonic treatment on the graft reaction between soy protein isolate and gum acacia and on the physicochemical properties of conjugates. *Journal of agricultural and food chemistry*, **58**(7), 4494-4499.

Misra, N., Kaur, S., Tiwari, B.K., Kaur, A., Singh, N., Cullen, P. 2015. Atmospheric pressure cold plasma (ACP) treatment of wheat flour. *Food Hydrocolloids*, **44**, 115-121

Noshad, M., Mohebbi, M., Koocheki, A., Shahidi, F. 2016. Influence of interfacial engineering on stability of emulsions stabilized with soy protein isolate. *Journal of Dispersion Science and Technology*, **37**(1), 56-65.

Pirestani, S., Nasirpour, A., Keramat, J., Desobry, S., Jasniewski, J. 2017. Effect of glycosylation with gum Arabic by Maillard reaction in a liquid system on the emulsifying properties of canola protein isolate. *Carbohydrate Polymers*, **157**, 1620-1627.

Pojić, M., Mišan, A., Tiwari, B. 2018. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology*, **75**, 93-104.

Sarangapani, C., O'Toole, G., Cullen, P., Bourke, P. 2017. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science & Emerging Technologies*, **44**, 235-241.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Sharifian, A., Soltanizadeh, N., Abbaszadeh, R. 2019. Effects of dielectric barrier discharge plasma on the physicochemical and functional properties of myofibrillar proteins. *Innovative food science & emerging technologies*, **54**, 1-8.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Tauseef, M., Sultan, M.S.S., Shahid, M. 2020. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Sullivan, G.A., Sebranek, J.G. 2012. Nitrosylation of myoglobin and nitrosation of cysteine by nitrite in a model system simulating meat curing. *Journal of agricultural and food chemistry*, **60**(7), 1748-1754.

Sun, W., Zhou, F., Sun, D.-W., Zhao, M. 2013. Effect of oxidation on the emulsifying properties of myofibrillar proteins. *Food and Bioprocess Technology*, **6**(7), 1703-1712.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28.

Wanapat, M., Huang, B., Viennasay, B., Totakul, P., Matra, M., Phesatcha, B., Ampapon, T. 2020. Rapeseed pod meal can replace concentrate and enhance utilization of feed on in vitro gas production and fermentation characteristics. *Tropical Animal Health and Production*, **52**(5), 2593-2598.

Wang, Z., Li, Y., Jiang, L., Qi, B., Zhou, L. 2014. Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry*, **2014**.

Wong, D.W., Camirand, W.M., Pavlath, A.E., Parris, N., Friedman, M. 1996. Structures and functionalities of milk proteins. *Critical Reviews in Food Science & Nutrition*, **36**(8), 807-844.

Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.

Yang, J., Liu, G., Zeng, H., Chen, L. 2018. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. *Food Hydrocolloids*, **83**, 275-286.

Zhang, D., Li, H., Emara, A., Hu, Y., Wang, Z., Wang, M., He, Z. 2020. Effect of in vitro oxidation on the water retention mechanism of myofibrillar proteins gel from pork muscles. *Food Chemistry*, **315**, 126226.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

Cao, X., Liang, J., Aluko, R.E., Thiyam-Holländer, U. 2019. In situ oxidation of canola meal sinapic acid by horseradish peroxidase (type II) and tyrosinase. *Journal of Food Biochemistry*, **43**(6), e12884.

Cheng, H.-H., Chen, S.-S., Yoshizuka, K., Chen, Y.-C. 2012. Degradation of phenolic compounds in water by non-thermal plasma treatment. *Journal of Water Chemistry and Technology*, **34**(4), 179-189.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Gurol, M.D., Vatistas, R. 1987. Oxidation of phenolic compounds by ozone and ozone + u.v. radiation: A comparative study. *Water Research*, **21**(8), 895-900.

Lukes, P., Locke, B.R. 2005. Plasmachemical oxidation processes in a hybrid gas–liquid electrical discharge reactor. *Journal of Physics D: Applied Physics*, **38**(22), 4074-4081.

Sugiarto, A.T., Sato, M. 2001. Pulsed plasma processing of organic compounds in aqueous solution. *Thin Solid Films*, **386**(2), 295-299.

Torres, B., Tiwari, B., Patras, A., Wijngaard, H.H., Brunton, N., Cullen, P., O'Donnell, C. 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. *Food Chemistry*, **124**(3), 721-726.

Yi, Z., Jia-qing, X., Ci, L., Jun, L., Hong-li, S. 2008. Mechanism of Oxidation of Aqueous Phenol by H2O2 Catalysed by a Macrocyclic Iron(II) Complex. *Progress in Reaction Kinetics and Mechanism*, **33**(3), 241-252.

ZHANG, J., CHEN, J., LI, X. 2009. Remove of Phenolic Compounds in Water by Low-Temperature Plasma: A Review of Current Research. *Journal of Water Resource and Protection*, **Vol.01No.02**, 11.

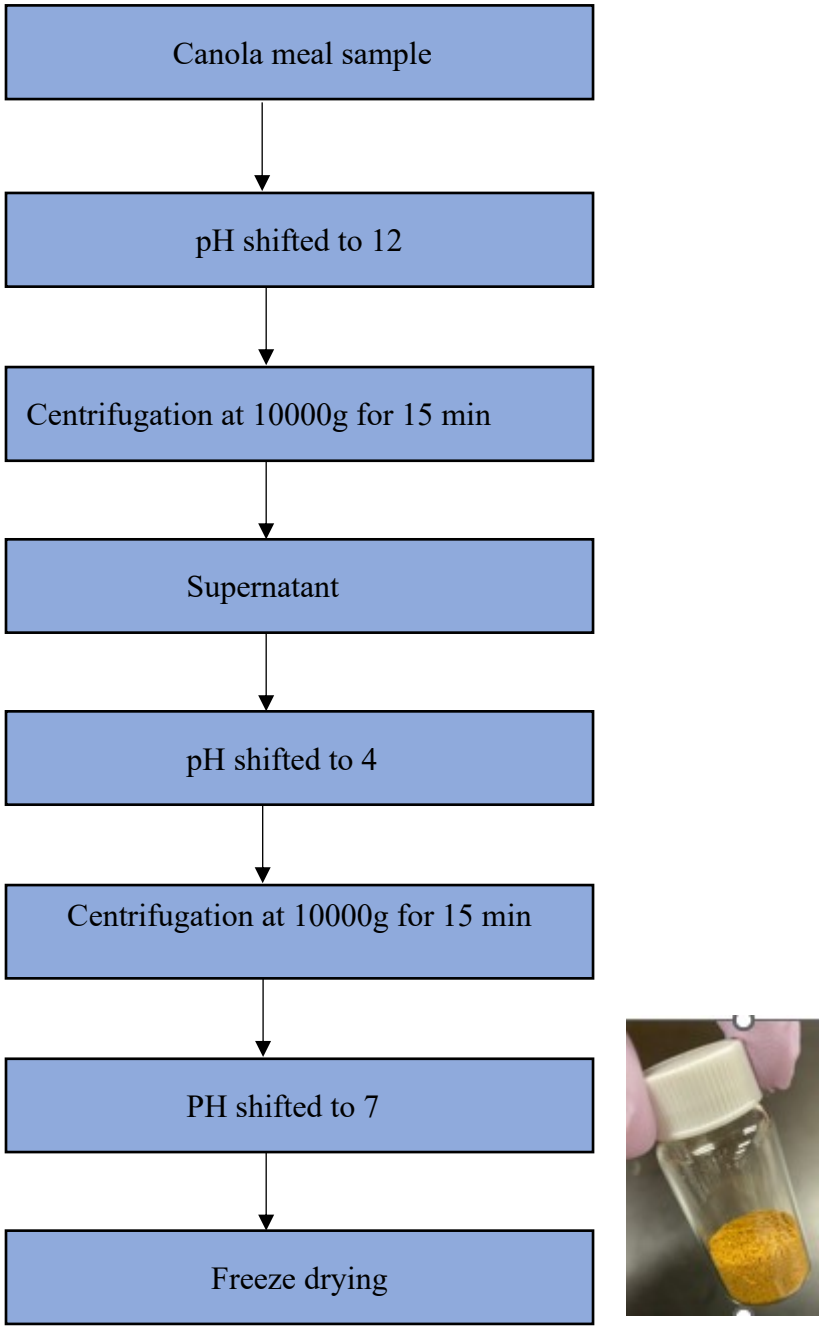


Figure 3.1. The flowchart representation of alkaline extraction method

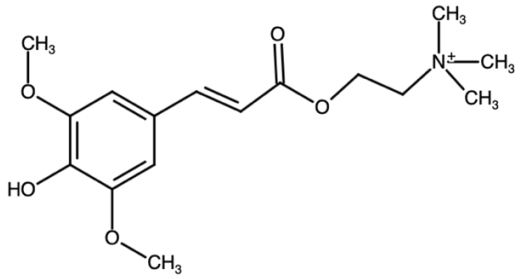


Figure 3.2. The chemical structure of sinapine

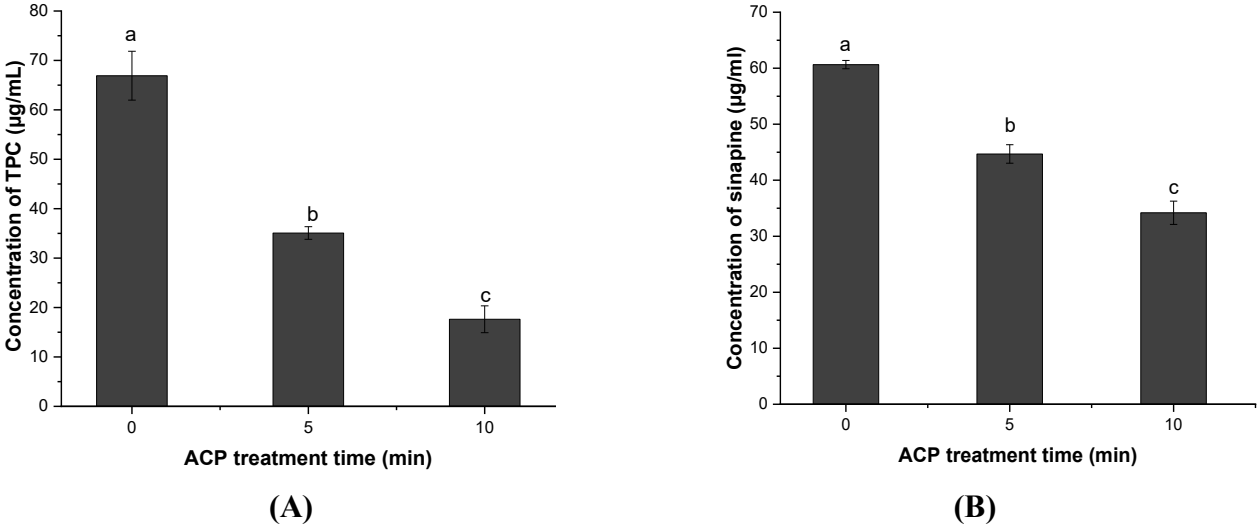


Figure 3.3. (A) The impact of ACP treatment on the reduction of TPC concentrations. **(B)** The impact of ACP treatment on the reduction of sinapine concentrations. Values with different letters are considered significantly distinct ($p<0.05$, $n\geq3$).

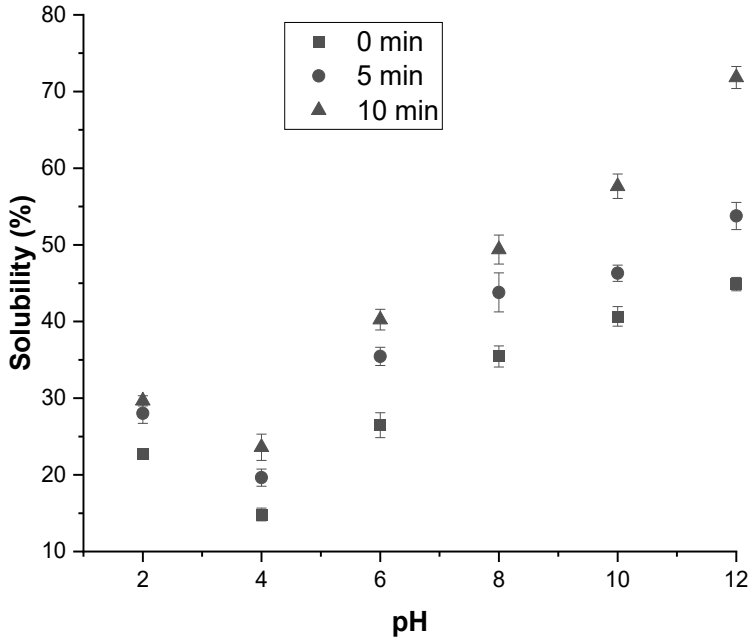


Figure 3.4. The impact of ACP treatment on the canola protein concentrate solubility at different pH.

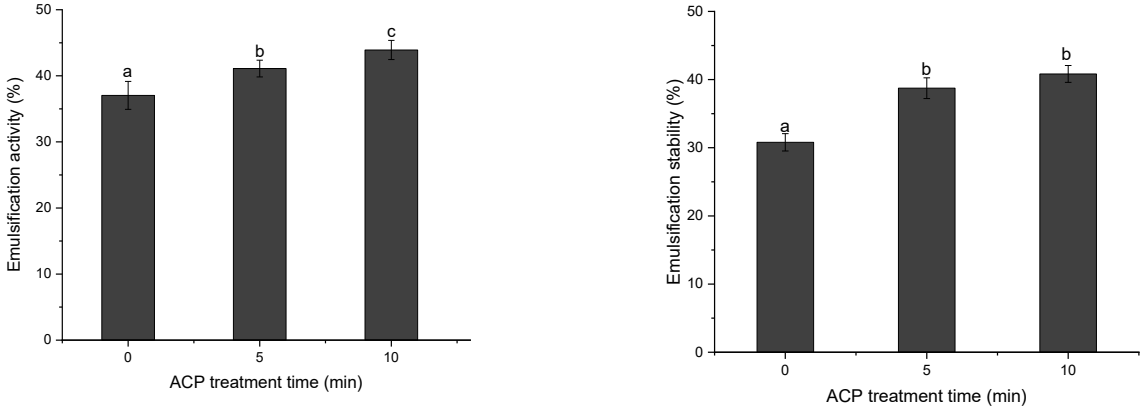
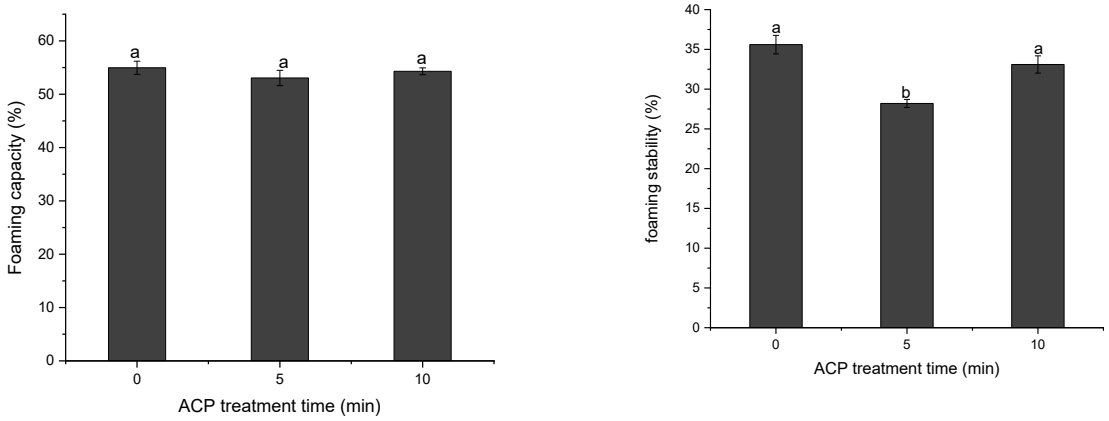


Figure 3.5. (A) The impact of ACP treatment on the emulsion activity and (B) emulsion stability of canola protein. Values with different letters are considered significantly distinct ($p<0.05$, $n\geq3$)



(A) **(B)**

Figure 3.6. The impact of ACP treatment on the foaming capacity and foaming stability of canola protein. Values with different letters are considered significantly distinct ($p<0.05$, $n\geq3$).

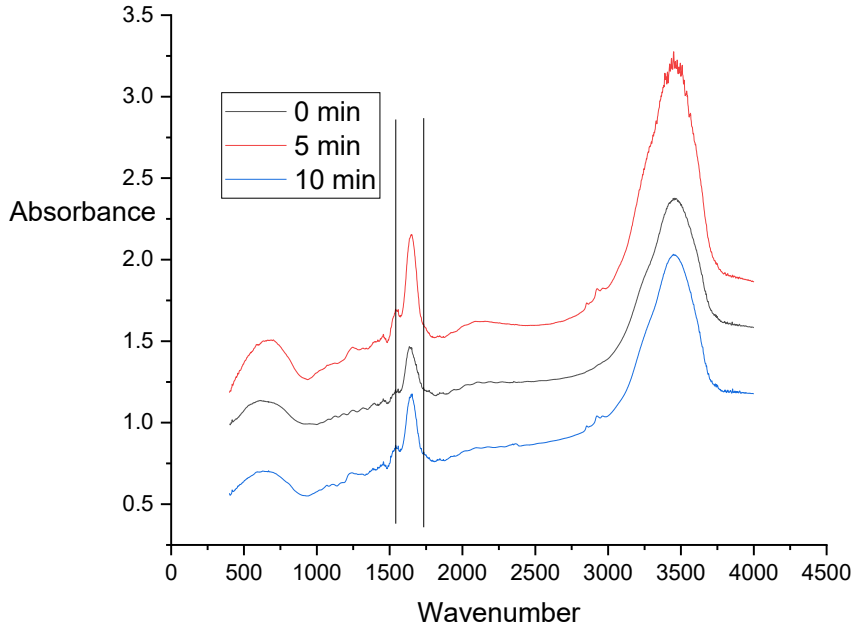


Figure 3.7. FTIR spectra of ACP treated and untreated samples of CPC.

Table 3.1 Effect of ACP treatment on secondary structure of CPC.

Treatment time (min)	Secondary structure			
	β sheet (%)	Random coil (%)	α helix (%)	β turn (%)
0	27.3	11.9	12.2	45.1
5	30.9	14.7	9.76	46.3
10	27.7	12.6	7.54	49.0

Table 3.2. Influence of ACP treatment on zeta potential of CPC suspension with or without ACP treatment.

ACP treatment	Zeta potential (mV)
0 min	-26.1±1.1[a]
5 min	-18.3±1.3[b]
10 min	-21.6±0.6[c]

Values within same column that are denoted by different letters are considered significantly distinct ($p<0.05$, $n\geq3$).

Table 3.3. The impact of ACP treatment on free -SH contents (total and exposed) in untreated and treated CPC solution.

Treatment time (min)	Free -SH contents (μmol/g protein)	
	Total	Exposed
0 min	14.6±0.7[a]	3.79±0.82[a]
5 min	10.6±0.5[b]	4.32±0.57[a]
10 min	9.20±0.42[c]	4.87±0.25[a]

Values within same column that are denoted by different letters are considered significantly distinct ($p<0.05$, $n\geq3$).

Chapter 4: Combine Effect of pH Shifting and ACP Treatment to Improve the Functional Properties of Canola Protein.

4.1 Introduction

There is an increasing demand for animal proteins, which is expected to be doubled by 2050 (Van de Noort, 2017). Plant protein is an essential part of the human diet. Moreover, the need for food proteins will continue to increase because the human population is expected to exceed 9 billion by the year 2050. This increased requirement can only be satisfied by the consumption of novel and sustainable proteins (Poore & Nemecek, 2018). However, plant proteins have poor functional properties than many animal-based proteins, that restricts their use in the food industry. Previous research has focused on improving plant protein functionalities with different approaches.

Canada is regarded as the largest producer of canola in the world (Aider & Barbana, 2011). Canola meal, which is leftover after oil extraction contains 50% of the protein in dry basis. Canola meal is utilized as animal and poultry feed (Chmielewska et al., 2021). There have been no reports on the use of canola meal as a source of human food in an industrial scale, though previous research has shown that canola protein has the potential to be used in human food in terms of amino acid profile (Fleddermann et al., 2013; Wanasundara et al., 2016b). A large number of companies are interested in introducing canola protein (obtained from the extraction of canola meal) to the market, and the technology for extracting canola protein is constantly improving (Mupondwa et al., 2018). All of these suggest that canola protein has the potential to be used effectively as a protein source and ingredient for food industry (Carré & Pouzet, 2014; Wanasundara et al., 2016b).

Atmospheric cold plasma (ACP) is a novel nonthermal technology, that was rigorously studied recently. Plasma formation results in the production of ROS and RNS. The general mechanisms of altering the different properties of proteins by cold plasma are the cleavage of covalent bonds inside the protein molecules and sulfur amino acid oxidation, causing the dissociation of disulfide (S-S) bonds. This may cause the formation of SH and SO groups, further altering the protein's structure and polypeptide chains (Dong et al., 2017b).

A method called pH-shifting allows protein to partially unfold at an alkaline pH, increasing the repulsions between charged side chain groups within the molecule before folding back into a molten globule shape, when the pH is brought back to neutral (Basak & Annapure, 2022). It has been demonstrated that pH shifting treatment was able to significantly improve the functional properties of plant proteins, for example, solubility, emulsification, and foaming properties (Jiang et al., 2017; Zhang et al., 2022). When canola protein concentrate (CPC) suspension is kept for a brief duration at an alkaline or acidic pH, the denaturation leads to structural unfolding. Then a brief incubation of protein suspension at the neutral pH leads to partial refolding (Kristinsson & Hultin, 2003).

The combination of ACP and pH shifting can act synergistically to improve the solubility and emulsification properties of CPC. The ACP treatment modifies the protein's surface, while pH shifting affects the protein's charge and conformation. The modified surface and altered protein structure by the combined ACP treatment and pH shifting, can possibly lead to enhanced functional properties such as solubility and emulsification, making it more suitable for various food applications. However, it is essential to optimize the specific conditions of cold plasma treatment and pH shifting to achieve the desired functional improvements without negatively impacting the overall quality of the proteins. The objective of this study was to analyze the degradation of sinapine

and improvement in the selected functional properties of CPC by the combined ACP and pH shifting treatments.

4.2 Materials and methods

4.2.1 Materials and chemicals

Canola meal (40.7% protein, 37.8% carbohydrates, 14% fat, 2% ash, 5.5% moisture) was commercially sourced in western Canada and it was from the 2019 harvest and processed in Alberta Canola meal was used to obtain the canola protein concentrate (CPC). Sinapine standard (2 mg) was purchased from Selleckchem (Ontario, Canada) and sodium dodecyl sulfate (SDS), acetonitrile (can), methanol, trifluoracetic acid (TFA), Folin-Ciocalteu (FC) reagent, trinitrobenzene sulfonic acid (TNBS), sodium phosphate buffer, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), glycine, ethylenediaminetetraacetic acid (EDTA), were purchased from Sigma-Aldrich (Sigma Aldrich, Mississauga, Canada), and remaining chemicals (sodium carbonate) were purchased from Fisher scientific.

4.2.2 Extraction of canola protein concentrate

The extraction of CPC was performed according to (Zhang et al., 2021b) with some modifications as described in Chapter 3. CPC (0.4 g) was dissolved in 10 mL of distilled water with 1% SDS to make 4% (w/v) solution and was used for all further analysis.

4.2.3 Atmospheric cold plasma and pH-shifting treatment

In this study, canola protein suspension was treated using a dielectric barrier discharge (DBD) plasma system (Advanced plasma solution, Malvern, USA) as described in Chapter 3.

Before ACP treatment the pH of the CPC suspension was adjusted to pH 6, 8 and 10. After pH adjustment, ACP treatment was carried out for 10 min and after the treatment, all the suspensions

were neutralized to pH 7 by adding of 1M HCL or 1M NaOH. The control samples were without ACP treatment but with the respective pH 6, 8 and 10.

4.2.4 Analysis of functional properties of canola protein and quantification of sinapine

The effect of combined ACP and pH shifting on the selected functional properties of canola protein, including solubility, emulsification, and foaming properties were analyzed as described in Chapter 3. Also, quantification of sinapine was determined by HPLC as described in Chapter 3.

4.2.5 Statistical analysis

The data was analyzed using SPSS V.27 (IBM SPSS, Armork, NY) through one-way analysis of variance (ANOVA), followed by the Duncan test for determining significance ($P<0.05$). The Results were represented as the mean value ± with the standard deviation based on triplicate samples.

4.3 Results and discussion

4.3.1 Degradation of sinapine

The combined effect of pH shifting with the 10 min of ACP treatment reduced the concentration of sinapine substantially, e.g., the sinapine concentration at pH 10 reduced from 94.6 µg/mL to 12.6 µg/mL, while in the first study the concentration of sinapine reduced after 5 min ACP treatment from 60.6 to 44.7 µg/mL and 10 min ACP treatment reduced it to 34.2 µg/mL without pH shifting treatment. However, in first study the concentration of sinapine without ACP and pH shifting treatment was 60 µg/mL. In addition, pH shifting with ACP treatment had a significant impact on degradation of sinapine. Figure 4.1 shows the concentration of sinapine is depending on the pH. The change in sinapine concentration during pH shifting treatment can be attributed to the pH-dependent solubility and stability. At alkaline pH, sinapine was more soluble, resulting in an increased concentration compared to the pH 6, 7 and 8. This suggests that the alkaline conditions favor the

dissolution of sinapine, resulting in a higher concentration. It is important to consider these results and it is required to conduct further research to learn the underlying mechanisms involved in the sinapine concentration changes at different pH The most significant species for the oxidative degradation of phenols are thought to be the OH radical and ozone (Lukes & Locke, 2005). The primary oxidation process for the breakdown of phenols under pulsed electrical discharges in an oxygen environment has been proposed to be hydroxylation. A phenol compound's aromatic ring can be attacked electrophilically by the OH radical and ozone to produce dihydroxy benzenes like catechol and hydroquinone (Lukes & Locke, 2005).

The greater reduction in sinapine concentration at alkaline pH rather than acidic pH after the combined ACP and pH shifting treatment can be attributed to several factors. One possible reason is that sinapine may be more susceptible to oxidative reactions under alkaline conditions. ACP treatment generates reactive oxygen and nitrogen species, which can lead to oxidation or degradation of sinapine. In an alkaline environment, these reactive species may have a stronger oxidative effect, resulting in a more significant reduction in sinapine concentration (Lukes & Locke, 2005). Additionally, the alkaline pH may also influence the stability and solubility of sinapine. At higher pH levels, sinapine may undergo hydrolysis or other chemical transformations, leading to its reduced concentration. The altered solubility and partitioning behaviour of sinapine in an alkaline environment can also contribute to its greater reduction after the combined ACP and pH shifting process. Furthermore, the ACP treatment may induce changes in the protein matrix of canola, affecting the binding or interaction of sinapine with the proteins. These changes in protein conformation, solubility, or aggregation behavior can be influenced by the pH of the system. Thus, the alkaline pH may facilitate greater disruption of sinapine-protein interactions, leading to a more significant reduction in sinapine concentration compared to acidic pH. It was worth noting that the

specific effects of ACP and pH shifting on sinapine reduction can vary depending on the parameters used, such as ACP treatment conditions, pH levels, treatment duration, and the specific characteristics of the canola protein matrix. Also, the pH shifting mechanism associated with sinapine degradation involves hydrolysis reactions. Sinapine contains ester linkages, and under certain conditions, these ester bonds can undergo hydrolysis, resulting in the breakdown of sinapine into its component parts, sinapic acid and choline. In an alkaline environment, the hydroxyl ions (OH^-) present can act as a nucleophile, attacking the ester bond of sinapine (Momen et al., 2021). This hydrolytic reaction cleaves the ester bond, leading to the release of sinapic acid and choline, leading to a decrease in the concentration of sinapine. The alkaline pH provides a favorable environment for the hydrolysis of sinapine because hydroxyl ions are more abundant and active under alkaline conditions. The increased concentration of hydroxyl ions and the acceleration of the hydrolysis reaction, led to a higher rate of sinapine degradation (Momen et al., 2021). On the other hand, under acidic conditions, the hydrolysis reaction is less favored. Acidic pH values result in a lower concentration of hydroxyl ions, which reduces the rate of ester bond cleavage, leading to lower sinapine degradation.

4.3.2 Protein solubility

Solubility of CPC was increased by the pH shifting treatment, particularly in alkaline conditions. Also, after ACP treatment solubility of CPC was increased compared to the control treatment. ACP treatment combined with pH shifting (pH 10) increased the canola protein solubility up to 74% (Fig 4.2). The interaction and bonding of water molecules to the protein surface are improved by the addition of certain hydrophilic groups because of the reactive species produced by ACP. Moreover, the increase of the solubility after ACP and pH shifting was probably due to the unfolding and refolding of the CPC structure under extreme alkaline pH, which exposed certain polar groups (Jiang

et al., 2010). However, the combined treatment of ACP and pH shifting resulted in improved solubility of CPC not only at pH 10 but also at other pH levels specifically at pH 6 and 8 is noteworthy since these values are relevant for many food applications. The solubility increased at these pH levels suggesting that the treatment effectively modified the CPC structure, leading to improved dispersion and dissolution in aqueous solutions. Improved solubility near-neutral pHs is particularly advantageous for food applications as it allows for better incorporation and dispersibility of canola protein in various food formulations. Another potential process impacting the solubility of proteins is surface etching. The active sites are produced on the protein surface after the bombardment of plasma reactive species (Campbell et al., 2016). The pH of the protein solution decreased by the electrolysis of atmospheric water vapour, which can add an acidic group to the surface. This improves the surface polarity even more, which has a significant impact on the solubility (Basak & Annapure, 2022). The impact of pH on CPC can be significant, as it can affect the protein's structure, solubility, and functionality. CPC like most proteins, has an optimal pH range at which its highest stability and functionality are achieved. Canola protein is typically stable and soluble under alkaline conditions. However, when the pH becomes more acidic or near the isoelectric point (pI), the CPC tends to undergo structural changes, leading to aggregation and precipitation. At acidic pH, the increased concentration of OH^- can cause the protein molecules to undergo denaturation or unfolding. This denaturation disrupts the protein's tertiary and quaternary structure, resulting in a loss of its functional properties such as emulsification, and foaming properties. Also, by combining ACP and pH shifting process, the modified protein structure and increased surface hydrophilicity can contribute to improved solubility. However, in previous chapter ACP treatment alone modify the protein solubility to some extent compared to the control treatment. ACP treatment alone can disrupt the protein-protein interactions and unfold protein structure, leading to increase

the solubility. The combination of ACP and pH shifting treatment can further enhance solubility by utilizing the pH adjustment to optimize protein-protein and protein-protein water interactions. Previous studies also reported that the ACP treatment has the ability to improve the solubility of peanut protein (Ji et al., 2018), zein protein (Dong et al., 2017a), and grass pea protein isolate (GPPI) (Mehr & Koocheki, 2020).

4.3.3 Emulsification activity (EA) and stability (ES)

Two important characteristics of plant proteins for their use in various food systems are emulsification and foaming properties. Protein's interfacial activity refers to their capacity to adsorb at the interface between oil and water, creating stable films around bubbles that possess appropriate elasticity (Zhang et al., 2014). For proteins to exhibit effective emulsifying and foaming properties, they should have the ability to readily absorb the adjacent molecules at the interface between water and oil (Basak & Annapure, 2022). pH shifting treatment can affect the net charge of canola protein molecules. At alkaline pH range, the proteins might carry a higher net negative charge due to ionization of specific amino acid residues. This increased negative charge can promote better electrostatic repulsion between protein molecules, preventing their aggregation and contributing to improve emulsion stability (Lima et al., 2023). While atmospheric cold plasma can promote the development of a flexible structure in proteins, enabling them to generate films at the oil-water interface and this structural change has a beneficial impact on emulsifying and foaming properties. Compared to the control treatment, the 10 min ACP with pH shifting at pH values of 6, 8 and 10 considerably increased the EA and ES of the CPC (Fig 4.3). In addition, the most notable increase of EA and ES after ACP treatment was observed at pH 10, where EA increased to 57.9% compared to the control of 51.2%, and compared to the without ACP and pH shifting treatment in first study which was 37.04%. The increase in EA may be due to the result of the protein's partial denaturation

and unfolding and the development of a more disordered structure after ACP treatment (Pirestani et al., 2017). However, the highest increase in ES of CPC after ACP treatment was found at pH 10 (54.5%) compared to the without ACP and pH shifting treatment from previous study that have 30% FS. The high voltage by the plasma introduces RNS and ROS, which react with the water in the liquid phase leading to net negative charges. The introduction of a high negative charge through ACP induces electrostatic repulsion within the proteins, which positively impacts the stability of the emulsion (Chmielewska et al., 2021). As previously reported in the literature, oxidation plays an important role in increasing protein surface hydrophobicity. As the protein oxidation is increased during the ACP treatment, the buried hydrophobic amino acids become more exposed to the surface, leading to an increase in surface hydrophobicity. The increase in emulsification activity after ACP treatment might be due to protein denaturation and protein hydrophobic group exposure, which enhanced the capacity of the protein to interact at the oil-water interface (Sun et al., 2013). The ACP reactive species probably increased protein oxidation and structural modifications, which probably caused the exposure of hydrophobic groups of CPC, increasing the emulsification capacity (Sharifian et al., 2019).

The combined ACP and pH shifting improved the emulsion capacity depending on pH of the system. Under alkaline conditions, a greater improvement in emulsion capacity was observed compared to the acidic conditions. The ACP with pH shifting at pH 10 enhanced EA and ES compared to the ACP treatment at neutral pH. Additionally, the correlation between EA and pH for canola protein closely resembled the relationship observed between protein solubility and pH. These findings were consistent with previous studies that have identified a strong correlation between EA and protein solubility (Zhang et al., 2021a) Yanjun et al., 2014). Dependence of emulsion capacity on pH was expected, as it is known that the emulsion capacity of a total protein depends upon the hydrophilic-

hydrophobic balance, which is affected by pH, particularly by the ACP treatment at a more basic pH (Basak & Annapure, 2022). The combined effect of pH shifting and ACP treatment probably enabled protein unfolding and the more flexible structures can realign at the oil-water/air-water interfaces (Zhang et al., 2021a).

4.3.3. Foaming capacity (FC) and stability (FS)

The foaming properties of CPC were significantly increased when exposed to ACP for 10 min followed by pH shifting to 6, 8 and 10. The stability of foam relies on the formation of a dense and cohesive layer that encloses the air bubble, whereas the ability to form foam is determined by the protein's diffusion at the air-water interface, facilitated by its structural unfolding. After applying a combined treatment of ACP (10 min) and pH shifting to neutral at pH 10, the FC of CPC exhibited a substantial increase. The foaming capacity increased to 86.63%, in contrast to the control which only achieved 81.48%, comparing to the without ACP and pH shifting treatment FC values which is 55% in first study While the FS found 75.66% compared to the control 68% at pH 10 with combined both treatments (Fig 4.4) comparing to the without ACP and pH shifting treatment values which is 35% in first study. However, the previous chapter revealed that ACP treatment alone did not result in any notable improvement in foaming capacity and stability. The second study demonstrated that combining ACP with pH shifting treatment at alkaline conditions exhibited a significant enhancement in the foaming properties of canola protein. The reason behind this alkaline pH shifting facilitated hydrophobic interactions, while the ACP treatment induced structural modifications (Zhao et al., 2022). As described above, the pH shifting promotes solubility of proteins and ACP treatment can result in oxidation leading to unfolding and exposure of hydrophobic groups. This makes protein structure flexible so it can organize and rearrange into a film at the air-water interface, enhancing foam stability that produces the foam denaturation (Sharifian et al., 2019). At

alkaline pH the protein becomes partially unfolded leading to its better solubility compared to neutral pH- further, the additional negative charge introduction by ACP treatment enhanced hydrophilic-hydrophobic balance leading to a more flexible structure, increasing solubility in aqueous solutions, and promoting emulsifying properties. These effects may lead to strong interactions at the air-water interface causing an increase in foaming capabilities. The outcomes of this study align with the findings on oat protein treated by cold plasma (Eazhumalai et al., 2023). This study revealed a positive correlation between FC and both the duration of plasma (30 min) and the voltage applied. In comparison to the control treatment, the CPC treated with ACP exhibited the highest FC, with a notable improvement ranging from 60.16% to 73% after treatment. ACP led to the partial unfolding of protein molecules at the interface between air and liquid, enhancing their ability to spread across the air-water interface and effectively enclose air bubbles. This process resulted in an improved foaming capacity. Simultaneously, pH-shifting also induced the exposure of hydrophobic protein groups. Compared to the control treatment, all the treated CPC samples showed improvements in FS (Fig 4.5B). Foaming properties of canola protein were increased at alkaline pH due to several factors one of which is the alkaline conditions can modify the electrical charge of the protein molecules, resulting in reduced repulsion between protein molecules. This allows them to come closer together and form a cohesive protein network that can trap and retain gas bubbles, leading to increased foaming properties. Different studies of canola protein isolate (CPI) by ultrasound treatment (Flores-Jiménez et al., 2019) showed similar results. The author reported that the foaming properties of CPI after ultrasound treatment at pH 4, 6 and 8 increased significantly compared to the control treatment Ultrasound treatment resulted in partial denaturation of proteins, creating better flexible protein structure in the solutions, and improving their association at the air-water interphase.

5. Conclusions

This is the first study to examine the effect of a combined pH shifting and ACP treatment on the antinutritional compounds of canola protein, mainly sinapine degradation and the other functional properties including solubility, emulsification, foaming capacity, and stability. The results indicate that the combined pH shifting and ACP treatment can be a useful method for canola protein functionality improvement. Canola protein concentrate solubility, emulsification, and foaming properties were enhanced by pH shifting followed by ACP treatment, particularly at alkaline conditions. This study broadens the scope of ACP technology for food protein applications. However, more research is required to determine the actual mechanism of sinapine degradation and the improvement of functional properties by combined pH shifting and ACP treatment.

4.4 References

Ahmadnia, M., Sadeghi, M., Abbaszadeh, R., Ghomi Marzdashti, H.R. 2021. Decontamination of whole strawberry via dielectric barrier discharge cold plasma and effects on quality attributes. *Journal of Food Processing and Preservation*, **45**(1), e15019.

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity–A practical and critical review. *Trends in food science & technology*, **22**(1), 21-39.

Aiking, H. 2011. Future protein supply. *Trends in Food Science & Technology*, **22**(2-3), 112-120.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Aschemann-Witzel, J., Gantriis, R.F., Fraga, P., Perez-Cueto, F.J. 2021. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**(18), 3119-3128.

Basak, S., Annapure, U.S. 2022. Recent trends in the application of cold plasma for the modification of plant proteins-A review. *Future Foods*, 100119.

Bhatty, R., McKenzie, S., Finlayson, A. 1968. The proteins of rapeseed (Brassica napus L.) soluble in salt solutions. *Canadian journal of biochemistry*, **46**(10), 1191-1197.

Bueno, A.S., Pereira, C.M., Menegassi, B., Arêas, J.A.G., Castro, I.A. 2009. Effect of extrusion on the emulsifying properties of soybean proteins and pectin mixtures modelled by response surface methodology. *Journal of food engineering*, **90**(4), 504-510.

Burgess, D.A. 1991. Purification, characterization, and the micelle response of the 12S canola globulin.

Cao, X., Liang, J., Aluko, R.E., Thiyam-Holländer, U. 2019. In situ oxidation of canola meal sinapic acid by horseradish peroxidase (type II) and tyrosinase. *Journal of Food Biochemistry*, **43**(6), e12884.

Capper, J.L. 2020. Opportunities and Challenges in Animal Protein Industry Sustainability: The Battle Between Science and Consumer Perception. *Animal Frontiers*, **10**(4), 7-13.

Carré, P., Pouzet, A. 2014. Rapeseed market, worldwide and in Europe. *Ocl*, **21**(1), D102.

Chang, C., Tu, S., Ghosh, S., Nickerson, M. 2015. Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, **77**, 360-367.

Chang, P.G., Gupta, R., Timilsena, Y.P., Adhikari, B. 2016. Optimisation of the complex coacervation between canola protein isolate and chitosan. *Journal of Food Engineering*, **191**, 58-66.

Chen, C., Liu, C., Jiang, A., Guan, Q., Sun, X., Liu, S., Hao, K., Hu, W. 2019. The effects of cold plasma-activated water treatment on the microbial growth and antioxidant properties of fresh-cut pears. *Food and Bioprocess Technology*, **12**(11), 1842-1851.

Cheng, H.-H., Chen, S.-S., Yoshizuka, K., Chen, Y.-C. 2012. Degradation of phenolic compounds in water by non-thermal plasma treatment. *Journal of Water Chemistry and Technology*, **34**(4), 179-189.

Campbell, L., Rempel, C.B., Wanasundara, J.P. 2016. Canola/rapeseed protein: Future opportunities and directions—Workshop proceedings of IRC 2015, MDPI.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Coffmann, C., Garciaj, V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, **12**(5), 473-484.

Coutinho, N.M., Silveira, M.R., Fernandes, L.M., Moraes, J., Pimentel, T.C., Freitas, M.Q., Silva, M.C., Raices, R.S., Ranadheera, C.S., Borges, F.O. 2019. Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry*, **278**, 276-283.

Croguennec, T., Renault, A., Bouhallab, S., Pezennec, S. 2006. Interfacial and foaming properties of sulfydryl-modified bovine β-lactoglobulin. *Journal of Colloid and Interface Science*, **302**(1), 32-39.

Cumby, N., Zhong, Y., Naczk, M., Shahidi, F. 2008. Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food chemistry*, **109**(1), 144-148.

de Castro, D.R.G., Mar, J.M., da Silva, L.S., da Silva, K.A., Sanches, E.A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F.A., Campelo, P.H. 2020. Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, **131**, 109044.

Dong, S., Gao, A., Xu, H., Chen, Y. 2017a. Effects of dielectric barrier discharges (DBD) cold plasma treatment on physicochemical and structural properties of zein powders. *Food and Bioprocess Technology*, **10**(3), 434-444.

Dong, S., Gao, A., Zhao, Y., Li, Y.-t., Chen, Y. 2017b. Characterization of physicochemical and structural properties of atmospheric cold plasma (ACP) modified zein. *Food and Bioproducts Processing*, **106**, 65-74.

Dong, S., Wang, J.-m., Cheng, L.-m., Lu, Y.-l., Li, S.-h., Chen, Y. 2017c. Behavior of zein in aqueous ethanol under atmospheric pressure cold plasma treatment. *Journal of agricultural and food chemistry*, **65**(34), 7352-7360.

Eaton, P. 2006. Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. *Free Radical Biology and Medicine*, **40**(11), 1889-1899.

Eazhumalai, G., Kalaivendan, R.G.T., Annapure, U.S. 2023. Effect of atmospheric pin-to-plate cold plasma on oat protein: Structural, chemical, and foaming characteristics. *International Journal of Biological Macromolecules*, 125103.

Fleddermann, M., Fechner, A., Rößler, A., Bähr, M., Pastor, A., Liebert, F., Jahreis, G. 2013. Nutritional evaluation of rapeseed protein compared to soy protein for quality, plasma amino acids, and nitrogen balance–a randomized cross-over intervention study in humans. *Clinical Nutrition*, **32**(4), 519-526.

Flores-Jiménez, N.T., Ulloa, J.A., Silvas, J.E.U., Ramírez, J.C.R., Ulloa, P.R., Rosales, P.U.B., Carrillo, Y.S., Leyva, R.G. 2019. Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (Brassica napus L.) protein isolate. *Food Research International*, **121**, 947-956.

Fox, P. 1989. The milk protein system. *Developments in dairy chemistry. 4. Functional milk proteins.*, 1-53.

Fujihara, S., Kasuga, A., Aoyagi, Y. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science*, **66**(3), 412-415.

Friedman, M., Jürgens, H.S. 2000. Effect of pH on the stability of plant phenolic compounds. *Journal of agricultural and food chemistry*, **48**(6), 2101-2110.

Garofulić, I.E., Jambrak, A.R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., Herceg, Z. 2015. The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (Prunus cerasus var. Marasca) juice. *LWT-Food Science and Technology*, **62**(1), 894-900.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food bioscience*, **12**, 128-138.

Gurol, M.D., Vatistas, R. 1987. Oxidation of phenolic compounds by ozone and ozone + u.v. radiation: A comparative study. *Water Research*, **21**(8), 895-900.

Haris, P.I., Severcan, F. 1999. FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. *Journal of Molecular Catalysis B: Enzymatic*, **7**(1-4), 207-221.

Hristov, A., Domitrovich, C., Wachter, A., Cassidy, T., Lee, C., Shingfield, K., Kairenius, P., Davis, J., Brown, J. 2011. Effect of replacing solvent-extracted canola meal with high-oil traditional canola, high-oleic acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. *Journal of dairy science*, **94**(8), 4057-4074.

Hüsken, A., Baumert, A., Milkowski, C., Becker, H.C., Strack, D., Möllers, C. 2005. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (Brassica napus L.). *Theoretical and Applied Genetics*, **111**, 1553-1562.

Ismail, F., Vaisey-Genser, M., Fyfe, B. 1981. Bitterness and astringency of sinapine and its components. *Journal of Food Science*, **46**(4), 1241-1244.

Itkonen, S.T., Päivärinta, E., Pellinen, T., Viitakangas, H., Risteli, J., Erkkola, M., Lamberg-Allardt, C., Pajari, A.-M. 2021. Partial replacement of animal proteins with plant proteins for 12 weeks accelerates bone turnover among healthy adults: A randomized clinical trial. *The Journal of Nutrition*, **151**(1), 11-19.

Jensen, S., Olsen, H., Sørensen, H. 1990. Aqueous enzymatic processing of rapeseed for production of high quality products. *Canola and rapeseed: production, chemistry, nutrition and processing technology*, 331-343.

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., Chen, Y. 2018. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, **11**(2), 344-354.

Ji, H., Han, F., Peng, S., Yu, J., Li, L., Liu, Y., Chen, Y., Li, S., Chen, Y. 2019. Behavioral solubilization of peanut protein isolate by atmospheric pressure cold plasma (ACP) treatment. *Food and bioprocess technology*, **12**(12), 2018-2027.

Jiang, J., Wang, Q., Xiong, Y.L. 2018. A pH shift approach to the improvement of interfacial properties of plant seed proteins. *Current opinion in food science*, **19**, 50-56.

Jiang, J., Xiong, Y.L., Chen, J. 2010. pH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions. *Journal of agricultural and food chemistry*, **58**(13), 8035-8042.

Jiang, S., Ding, J., Andrade, J., Rababah, T.M., Almajwal, A., Abulmeaty, M.M., Feng, H. 2017. Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics Sonochemistry*, **38**, 835-842.

Karaca, A.C., Low, N., Nickerson, M. 2011. Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International*, **44**(9), 2991-2998.

Kozlowska, H., Naczk, M., Shahidi, F., Zadernowski, R. 1990. Phenolic acids and tannins in rapeseed and canola. *Canola and rapeseed*, 193-210.

Kristinsson, H.G., Hultin, H.O. 2003. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. *Journal of Agricultural and Food Chemistry*, **51**(24), 7187-7196.

Krygier, K., Sosulski, F., Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, **30**(2), 330-334.

Law, H.-Y., Choi, S.-M., Ma, C.-Y. 2008. Study of conformation of vicilin from Dolichos lablab and Phaseolus calcaratus by Fourier-transform infrared spectroscopy and differential scanning calorimetry. *Food research international*, **41**(7), 720-729.

Li, M., Li, X., Han, C., Ji, N., Jin, P., Zheng, Y. 2019. Physiological and metabolomic analysis of cold plasma treated fresh-cut strawberries. *Journal of agricultural and food chemistry*, **67**(14), 4043-4053.

Li, Y., Cheng, Y., Zhang, Z., Wang, Y., Mintah, B.K., Dabbour, M., Jiang, H., He, R., Ma, H. 2020. Modification of rapeseed protein by ultrasound-assisted pH shift treatment: Ultrasonic mode and frequency screening, changes in protein solubility and structural characteristics. *Ultrasonics Sonochemistry*, **69**, 105240.

Lima, R.R., Stephani, R., Perrone, Í.T., de Carvalho, A.F. 2023. Plant-based proteins: A review of factors modifying the protein structure and affecting emulsifying properties. *Food Chemistry Advances*, 100397.

Lukes, P., Locke, B.R. 2005. Plasmachemical oxidation processes in a hybrid gas–liquid electrical discharge reactor. *Journal of Physics D: Applied Physics*, **38**(22), 4074-4081.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Malabat, C., nchez-Vioque, R.I., Rabiller, C., Gu guen, J. 2001. Emulsifying and foaming properties of native and chemically modified peptides from the 2S and 12S proteins of rapeseed (Brassica napus L.). *Journal of the American Oil Chemists' Society*, **78**(3), 235-242.

Mann, B., Malik, R. 1996. Studies on some functional characteristics of whey protein-polysaccharide complex. *Journal of food science and technology (Mysore)*, **33**(3), 202-206.

Mansour, E., Dworschák, E., Lugasi, A., Gaál, Ö., Barna, É., Gergely, A. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food chemistry*, **47**(3), 247-252.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Mills, J., Chong, J. 1977. Ultrastructure and mineral distribution in heat-damaged rapeseed. *Canadian Journal of Plant Science*, **57**(1), 21-30.

Mupondwa, E., Li, X., Wanasundara, J.P. 2018. Technoeconomic prospects for commercialization of Brassica (cruciferous) plant proteins. *Journal of the American Oil Chemists' Society*, **95**(8), 903-922.

Momen, S., Alavi, F., Aider, M. 2021. Alkali-mediated treatments for extraction and functional modification of proteins: Critical and application review. *Trends in Food Science & Technology*, **110**, 778-797.

Naczk, M., Amarowicz, R., Shahidi, F. 1998. Role of phenolics in flavor of rapeseed protein products. in: *Developments in food science*, Vol. 40, Elsevier, pp. 597-613.

Noshad, M., Mohebbi, M., Koocheki, A., Shahidi, F. 2016. Influence of interfacial engineering on stability of emulsions stabilized with soy protein isolate. *Journal of Dispersion Science and Technology*, **37**(1), 56-65.

Ostrowska, A., Kozlowska, M., Rachwal, D., Wnukowski, P., Nebesny, E., Rosicka-Kaczmarek, J. 2018. Rapeseed protein-fibre concentrate: Chemical composition and functional properties. *Żywność Nauka Technologia Jakość*, **25**(4).

Pirestani, S., Nasirpour, A., Keramat, J., Desobry, S. 2017. Preparation of chemically modified canola protein isolate with gum Arabic by means of Maillard reaction under wet-heating conditions. *Carbohydrate Polymers*, **155**, 201-207.

Pojić, M., Mišan, A., Tiwari, B. 2018. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology*, **75**, 93-104.

Poore, J., Nemecek, T. 2018. Reducing food's environmental impacts through producers and consumers. *Science*, **360**(6392), 987-992.

Prakash, V., Narasinga Rao, M. 1986. Physicochemical properties of oilseed protein. *Critical Reviews in Biochemistry*, **20**(3), 265-363.

Rout, S., Srivastav, P.P. 2023. Effect of cold plasma on the technological and functional modification of plant proteins and enzymes. Innovative Food Science & Emerging Technologies, 103447.

Sarangapani, C., O'Toole, G., Cullen, P., Bourke, P. 2017. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science & Emerging Technologies*, **44**, 235-241.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Sharafodin, H., Soltanizadeh, N. 2022. Potential application of DBD Plasma Technique for modifying structural and physicochemical properties of Soy Protein Isolate. *Food Hydrocolloids*, **122**, 107077.

Sharifian, A., Soltanizadeh, N., Abbaszadeh, R. 2019. Effects of dielectric barrier discharge plasma on the physicochemical and functional properties of myofibrillar proteins. *Innovative food science & emerging technologies*, **54**, 1-8.

Shi, W., Dumont, M.-J. 2014. Processing and physical properties of canola protein isolate-based films. *Industrial Crops and Products*, **52**, 269-277.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Sultan, M.T., Saddique, M.S., Shahid, M. 2020a. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Tauseef, M., Sultan, M.S.S., Shahid, M. 2020b. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Song, L., Thornalley, P.J. 2007. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and chemical toxicology*, **45**(2), 216-224.

Sugiarto, A.T., Sato, M. 2001. Pulsed plasma processing of organic compounds in aqueous solution. *Thin Solid Films*, **386**(2), 295-299.

Sullivan, G.A., Sebranek, J.G. 2012. Nitrosylation of myoglobin and nitrosation of cysteine by nitrite in a model system simulating meat curing. *Journal of agricultural and food chemistry*, **60**(7), 1748-1754.

Sun, W., Zhou, F., Sun, D.-W., Zhao, M. 2013. Effect of oxidation on the emulsifying properties of myofibrillar proteins. *Food and Bioprocess Technology*, **6**(7), 1703-1712.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011a. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011b. Extraction and characterization of protein fractions from Australian canola meals. *Food Research International*, **44**(4), 1075-1082.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O., Day, L. 2014. Gelling properties of protein fractions and protein isolate extracted from Australian canola meal. *Food research international*, **62**, 819-828.

Thompson, L.U., Serraino, M.R. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. *Journal of agricultural and food chemistry*, **34**(3), 468-469.

Tomé, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements—functional aspects. *Food and nutrition bulletin*, **34**(2), 272-274.

Torres, B., Tiwari, B., Patras, A., Wijngaard, H.H., Brunton, N., Cullen, P., O'Donnell, C. 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. *Food Chemistry*, **124**(3), 721-726.

Uppström, B., Svensson, R. 1980. Determination of phytic acid in rapeseed meal. *Journal of the Science of Food and Agriculture*, **31**(7), 651-656.

Van de Noort, M. 2017. Lupin: An important protein and nutrient source. in: *Sustainable protein sources*, Elsevier, pp. 165-183.

Velasco, L., Möllers, C. 1998. Nondestructive assessment of sinapic acid esters in Brassica species: II. Evaluation of germplasm and identification of phenotypes with reduced levels. *Crop Science*, **38**(6), 1650-1654.

Venkataratnam, H., Sarangapani, C., Cahill, O., Ryan, C.B. 2019. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, **52**, 368-375.

Von Der Haar, D., Müller, K., Bader-Mittermaier, S., Eisner, P. 2014. Rapeseed proteins–Production methods and possible application ranges. *Ocl*, **21**(1), D104.

Vuorela, S. 2005. Analysis, isolation, and bioactivities of rapeseed phenolics.

Wanapat, M., Huang, B., Viennasay, B., Totakul, P., Matra, M., Phesatcha, B., Ampapon, T. 2020. Rapeseed pod meal can replace concentrate and enhance utilization of feed on in vitro gas production and fermentation characteristics. *Tropical Animal Health and Production*, **52**(5), 2593-2598.

Wanasundara, J., McIntosh, T., Perera, S., Withana-Gamage, T., Mitra, P. 2016a. Canola/rapeseed protein-functionality and nutrition. OCL 23. *D407*.

Wanasundara, J., Tan, S., Alashi, A., Pudel, F., Blanchard, C. 2017. Proteins from canola/rapeseed: Current status. in: *Sustainable protein sources*, Elsevier, pp. 285-304.

Wanasundara, J.P. 2011. Proteins of Brassicaceae oilseeds and their potential as a plant protein source. *Critical reviews in* food *science and nutrition*, **51**(7), 635-677.

Wanasundara, J.P., Abeysekara, S.J., McIntosh, T.C., Falk, K.C. 2012. Solubility differences of major storage proteins of Brassicaceae oilseeds. *Journal of the American Oil Chemists' Society*, **89**, 869-881.

Wanasundara, J.P., McIntosh, T.C., Perera, S.P., Withana-Gamage, T.S., Mitra, P. 2016b. Canola/rapeseed protein-functionality and nutrition. *OCl*, **23**(4), D407.

Wang, Z., Li, Y., Jiang, L., Qi, B., Zhou, L. 2014. Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry*, **2014**.

Wong, D.W., Camirand, W.M., Pavlath, A.E., Parris, N., Friedman, M. 1996. Structures and functionalities of milk proteins. *Critical Reviews in Food Science & Nutrition*, **36**(8), 807-844.

Wu, J., Aluko, R.E., Muir, A.D. 2008. Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food chemistry*, **111**(4), 942-950.

Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.

Yang, J., Liu, G., Zeng, H., Chen, L. 2018. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. *Food Hydrocolloids*, **83**, 275-286.

Yanjun, S., Jianhang, C., Shuwen, Z., Hongjuan, L., Jing, L., Lu, L., Uluko, H., Yanling, S., Wenming, C., Wupeng, G. 2014. Effect of power ultrasound pre-treatment on the physical and functional properties of reconstituted milk protein concentrate. *Journal of Food Engineering*, **124**, 11-18.

Yi, Z., Jia-qing, X., Ci, L., Jun, L., Hong-li, S. 2008. Mechanism of Oxidation of Aqueous Phenol by H2O2 Catalysed by a Macrocyclic Iron(II) Complex. *Progress in Reaction Kinetics and Mechanism*, **33**(3), 241-252.

Yiu, S., Poon, H., Fulcher, R., Altosaar, I. 1982. The microscopic structure and chemistry of rapeseed and its products. *Food Structure*, **1**(2), 4.

Yoon, J.H., Thompson, L.U., Jenkins, D. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *The American journal of clinical nutrition*, **38**(6), 835-842.

Zhang, D., Li, H., Emara, A., Hu, Y., Wang, Z., Wang, M., He, Z. 2020. Effect of in vitro oxidation on the water retention mechanism of myofibrillar proteins gel from pork muscles. *Food Chemistry*, **315**, 126226.

ZHANG, J., CHEN, J., LI, X. 2009. Remove of Phenolic Compounds in Water by Low-Temperature Plasma: A Review of Current Research. *Journal of Water Resource and Protection*, **Vol.01No.02**, 11.

Zhang, Q.-T., Tu, Z.-C., Xiao, H., Wang, H., Huang, X.-Q., Liu, G.-X., Liu, C.-M., Shi, Y., Fan, L.-L., Lin, D.-R. 2014. Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. *Food and Bioproducts Processing*, **92**(1), 30-37.

Zhang, Q., Cheng, Z., Zhang, J., Nasiru, M.M., Wang, Y., Fu, L. 2021a. Atmospheric cold plasma treatment of soybean protein isolate: Insights into the structural, physicochemical, and allergenic characteristics. *Journal of Food Science*, **86**(1), 68-77.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021b. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

Zhang, S., Huang, W., Roopesh, M., Chen, L. 2022. Pre-treatment by combining atmospheric cold plasma and pH-shifting to prepare pea protein concentrate powders with improved gelling properties. *Food Research International*, **154**, 111028.

Zum Felde, T., Baumert, A., Strack, D., Becker, H., Möllers, C. 2007. Genetic variation for sinapate ester content in winter rapeseed (Brassica napus L.) and development of NIRS calibration equations. *Plant Breeding*, **126**(3), 291-296.

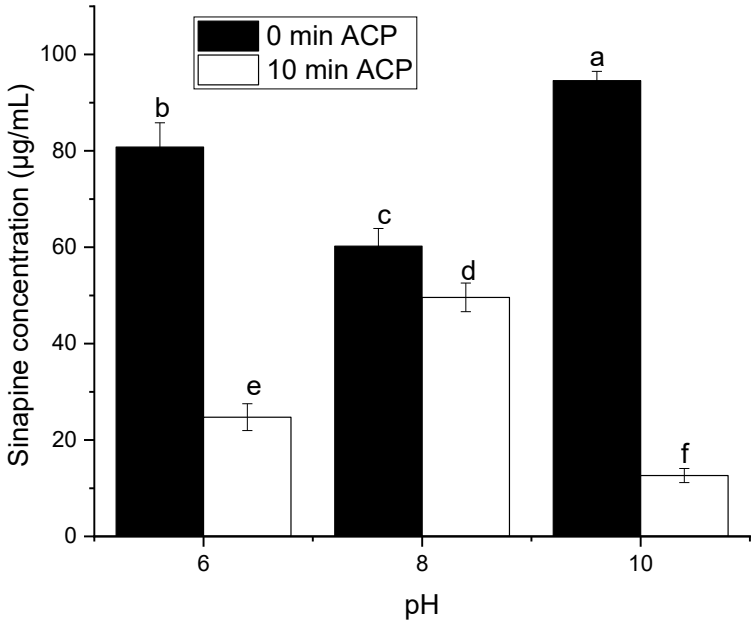


Figure 4.1. Influence of ACP treatment followed by pH shifting to selected pH on sinapine concentration. Values with different letters are significantly different ($p<0.05$, $n\geq3$).

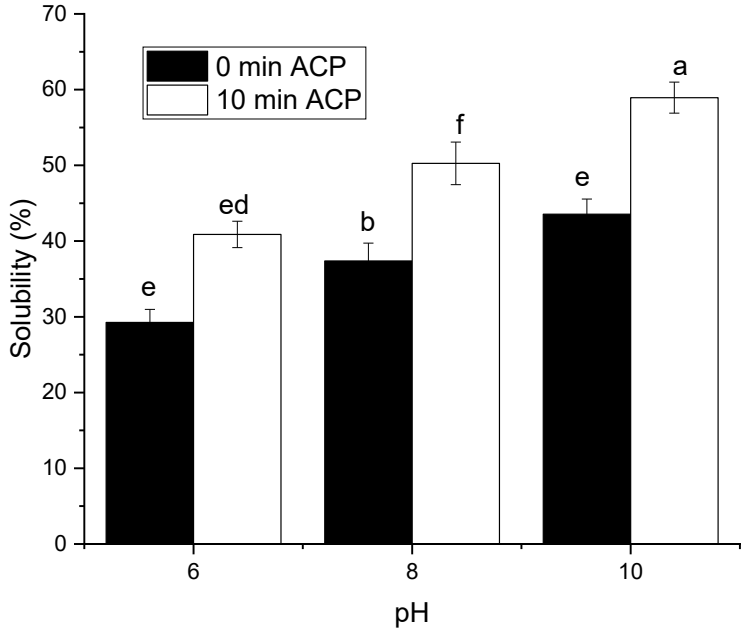


Figure 4.2. Influence of ACP treatment followed by pH shifting to selected pH on the canola protein concentrate solubility. Values with different letters are significantly different ($p<0.05$, $n\geq3$).

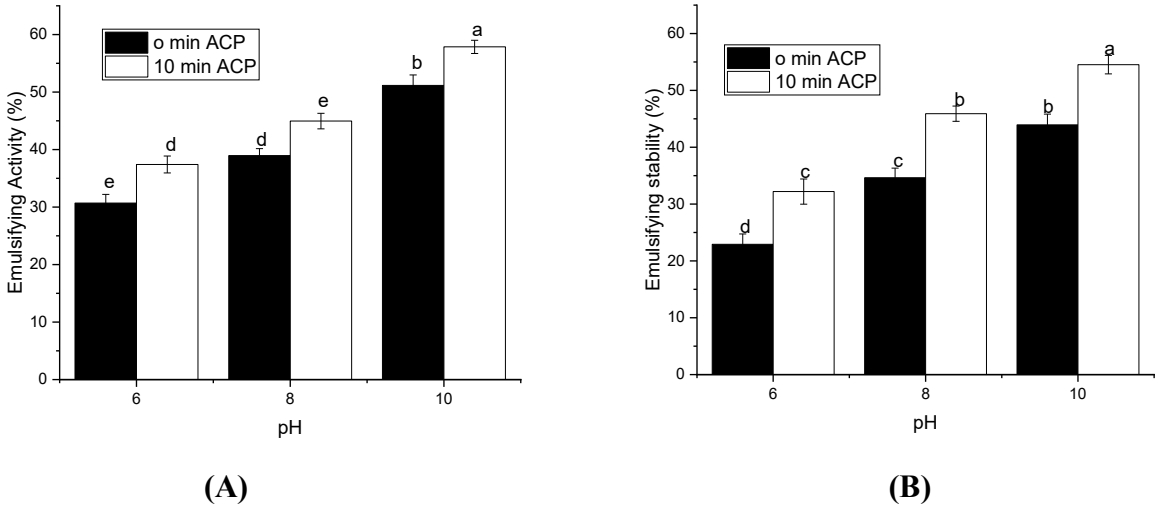
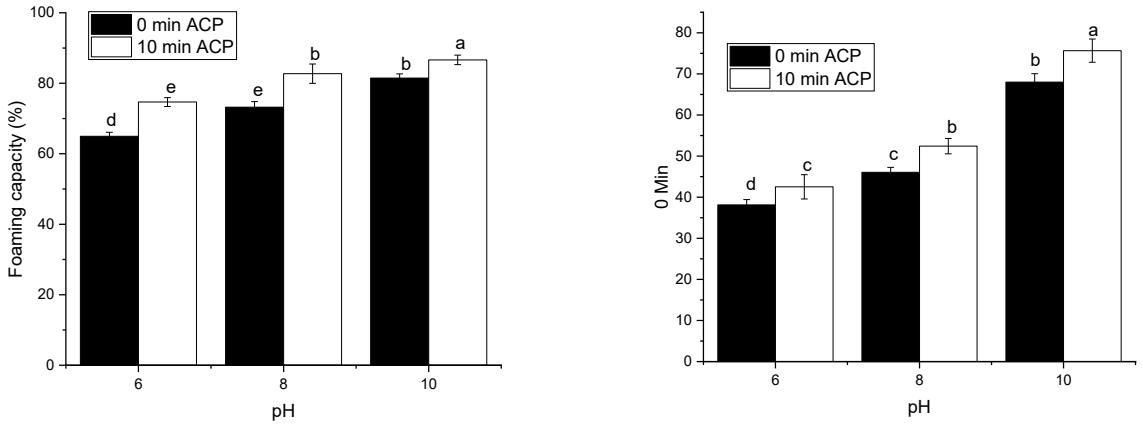


Figure 4.3 (A) Influence of ACP treatment followed by pH shifting on the emulsion activity and (B) emulsion stability of canola protein. Values with different letters are considered significantly different ($p<0.05$, $n\geq3$)



(A) **(B)**

Figure 4.4. (A) Influence of ACP treatment followed by pH shifting on the foaming capacity and (B) foaming stability of canola protein. Values with different letters are considered significantly different ($p<0.05$, $n\geq3$)

Chapter 5: Conclusion and Recommendation

5.1 Overall conclusion

In this research, atmospheric cold plasma (ACP) was used to degrade the antinutritional phenolic compounds from canola protein concentrate (CPC) and improve its functional properties. There are limited number of studies in the literature regarding the enhancement of various functional properties in canola protein through non-thermal treatments. This study is unique as it focused on the utilization of ACP to enhance the functional properties of canola protein. In addition, no study has been conducted so far on the degradation of antinutritional phenolic compounds from canola protein using ACP treatment. The reduction of antinutritional phenolic compounds in canola protein through pH shifting and ACP presents a promising approach to enhance its safety and nutritional quality. By mitigating the presence of these compounds, which can inhibit nutrient absorption or contribute to the adverse health effects, the suitability of canola protein for human consumption is improved.

In chapter 3 of this study, the total phenolic compounds in canola protein were decreased after 5 and 10 min ACP treatment. For instance, the concentration of total phenolic compounds decreased from 66.9 to 17.6 μg/mL after 10 min of ACP treatment.

The solubility and functional properties of canola protein after ACP treatment were studied. This result showed that the solubility improvement in CPC after ACP treatment was pH dependent. The ACP treatment promoted exposure of hydrophobic groups, leading to enhancement in functional properties like emulsification and foaming properties. For instance, the maximum solubility of CPC at pH 12 was 71.8% after 10 min of ACP treatment.

Further, the ACP was able to significantly improve the emulsification activity and emulsification stability. Short-term ACP treatment can modify the protein structure and make it more flexible and capable of producing films at the oil-water or air-water interface, which has a good impact on the emulsifying and foaming properties. Fourier transform infrared spectroscopy revealed that the ACP treatment decreased the α-helix but increased the β-sheets and random coil by protein denaturation which may explain its higher adsorption at the oil-water interface.

In chapter 4 of this study, the aim was to evaluate the combined effect of pH shifting and ACP to improve the functional properties by degrading the antinutritional compounds from CPC, without increasing the ACP treatment time. Changing the pH prior to ACP treatment significantly improved the solubility of the CPC. The maximum solubility was observed at pH 10 after 10 min ACP treatment. The solubility of the canola protein concentrate increased with increasing the pH before ACP treatment. The ACP treatment with pH shifting significantly improved the emulsification activity and stability at pH 6, 8 and 10. Possibly the electrostatic repulsion, partial unfolding, and degradation of protein aggregates by ACP had a positive effect on emulsification activity and emulsification stability.

Furthermore, ACP with pH shifting treatment significantly reduced the antinutritional sinapine concentration, most probably by promoting protein solubility and decoupling of sinapine from protein.

Future recommendations

There are different areas that can be further investigated in future:

1) The preliminary antinutritional phenolic compound degradation was performed in this study. However, some other undesirable antinutritional compounds like glucosinolates, phytates, and fibres need to be degraded because these compounds can influence the

functional properties and hence the utilization of canola protein for food application. The effect of ACP on the degradation of these antinutritional compounds needs to be investigated.

2) More understanding on the phenolic degradation mechanisms with the combined effect of ACP and pH shifting treatment is required.
3) Investigation on the digestibility of canola protein concentrate after ACP treatment, as the antinutritional compounds can be responsible for the reduced digestibility.
4) The structural properties of canola protein concentrate will need to be studied to determine how the combined effect of ACP with pH shifting changes the protein morphology, its molecular size, and the hydrophilic-hydrophobic balance.

Bibliography

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity–A practical and critical review. *Trends in food science & technology*, **22**(1), 21-39.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Alashi, A.M., Blanchard, C.L., Mailer, R.J., Agboola, S.O. 2013. Technological and bioactive functionalities of canola meal proteins and hydrolysates. *Food Reviews International*, **29**(3), 231-260.

Aiking, H. 2011. Future protein supply. *Trends in Food Science & Technology*, **22**(2-3), 112-120.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Aschemann-Witzel, J., Gantriis, R.F., Fraga, P., Perez-Cueto, F.J. 2021. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**(18), 3119-3128.

Ahmadnia, M., Sadeghi, M., Abbaszadeh, R., Ghomi Marzdashti, H.R. 2021. Decontamination of whole strawberry via dielectric barrier discharge cold plasma and effects on quality attributes. *Journal of Food Processing and Preservation*, **45**(1), e15019.

Aider, M., Barbana, C. 2011. Canola proteins: composition, extraction, functional properties, bioactivity, applications as a food ingredient and allergenicity–A practical and critical review. *Trends in food science & technology*, **22**(1), 21-39.

Aiking, H. 2011. Future protein supply. *Trends in Food Science & Technology*, **22**(2-3), 112-120.

Akharume, F.U., Aluko, R.E., Adedeji, A.A. 2021. Modification of plant proteins for improved functionality: A review. *Comprehensive Reviews in Food Science and Food Safety*, **20**(1), 198-224.

Aschemann-Witzel, J., Gantriis, R.F., Fraga, P., Perez-Cueto, F.J. 2021. Plant-based food and protein trend from a business perspective: markets, consumers, and the challenges and opportunities in the future. *Critical Reviews in Food Science and Nutrition*, **61**(18), 3119-3128.

Bhatty, R., McKenzie, S., Finlayson, A. 1968. The proteins of rapeseed (Brassica napus L.) soluble in salt solutions. *Canadian journal of biochemistry*, **46**(10), 1191-1197.

Brandenburg, R., Ehlbeck, J., Stieber, M., v. Woedtke, T., Zeymer, J., Schlüter, O., Weltmann, K.D. 2007. Antimicrobial treatment of heat sensitive materials by means of atmospheric pressure Rf-driven plasma jet. *Contributions to Plasma Physics*, **47**(1-2), 72-79.

Burgess, D.A. 1991. Purification, characterization, and the micelle response of the 12S canola globulin.

Basak, S., Annapure, U.S. 2022. Recent trends in the application of cold plasma for the modification of plant proteins-A review. *Future Foods*, 100119.

Bhatty, R., McKenzie, S., Finlayson, A. 1968. The proteins of rapeseed (Brassica napus L.) soluble in salt solutions. *Canadian journal of biochemistry*, **46**(10), 1191-1197.

Bueno, A.S., Pereira, C.M., Menegassi, B., Arêas, J.A.G., Castro, I.A. 2009. Effect of extrusion on the emulsifying properties of soybean proteins and pectin mixtures modelled by response surface methodology. *Journal of food engineering*, **90**(4), 504-510.

Burgess, D.A. 1991. Purification, characterization, and the micelle response of the 12S canola globulin

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Caessens, P.W., De Jongh, H.H., Norde, W., Gruppen, H. 1999. The adsorption-induced secondary structure of β-casein and of distinct parts of its sequence in relation to foam and emulsion properties. *Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology*, **1430**(1), 73-83.

Capper, J.L. 2020. Opportunities and Challenges in Animal Protein Industry Sustainability: The Battle Between Science and Consumer Perception. *Animal Frontiers*, **10**(4), 7-13.

Chang, C., Tu, S., Ghosh, S., Nickerson, M. 2015. Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, **77**, 360-367.

Chen, C., Liu, C., Jiang, A., Guan, Q., Sun, X., Liu, S., Hao, K., Hu, W. 2019. The effects of cold plasma-activated water treatment on the microbial growth and antioxidant properties of fresh-cut pears. *Food and Bioprocess Technology*, **12**(11), 1842-1851

Coutinho, N.M., Silveira, M.R., Fernandes, L.M., Moraes, J., Pimentel, T.C., Freitas, M.Q., Silva, M.C., Raices, R.S., Ranadheera, C.S., Borges, F.O. 2019. Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry*, **278**, 276-283

Chang, P.G., Gupta, R., Timilsena, Y.P., Adhikari, B. 2016. Optimisation of the complex coacervation between canola protein isolate and chitosan. *Journal of Food Engineering*, **191**, 58-66.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Coffmann, C., Garciaj, V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, **12**(5), 473-484.

Cao, X., Liang, J., Aluko, R.E., Thiyam-Holländer, U. 2019. In situ oxidation of canola meal sinapic acid by horseradish peroxidase (type II) and tyrosinase. *Journal of Food Biochemistry*, **43**(6), e12884.

Cheng, H.-H., Chen, S.-S., Yoshizuka, K., Chen, Y.-C. 2012. Degradation of phenolic compounds in water by non-thermal plasma treatment. *Journal of Water Chemistry and Technology*, **34**(4), 179-189.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Cao, X., Liang, J., Aluko, R.E., Thiyam-Holländer, U. 2019. In situ oxidation of canola meal sinapic acid by horseradish peroxidase (type II) and tyrosinase. *Journal of Food Biochemistry*, **43**(6), e12884.

Capper, J.L. 2020. Opportunities and Challenges in Animal Protein Industry Sustainability: The Battle Between Science and Consumer Perception. *Animal Frontiers*, **10**(4), 7-13.

Carré, P., Pouzet, A. 2014. Rapeseed market, worldwide and in Europe. *Ocl*, **21**(1), D102.

Chang, C., Tu, S., Ghosh, S., Nickerson, M. 2015. Effect of pH on the inter-relationships between the physicochemical, interfacial and emulsifying properties for pea, soy, lentil and canola protein isolates. *Food Research International*, **77**, 360-367.

Chang, P.G., Gupta, R., Timilsena, Y.P., Adhikari, B. 2016. Optimisation of the complex coacervation between canola protein isolate and chitosan. *Journal of Food Engineering*, **191**, 58-66.

Chen, C., Liu, C., Jiang, A., Guan, Q., Sun, X., Liu, S., Hao, K., Hu, W. 2019. The effects of cold plasma-activated water treatment on the microbial growth and antioxidant properties of fresh-cut pears. *Food and Bioprocess Technology*, **12**(11), 1842-1851.

Cheng, H.-H., Chen, S.-S., Yoshizuka, K., Chen, Y.-C. 2012. Degradation of phenolic compounds in water by non-thermal plasma treatment. *Journal of Water Chemistry and Technology*, **34**(4), 179-189.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, **61**(22), 3836-3856.

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., Rosicka-Kaczmarek, J. 2020. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition*, 1-21.

Coffmann, C., Garciaj, V. 1977. Functional properties and amino acid content of a protein isolate from mung bean flour. *International Journal of Food Science & Technology*, **12**(5), 473-484.

Coutinho, N.M., Silveira, M.R., Fernandes, L.M., Moraes, J., Pimentel, T.C., Freitas, M.Q., Silva, M.C., Raices, R.S., Ranadheera, C.S., Borges, F.O. 2019. Processing chocolate milk drink by low-pressure cold plasma technology. *Food Chemistry*, **278**, 276-283.

Croguennec, T., Renault, A., Bouhallab, S., Pezennec, S. 2006. Interfacial and foaming properties of sulfydryl-modified bovine β-lactoglobulin. *Journal of Colloid and Interface Science*, **302**(1), 32-39.

Cumby, N., Zhong, Y., Naczk, M., Shahidi, F. 2008. Antioxidant activity and water-holding capacity of canola protein hydrolysates. *Food chemistry*, **109**(1), 144-148.

Dong, S., Gao, A., Zhao, Y., Li, Y.-t., Chen, Y. 2017. Characterization of physicochemical and structural properties of atmospheric cold plasma (ACP) modified zein. *Food and Bioproducts Processing*, **106**, 65-74.

Dong, S., Gao, A., Xu, H., Chen, Y. 2017a. Effects of dielectric barrier discharges (DBD) cold plasma treatment on physicochemical and structural properties of zein powders. *Food and Bioprocess Technology*, **10**(3), 434-444.

de Castro, D.R.G., Mar, J.M., da Silva, L.S., da Silva, K.A., Sanches, E.A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F.A., Campelo, P.H. 2020. Dielectric barrier atmospheric cold

plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, **131**, 109044.

Dong, S., Gao, A., Zhao, Y., Li, Y.-t., Chen, Y. 2017b. Characterization of physicochemical and structural properties of atmospheric cold plasma (ACP) modified zein. *Food and Bioproducts Processing*, **106**, 65-74.

de Castro, D.R.G., Mar, J.M., da Silva, L.S., da Silva, K.A., Sanches, E.A., de Araújo Bezerra, J., Rodrigues, S., Fernandes, F.A., Campelo, P.H. 2020. Dielectric barrier atmospheric cold plasma applied on camu-camu juice processing: Effect of the excitation frequency. *Food Research International*, **131**, 109044.

Dong, S., Wang, J.-m., Cheng, L.-m., Lu, Y.-l., Li, S.-h., Chen, Y. 2017c. Behavior of zein in aqueous ethanol under atmospheric pressure cold plasma treatment. *Journal of agricultural and food chemistry*, **65**(34), 7352-7360.

Eaton, P. 2006. Protein thiol oxidation in health and disease: techniques for measuring disulfides and related modifications in complex protein mixtures. *Free Radical Biology and Medicine*, **40**(11), 1889-1899.

Feizollahi, E., N.N. Misra., & M. S. Roopesh (2021) Factors influencing the antimicrobial efficacy of Dielectric Barrier Discharge (DBD) Atmospheric Cold Plasma (ACP) in food processing applications, Critical Reviews in Food Science and Nutrition, 61:4, 666-689, DOI: 10.1080/10408398.2020.1743967

Flores-Jiménez, N.T., Ulloa, J.A., Silvas, J.E.U., Ramírez, J.C.R., Ulloa, P.R., Rosales, P.U.B., Carrillo, Y.S., Leyva, R.G. 2019. Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (Brassica napus L.) protein isolate. *Food Research International*, **121**, 947-956.

Fox, P. 1989. The milk protein system. *Developments in dairy chemistry. 4. Functional milk proteins.*, 1-53.

Fujihara, S., Kasuga, A., Aoyagi, Y. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science*, **66**(3), 412-415.

Fleddermann, M., Fechner, A., Rößler, A., Bähr, M., Pastor, A., Liebert, F., Jahreis, G. 2013. Nutritional evaluation of rapeseed protein compared to soy protein for quality, plasma amino acids, and nitrogen balance–a randomized cross-over intervention study in humans. *Clinical Nutrition*, **32**(4), 519-526.

Flores-Jiménez, N.T., Ulloa, J.A., Silvas, J.E.U., Ramírez, J.C.R., Ulloa, P.R., Rosales, P.U.B., Carrillo, Y.S., Leyva, R.G. 2019. Effect of high-intensity ultrasound on the compositional, physicochemical, biochemical, functional and structural properties of canola (Brassica napus L.) protein isolate. *Food Research International*, **121**, 947-956.

Fox, P. 1989. The milk protein system. *Developments in dairy chemistry. 4. Functional milk proteins.*, 1-53.

Fujihara, S., Kasuga, A., Aoyagi, Y. 2001. Nitrogen-to-protein conversion factors for common vegetables in Japan. *Journal of Food Science*, **66**(3), 412-415.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food Bioscience*, **12**, 128-138.

Garofulić, I.E., Jambrak, A.R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., Herceg, Z. 2015. The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (Prunus cerasus var. Marasca) juice. *LWT-Food Science and Technology*, **62**(1), 894-900.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food bioscience*, **12**, 128-138.

Grzegorzewski, F., Ehlbeck, J., Schlüter, O., Kroh, L.W., Rohn, S. 2011. Treating lamb's lettuce with a cold plasma–Influence of atmospheric pressure Ar plasma immanent species on the phenolic profile of Valerianella locusta. *LWT-Food Science and Technology*, **44**(10), 2285-2289.

Gurol, M.D., Vatistas, R. 1987. Oxidation of phenolic compounds by ozone and ozone + u.v. radiation: A comparative study. *Water Research*, **21**(8), 895-900.

Garofulić, I.E., Jambrak, A.R., Milošević, S., Dragović-Uzelac, V., Zorić, Z., Herceg, Z. 2015. The effect of gas phase plasma treatment on the anthocyanin and phenolic acid content of sour cherry Marasca (Prunus cerasus var. Marasca) juice. *LWT-Food Science and Technology*, **62**(1), 894-900.

Gerzhova, A., Mondor, M., Benali, M., Aider, M. 2015. Study of the functional properties of canola protein concentrates and isolates extracted by electro-activated solutions as non-invasive extraction method. *Food bioscience*, **12**, 128-138.

Gurol, M.D., Vatistas, R. 1987. Oxidation of phenolic compounds by ozone and ozone + u.v. radiation: A comparative study. *Water Research*, **21**(8), 895-900.

Hüsken, A., Baumert, A., Milkowski, C., Becker, H.C., Strack, D., Möllers, C. 2005. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (Brassica napus L.). *Theoretical and Applied Genetics*, **111**, 1553-1562.

Hristov, A., Domitrovich, C., Wachter, A., Cassidy, T., Lee, C., Shingfield, K., Kairenius, P., Davis, J., Brown, J. 2011. Effect of replacing solvent-extracted canola meal with high-oil traditional canola, high-oleic acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. *Journal of dairy science*, **94**(8), 4057-4074.

Haris, P.I., Severcan, F. 1999. FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. *Journal of Molecular Catalysis B: Enzymatic*, **7**(1-4), 207-221.

Hristov, A., Domitrovich, C., Wachter, A., Cassidy, T., Lee, C., Shingfield, K., Kairenius, P., Davis, J., Brown, J. 2011. Effect of replacing solvent-extracted canola meal with high-oil traditional canola, high-oleic acid canola, or high-erucic acid rapeseed meals on rumen fermentation, digestibility, milk production, and milk fatty acid composition in lactating dairy cows. *Journal of dairy science*, **94**(8), 4057-4074.

Hüsken, A., Baumert, A., Milkowski, C., Becker, H.C., Strack, D., Möllers, C. 2005. Resveratrol glucoside (Piceid) synthesis in seeds of transgenic oilseed rape (Brassica napus L.). *Theoretical and Applied Genetics*, **111**, 1553-1562.

Ismail, F., Vaisey-Genser, M., Fyfe, B. 1981. Bitterness and astringency of sinapine and its components. *Journal of Food Science*, **46**(4), 1241-1244.

Itkonen, S.T., Päivärinta, E., Pellinen, T., Viitakangas, H., Risteli, J., Erkkola, M., Lamberg-Allardt, C., Pajari, A.-M. 2021. Partial replacement of animal proteins with plant proteins for 12 weeks accelerates bone turnover among healthy adults: A randomized clinical trial. *The Journal of Nutrition*, **151**(1), 11-19.

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., Chen, Y. 2018. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, **11**(2), 344-354.

Jiang, J., Wang, Q., Xiong, Y.L. 2018. A pH shift approach to the improvement of interfacial properties of plant seed proteins. *Current opinion in food science*, **19**, 50-56.

Jensen, S., Olsen, H., Sørensen, H. 1990. Aqueous enzymatic processing of rapeseed for production of high quality products. *Canola and rapeseed: production, chemistry, nutrition and processing technology*, 331-343.

Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., Chen, Y. 2018. Effects of dielectric barrier discharge (DBD) cold plasma treatment on physicochemical and functional properties of peanut protein. *Food and Bioprocess Technology*, **11**(2), 344-354.

Ji, H., Han, F., Peng, S., Yu, J., Li, L., Liu, Y., Chen, Y., Li, S., Chen, Y. 2019. Behavioral solubilization of peanut protein isolate by atmospheric pressure cold plasma (ACP) treatment. *Food and bioprocess technology*, **12**(12), 2018-2027.

Jiang, J., Wang, Q., Xiong, Y.L. 2018. A pH shift approach to the improvement of interfacial properties of plant seed proteins. *Current opinion in food science*, **19**, 50-56.

Jiang, J., Xiong, Y.L., Chen, J. 2010. pH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions. *Journal of agricultural and food chemistry*, **58**(13), 8035-8042.

Jiang, S., Ding, J., Andrade, J., Rababah, T.M., Almajwal, A., Abulmeaty, M.M., Feng, H. 2017. Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments. *Ultrasonics Sonochemistry*, **38**, 835-842.

Jensen, S., Olsen, H., Sørensen, H. 1990. Aqueous enzymatic processing of rapeseed for production of high quality products. *Canola and rapeseed: production, chemistry, nutrition and processing technology*, 331-343.

Kristinsson, H.G., Hultin, H.O. 2003. Changes in conformation and subunit assembly of cod myosin at low and high pH and after subsequent refolding. *Journal of Agricultural and Food Chemistry*, **51**(24), 7187-7196.

Krygier, K., Sosulski, F., Hogge, L. 1982. Free, esterified, and insoluble-bound phenolic acids. 1. Extraction and purification procedure. *Journal of Agricultural and Food Chemistry*, **30**(2), 330-334.

Kozlowska, H., Naczk, M., Shahidi, F., Zadernowski, R. 1990. Phenolic acids and tannins in rapeseed and canola. *Canola and rapeseed*, 193-210.

Karaca, A.C., Low, N., Nickerson, M. 2011. Emulsifying properties of canola and flaxseed protein isolates produced by isoelectric precipitation and salt extraction. *Food Research International*, **44**(9), 2991-2998.

Law, H.-Y., Choi, S.-M., Ma, C.-Y. 2008. Study of conformation of vicilin from Dolichos lablab and Phaseolus calcaratus by Fourier-transform infrared spectroscopy and differential scanning calorimetry. *Food research international*, **41**(7), 720-729.

Li, M., Li, X., Han, C., Ji, N., Jin, P., Zheng, Y. 2019. Physiological and metabolomic analysis of cold plasma treated fresh-cut strawberries. *Journal of agricultural and food chemistry*, **67**(14), 4043-4053.

Lukes, P., Locke, B.R. 2005. Plasmachemical oxidation processes in a hybrid gas–liquid electrical discharge reactor. *Journal of Physics D: Applied Physics*, **38**(22), 4074-4081.

Li, Y., Cheng, Y., Zhang, Z., Wang, Y., Mintah, B.K., Dabbour, M., Jiang, H., He, R., Ma, H. 2020. Modification of rapeseed protein by ultrasound-assisted pH shift treatment: Ultrasonic mode and frequency screening, changes in protein solubility and structural characteristics. *Ultrasonics Sonochemistry*, **69**, 105240.

Law, H.-Y., Choi, S.-M., Ma, C.-Y. 2008. Study of conformation of vicilin from Dolichos lablab and Phaseolus calcaratus by Fourier-transform infrared spectroscopy and differential scanning calorimetry. *Food research international*, **41**(7), 720-729.

Li, M., Li, X., Han, C., Ji, N., Jin, P., Zheng, Y. 2019. Physiological and metabolomic analysis of cold plasma treated fresh-cut strawberries. *Journal of agricultural and food chemistry*, **67**(14), 4043-4053.

Lukes, P., Locke, B.R. 2005. Plasmachemical oxidation processes in a hybrid gas–liquid electrical discharge reactor. *Journal of Physics D: Applied Physics*, **38**(22), 4074-4081.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Mansour, E., Dworschák, E., Lugasi, A., Gaál, Ö., Barna, É., Gergely, A. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food chemistry*, **47**(3), 247-252.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Malabat, C., nchez-Vioque, R.I., Rabiller, C., Gu guen, J. 2001. Emulsifying and foaming properties of native and chemically modified peptides from the 2S and 12S proteins of rapeseed (Brassica napus L.). *Journal of the American Oil Chemists' Society*, **78**(3), 235-242.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Makris, D.P., Rossiter, J.T. 2002. Hydroxyl free radical-mediated oxidative degradation of quercetin and morin: a preliminary investigation. *Journal of Food Composition and Analysis*, **15**(1), 103-113.

Mann, B., Malik, R. 1996. Studies on some functional characteristics of whey protein-polysaccharide complex. *Journal of food science and technology (Mysore)*, **33**(3), 202-206.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Mu, L., Zhao, M., Yang, B., Zhao, H., Cui, C., Zhao, Q. 2010. Effect of ultrasonic treatment on the graft reaction between soy protein isolate and gum acacia and on the physicochemical properties of conjugates. *Journal of agricultural and food chemistry*, **58**(7), 4494-4499

Mills, J., Chong, J. 1977. Ultrastructure and mineral distribution in heat-damaged rapeseed. *Canadian Journal of Plant Science*, **57**(1), 21-30.

Mupondwa, E., Li, X., Wanasundara, J.P. 2018. Technoeconomic prospects for commercialization of Brassica (cruciferous) plant proteins. *Journal of the American Oil Chemists' Society*, **95**(8), 903-922.

Mailer, R.J., McFadden, A., Ayton, J., Redden, B. 2008. Anti-nutritional components, fibre, sinapine and glucosinolate content, in Australian canola (Brassica napus L.) meal. *Journal of the American Oil Chemists' Society*, **85**(10), 937-944.

Malabat, C., nchez-Vioque, R.I., Rabiller, C., Gu guen, J. 2001. Emulsifying and foaming properties of native and chemically modified peptides from the 2S and 12S proteins of rapeseed (Brassica napus L.). *Journal of the American Oil Chemists' Society*, **78**(3), 235-242.

Mann, B., Malik, R. 1996. Studies on some functional characteristics of whey protein-polysaccharide complex. *Journal of food science and technology (Mysore)*, **33**(3), 202-206.

Mansour, E., Dworschák, E., Lugasi, A., Gaál, Ö., Barna, É., Gergely, A. 1993. Effect of processing on the antinutritive factors and nutritive value of rapeseed products. *Food chemistry*, **47**(3), 247-252.

Mehr, H.M., Koocheki, A. 2020. Effect of atmospheric cold plasma on structure, interfacial and emulsifying properties of Grass pea (Lathyrus sativus L.) protein isolate. *Food Hydrocolloids*, **106**, 105899.

Mills, J., Chong, J. 1977. Ultrastructure and mineral distribution in heat-damaged rapeseed. *Canadian Journal of Plant Science*, **57**(1), 21-30.

Mupondwa, E., Li, X., Wanasundara, J.P. 2018. Technoeconomic prospects for commercialization of Brassica (cruciferous) plant proteins. *Journal of the American Oil Chemists' Society*, **95**(8), 903-922.

Noshad, M., Mohebbi, M., Koocheki, A., Shahidi, F. 2016. Influence of interfacial engineering on stability of emulsions stabilized with soy protein isolate. *Journal of Dispersion Science and Technology*, **37**(1), 56-65.

Naczk, M., Amarowicz, R., Shahidi, F. 1998. Role of phenolics in flavor of rapeseed protein products. in: *Developments in food science*, Vol. 40, Elsevier, pp. 597-613.

Noshad, M., Mohebbi, M., Koocheki, A., Shahidi, F. 2016. Influence of interfacial engineering on stability of emulsions stabilized with soy protein isolate. *Journal of Dispersion Science and Technology*, **37**(1), 56-65.

Ostrowska, A., Kozlowska, M., Rachwal, D., Wnukowski, P., Nebesny, E., Rosicka-Kaczmarek, J. 2018. Rapeseed protein-fibre concentrate: Chemical composition and functional properties. *Żywność Nauka Technologia Jakość*, **25**(4).

Prakash, V., Narasinga Rao, M. 1986. Physicochemical properties of oilseed protein. *Critical Reviews in Biochemistry*, **20**(3), 265-363.

Pirestani, S., Nasirpour, A., Keramat, J., Desobry, S. 2017. Preparation of chemically modified canola protein isolate with gum Arabic by means of Maillard reaction under wet-heating conditions. *Carbohydrate Polymers*, **155**, 201-207.

Pojić, M., Mišan, A., Tiwari, B. 2018. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends in Food Science & Technology*, **75**, 93-104.

Poore, J., Nemecek, T. 2018. Reducing food's environmental impacts through producers and consumers. *Science*, **360**(6392), 987-992.

Prakash, V., Narasinga Rao, M. 1986. Physicochemical properties of oilseed protein. *Critical Reviews in Biochemistry*, **20**(3), 265-363.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Shi, W., Dumont, M.-J. 2014. Processing and physical properties of canola protein isolate-based films. *Industrial Crops and Products*, **52**, 269-277.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Sultan, M.T., Saddique, M.S., Shahid, M. 2020. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Sarangapani, C., O'Toole, G., Cullen, P., Bourke, P. 2017. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science & Emerging Technologies*, **44**, 235-241.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Sugiarto, A.T., Sato, M. 2001. Pulsed plasma processing of organic compounds in aqueous solution. *Thin Solid Films*, **386**(2), 295-299.

Sharifian, A., Soltanizadeh, N., Abbaszadeh, R. 2019. Effects of dielectric barrier discharge plasma on the physicochemical and functional properties of myofibrillar proteins. *Innovative food science & emerging technologies*, **54**, 1-8.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Tauseef, M., Sultan, M.S.S., Shahid, M. 2020. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Sullivan, G.A., Sebranek, J.G. 2012. Nitrosylation of myoglobin and nitrosation of cysteine by nitrite in a model system simulating meat curing. *Journal of agricultural and food chemistry*, **60**(7), 1748-1754.

Sun, W., Zhou, F., Sun, D.-W., Zhao, M. 2013. Effect of oxidation on the emulsifying properties of myofibrillar proteins. *Food and Bioprocess Technology*, **6**(7), 1703-1712.

Song, L., Thornalley, P.J. 2007. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and chemical toxicology*, **45**(2), 216-224.

Sarangapani, C., O'Toole, G., Cullen, P., Bourke, P. 2017. Atmospheric cold plasma dissipation efficiency of agrochemicals on blueberries. *Innovative Food Science & Emerging Technologies*, **44**, 235-241.

Shahidi, F., Naczk, M. 1992. An overview of the phenolics of canola and rapeseed: chemical, sensory and nutritional significance. *Journal of the American Oil Chemists' Society*, **69**(9), 917-924.

Sharafodin, H., Soltanizadeh, N. 2022. Potential application of DBD Plasma Technique for modifying structural and physicochemical properties of Soy Protein Isolate. *Food Hydrocolloids*, **122**, 107077.

Sharifian, A., Soltanizadeh, N., Abbaszadeh, R. 2019. Effects of dielectric barrier discharge plasma on the physicochemical and functional properties of myofibrillar proteins. *Innovative food science & emerging technologies*, **54**, 1-8.

Shi, W., Dumont, M.-J. 2014. Processing and physical properties of canola protein isolate-based films. *Industrial Crops and Products*, **52**, 269-277.

Sibt-e-Abbas, M., Butt, M.S., Khan, M.R., Sultan, M.T., Saddique, M.S., Shahid, M. 2020a. Nutritional and functional characterization of defatted oilseed protein isolates. *Pakistan Journal of Agricultural Sciences*, **57**(1).

Song, L., Thornalley, P.J. 2007. Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and chemical toxicology*, **45**(2), 216-224.

Sugiarto, A.T., Sato, M. 2001. Pulsed plasma processing of organic compounds in aqueous solution. *Thin Solid Films*, **386**(2), 295-299.

Sullivan, G.A., Sebranek, J.G. 2012. Nitrosylation of myoglobin and nitrosation of cysteine by nitrite in a model system simulating meat curing. *Journal of agricultural and food chemistry*, **60**(7), 1748-1754.

Sun, W., Zhou, F., Sun, D.-W., Zhao, M. 2013. Effect of oxidation on the emulsifying properties of myofibrillar proteins. *Food and Bioprocess Technology*, **6**(7), 1703-1712.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011a. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011b. Extraction and characterization of protein fractions from Australian canola meals. *Food Research International*, **44**(4), 1075-1082.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28

Thompson, L.U., Serraino, M.R. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. *Journal of agricultural and food chemistry*, **34**(3), 468-469.

Tomé, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements—functional aspects. *Food and nutrition bulletin*, **34**(2), 272-274.

Torres, B., Tiwari, B., Patras, A., Wijngaard, H.H., Brunton, N., Cullen, P., O'Donnell, C. 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. *Food Chemistry*, **124**(3), 721-726.

Tan, S.H., Mailer, R.J., Blanchard, C.L., Agboola, S.O. 2011a. Canola proteins for human consumption: extraction, profile, and functional properties. *Journal of food science*, **76**(1), R16-R28.

Thompson, L.U., Serraino, M.R. 1986. Effect of phytic acid reduction on rapeseed protein digestibility and amino acid absorption. *Journal of agricultural and food chemistry*, **34**(3), 468-469.

Tomé, D. 2013. Digestibility issues of vegetable versus animal proteins: protein and amino acid requirements—functional aspects. *Food and nutrition bulletin*, **34**(2), 272-274.

Torres, B., Tiwari, B., Patras, A., Wijngaard, H.H., Brunton, N., Cullen, P., O'Donnell, C. 2011. Effect of ozone processing on the colour, rheological properties and phenolic content of apple juice. *Food Chemistry*, **124**(3), 721-726.

Uppström, B., Svensson, R. 1980. Determination of phytic acid in rapeseed meal. *Journal of the Science of Food and Agriculture*, **31**(7), 651-656.

Van de Noort, M. 2017. Lupin: An important protein and nutrient source. in: *Sustainable protein sources*, Elsevier, pp. 165-183.

Velasco, L., Möllers, C. 1998. Nondestructive assessment of sinapic acid esters in Brassica species: II. Evaluation of germplasm and identification of phenotypes with reduced levels. *Crop Science*, **38**(6), 1650-1654.

Venkataratnam, H., Sarangapani, C., Cahill, O., Ryan, C.B. 2019. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, **52**, 368-375.

Von Der Haar, D., Müller, K., Bader-Mittermaier, S., Eisner, P. 2014. Rapeseed proteins–Production methods and possible application ranges. *Ocl*, **21**(1), D104.
Vuorela, S. 2005. Analysis, isolation, and bioactivities of rapeseed phenolics.
Velasco, L., Möllers, C. 1998. Nondestructive assessment of sinapic acid esters in Brassica species: II. Evaluation of germplasm and identification of phenotypes with reduced levels. *Crop Science*, **38**(6), 1650-1654.
Venkataratnam, H., Sarangapani, C., Cahill, O., Ryan, C.B. 2019. Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1. *Innovative Food Science & Emerging Technologies*, **52**, 368-375.
Von Der Haar, D., Müller, K., Bader-Mittermaier, S., Eisner, P. 2014. Rapeseed proteins–Production methods and possible application ranges. *Ocl*, **21**(1), D104.
Wanasundara, J., McIntosh, T., Perera, S., Withana-Gamage, T., Mitra, P. 2016a. Canola/rapeseed protein-functionality and nutrition. OCL 23. *D407*.
Wanasundara, J., Tan, S., Alashi, A., Pudel, F., Blanchard, C. 2017. Proteins from canola/rapeseed: Current status. in: *Sustainable protein sources*, Elsevier, pp. 285-304.
Wanasundara, J.P. 2011. Proteins of Brassicaceae oilseeds and their potential as a plant protein source. *Critical reviews in food science and nutrition*, **51**(7), 635-677.
Wanasundara, J.P., Abeysekara, S.J., McIntosh, T.C., Falk, K.C. 2012. Solubility differences of major storage proteins of Brassicaceae oilseeds. *Journal of the American Oil Chemists' Society*, **89**, 869-881.
Wanasundara, J.P., McIntosh, T.C., Perera, S.P., Withana-Gamage, T.S., Mitra, P. 2016b. Canola/rapeseed protein-functionality and nutrition. *OCl*, **23**(4), D407.
Wu, J., Aluko, R.E., Muir, A.D. 2008. Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food chemistry*, **111**(4), 942-950.
Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.
Wanapat, M., Huang, B., Viennasay, B., Totakul, P., Matra, M., Phesatcha, B., Ampapon, T. 2020. Rapeseed pod meal can replace concentrate and enhance utilization of feed on in vitro gas production and fermentation characteristics. *Tropical Animal Health and Production*, **52**(5), 2593-2598.
Wang, Z., Li, Y., Jiang, L., Qi, B., Zhou, L. 2014. Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry*, **2014**.
Wong, D.W., Camirand, W.M., Pavlath, A.E., Parris, N., Friedman, M. 1996. Structures and functionalities of milk proteins. *Critical Reviews in Food Science & Nutrition*, **36**(8), 807-844.
Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.
Wanapat, M., Huang, B., Viennasay, B., Totakul, P., Matra, M., Phesatcha, B., Ampapon, T. 2020. Rapeseed pod meal can replace concentrate and enhance utilization of feed on in vitro gas production and fermentation characteristics. *Tropical Animal Health and Production*, **52**(5), 2593-2598.
Wanasundara, J., McIntosh, T., Perera, S., Withana-Gamage, T., Mitra, P. 2016a. Canola/rapeseed protein-functionality and nutrition. OCL 23. *D407*.

Wanasundara, J., Tan, S., Alashi, A., Pudel, F., Blanchard, C. 2017. Proteins from canola/rapeseed: Current status. in: *Sustainable protein sources*, Elsevier, pp. 285-304.

Wanasundara, J.P. 2011. Proteins of Brassicaceae oilseeds and their potential as a plant protein source. *Critical reviews in food science and nutrition*, **51**(7), 635-677.

Wanasundara, J.P., Abeysekara, S.J., McIntosh, T.C., Falk, K.C. 2012. Solubility differences of major storage proteins of Brassicaceae oilseeds. *Journal of the American Oil Chemists' Society*, **89**, 869-881.

Wanasundara, J.P., McIntosh, T.C., Perera, S.P., Withana-Gamage, T.S., Mitra, P. 2016b. Canola/rapeseed protein-functionality and nutrition. *OCl*, **23**(4), D407.

Wang, Z., Li, Y., Jiang, L., Qi, B., Zhou, L. 2014. Relationship between secondary structure and surface hydrophobicity of soybean protein isolate subjected to heat treatment. *Journal of Chemistry*, **2014**.

Wong, D.W., Camirand, W.M., Pavlath, A.E., Parris, N., Friedman, M. 1996. Structures and functionalities of milk proteins. *Critical Reviews in Food Science & Nutrition*, **36**(8), 807-844.

Wu, J., Aluko, R.E., Muir, A.D. 2008. Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food chemistry*, **111**(4), 942-950.

Wu, J., Muir, A. 2008. Comparative structural, emulsifying, and biological properties of 2 major canola proteins, cruciferin and napin. *Journal of food science*, **73**(3), C210-C216.

Yiu, S., Poon, H., Fulcher, R., Altosaar, I. 1982. The microscopic structure and chemistry of rapeseed and its products. *Food Structure*, **1**(2), 4.

Yoon, J.H., Thompson, L.U., Jenkins, D. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *The American journal of clinical nutrition*, **38**(6), 835-842.

Yi, Z., Jia-qing, X., Ci, L., Jun, L., Hong-li, S. 2008. Mechanism of Oxidation of Aqueous Phenol by H2O2 Catalysed by a Macrocyclic Iron(II) Complex. *Progress in Reaction Kinetics and Mechanism*, **33**(3), 241-252.

Yang, J., Liu, G., Zeng, H., Chen, L. 2018. Effects of high pressure homogenization on faba bean protein aggregation in relation to solubility and interfacial properties. *Food Hydrocolloids*, **83**, 275-286.

Yanjun, S., Jianhang, C., Shuwen, Z., Hongjuan, L., Jing, L., Lu, L., Uluko, H., Yanling, S., Wenming, C., Wupeng, G. 2014. Effect of power ultrasound pre-treatment on the physical and functional properties of reconstituted milk protein concentrate. *Journal of Food Engineering*, **124**, 11-18.

Yi, Z., Jia-qing, X., Ci, L., Jun, L., Hong-li, S. 2008. Mechanism of Oxidation of Aqueous Phenol by H2O2 Catalysed by a Macrocyclic Iron(II) Complex. *Progress in Reaction Kinetics and Mechanism*, **33**(3), 241-252.

Yiu, S., Poon, H., Fulcher, R., Altosaar, I. 1982. The microscopic structure and chemistry of rapeseed and its products. *Food Structure*, **1**(2), 4.

Yoon, J.H., Thompson, L.U., Jenkins, D. 1983. The effect of phytic acid on in vitro rate of starch digestibility and blood glucose response. *The American journal of clinical nutrition*, **38**(6), 835-842.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

Zum Felde, T., Baumert, A., Strack, D., Becker, H., Möllers, C. 2007. Genetic variation for sinapate ester content in winter rapeseed (Brassica napus L.) and development of NIRS calibration equations. *Plant Breeding*, **126**(3), 291-296.

Zhang, D., Li, H., Emara, A., Hu, Y., Wang, Z., Wang, M., He, Z. 2020. Effect of in vitro oxidation on the water retention mechanism of myofibrillar proteins gel from pork muscles. *Food Chemistry*, **315**, 126226.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

ZHANG, J., CHEN, J., LI, X. 2009. Remove of Phenolic Compounds in Water by Low-Temperature Plasma: A Review of Current Research. *Journal of Water Resource and Protection*, **Vol.01No.02**, 11.

Zhang, D., Li, H., Emara, A., Hu, Y., Wang, Z., Wang, M., He, Z. 2020. Effect of in vitro oxidation on the water retention mechanism of myofibrillar proteins gel from pork muscles. *Food Chemistry*, **315**, 126226.

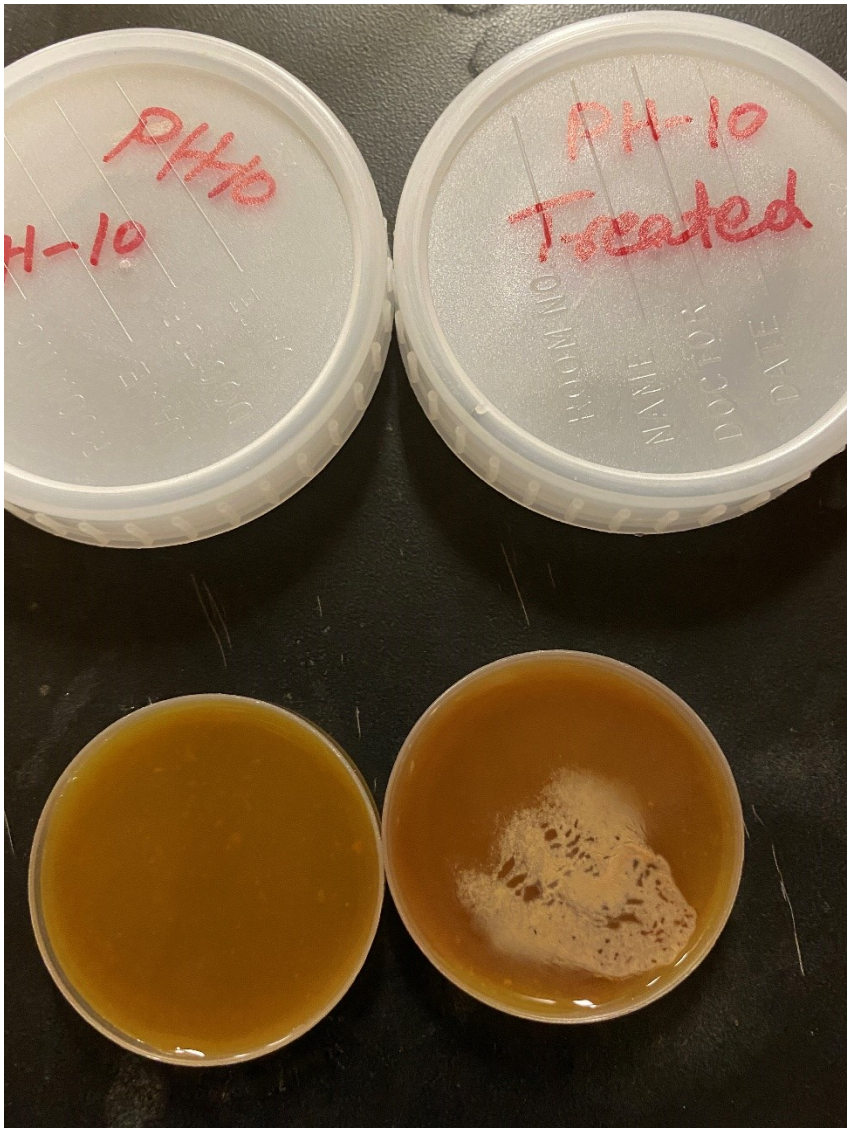
Zhang, Q.-T., Tu, Z.-C., Xiao, H., Wang, H., Huang, X.-Q., Liu, G.-X., Liu, C.-M., Shi, Y., Fan, L.-L., Lin, D.-R. 2014. Influence of ultrasonic treatment on the structure and emulsifying properties of peanut protein isolate. *Food and Bioproducts Processing*, **92**(1), 30-37.

Zhang, Q., Cheng, Z., Zhang, J., Nasiru, M.M., Wang, Y., Fu, L. 2021a. Atmospheric cold plasma treatment of soybean protein isolate: Insights into the structural, physicochemical, and allergenic characteristics. *Journal of Food Science*, **86**(1), 68-77.

Zhang, S., Huang, W., Feizollahi, E., Roopesh, M., Chen, L. 2021b. Improvement of pea protein gelation at reduced temperature by atmospheric cold plasma and the gelling mechanism study. *Innovative Food Science & Emerging Technologies*, **67**, 102567.

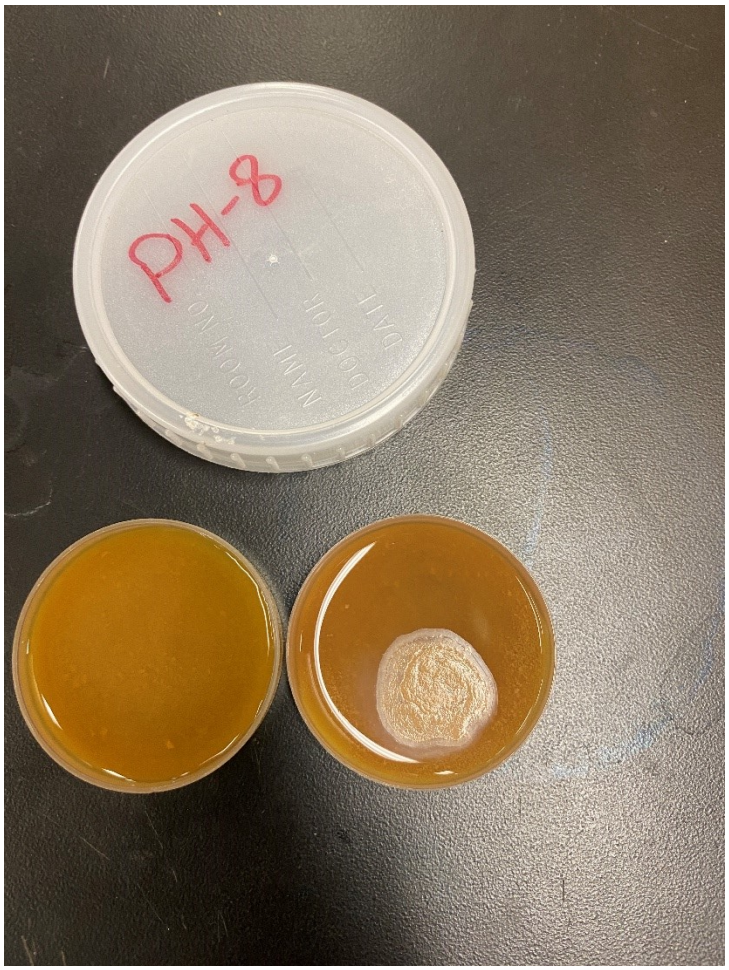
Zum Felde, T., Baumert, A., Strack, D., Becker, H., Möllers, C. 2007. Genetic variation for sinapate ester content in winter rapeseed (Brassica napus L.) and development of NIRS calibration equations. *Plant Breeding*, **126**(3), 291-296.

Appendix-1. Digital images of canola protein treated with or without ACP at different pH.



Picture 1: The canola protein sample at pH 10 was treated with ACP for 10 minutes (right side). Canola protein sample without ACP treatment (left side).

Picture 2: The canola protein sample at pH 6 was treated with ACP for 10 minutes (right side). Canola protein sample without ACP treatment (left side).



Picture 3: The canola protein sample at pH 8 was treated with ACP for 10 minutes (right side). Canola protein sample without ACP treatment (left side).