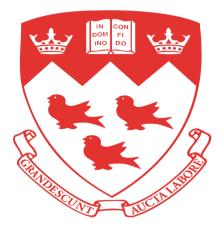
TECHNO-FUNCTIONAL, THERMAL AND RHEOLOGICAL PROPERTIES OF PEA PROTEIN PRODUCTS

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Department of Bioresource Engineering McGill University, Montréal, Canada AUGUST 2023 A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Master of Science

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DEDICATION

I dedicate this thesis to the almighty God. He alone made this work a huge success.

ABSTRACT

Pea protein products, which are popular due to their nutritional and functional properties, were the focus of this study. The study aimed to investigate the techno-functional, thermal, and rheological properties of pea protein products from different varieties of peas and predict these properties using a machine learning artificial neural network algorithm. The effect of the pea protein product's composition on these properties was evaluated, and data analysis was performed using Pareto charts, principal component analysis and cluster analysis. The study found that the techno-functional properties of the pea protein products, water absorption capacity, water solubility index, water absorption index, oil absorption index, protein solubility, emulsifying capacity, forming capacity, and forming stability, varied from 40.32% to 87.15% for protein solubility, 49.71% to 69.90% for water absorption capacity, 48.70% to 75.33% for water solubility index, 0.12% to 2.57% for water absorption index, 65.07% to 86.02% for oil absorption index, 23.07% to 49.11% for emulsifying capacity, 2.50% to 15.00% for forming capacity, and 1.00% to 5.00% for forming stability. The onset temperature ranged from 93.3°C to 166.19°C, peak temperature ranged from 128.97°C to 180.74°C, and gelatinization enthalpy ranged from 89.04 J/g to 280.42 J/g. The results showed that protein content had the most significant influence on water absorption capacity, oil absorption index, forming capacity, peak temperature, and enthalpy. The oil content showed the most significant influence on forming stability and onset temperature, while the interaction of oil content and protein content influenced water solubility index, water absorption index, and protein solubility the most. Principal component analysis and cluster analysis were used to identify unique varieties based on the cluster of techno-functional and thermal properties of the pea protein products. The rheological results showed that increasing the protein content led to a significant rise in storage modulus, loss modulus, and loss factor, while the complex viscosity decreased. An increase in oil content caused a decrease in storage modulus, loss factor, and complex viscosity. A rise in starch content led to a significant increase in the complex viscosity of pea protein products. Principal component analysis and cluster analysis were used to establish unique varieties based on the cluster of the rheological properties of the pea protein products. The machine learning artificial neural network algorithm results showed variations in the optimal number of neurons (100-200), iterations (10000-25000), and hidden layers (2-4) for different properties, as well as differences in Mean Absolute Error, coefficient of determination, Mean-Squared Error, and RMSE for training and test datasets. These findings provide valuable insights into the potential of machine learning algorithms for predicting the properties of pea protein products. In conclusion, this study provides valuable information for identifying key composition factors that affect the techno-functional, thermal, and rheological properties of pea protein products. It also assists in the selection of the most suitable pea protein product for use in product development and formulation and provides insights into the processing behavior and quality control of pea protein products.

RÉSUMÉ

Les produits à base de protéines de pois, qui sont populaires en raison de leurs propriétés nutritionnelles et fonctionnelles, ont été au centre de cette étude. L'étude visait à étudier les propriétés techno-fonctionnelles, thermiques et rhéologiques des produits à base de protéines de pois provenant de différentes variétés de pois, et à prédire ces propriétés à l'aide d'un algorithme de réseau neuronal artificiel d'apprentissage automatique. L'effet de la composition des produits à base de protéines de pois sur ces propriétés a été évalué, et l'analyse des données a été réalisée à l'aide de diagrammes de Pareto, d'une analyse en composantes principales et d'une analyse de regroupement. L'étude a révélé que les propriétés techno-fonctionnelles des produits à base de protéines de pois, telles que la capacité d'absorption d'eau, l'indice de solubilité dans l'eau, l'indice d'absorption d'eau, l'indice d'absorption d'huile, la solubilité des protéines, la capacité émulsifiante, la capacité de formation et la stabilité de formation, variaient de 40,32 % à 87,15 % pour la solubilité des protéines, de 49,71 % à 69,90 % pour la capacité d'absorption d'eau, de 48,70 % à 75,33 % pour l'indice de solubilité dans l'eau, de 0,12 % à 2,57 % pour l'indice d'absorption d'eau, de 65,07 % à 86,02 % pour l'indice d'absorption d'huile, de 23,07 % à 49,11 % pour la capacité émulsifiante, de 2,50 % à 15,00 % pour la capacité de formation, et de 1,00 % à 5,00 % pour la stabilité de formation. La température de début variait de 93,3°C à 166,19°C, la température de pic variait de 128,97°C à 180,74°C, et l'enthalpie de gélatinisation variait de 89,04 J/g à 280,42 J/g. Les résultats ont montré que la teneur en protéines avait la plus grande influence sur la capacité d'absorption d'eau, l'indice d'absorption d'huile, la capacité de formation, la température de pic et l'enthalpie. La teneur en huile a montré la plus grande influence sur la stabilité de formation et la température de début, tandis que l'interaction entre la teneur en huile et la teneur en protéines a eu le plus d'influence sur l'indice de solubilité dans l'eau, l'indice d'absorption d'eau et la solubilité des protéines.

L'analyse en composantes principales et l'analyse de regroupement ont été utilisées pour identifier des variétés uniques basées sur le regroupement des propriétés techno-fonctionnelles et thermiques des produits à base de protéines de pois. Les résultats rhéologiques ont montré qu'une augmentation de la teneur en protéines entraînait une augmentation significative du module de stockage, du module de perte et du facteur de perte, tandis que la viscosité complexe diminuait. Une augmentation de la teneur en huile entraînait une diminution du module de stockage, du

facteur de perte et de la viscosité complexe. Une augmentation de la teneur en amidon entraînait une augmentation significative de la viscosité complexe des produits à base de protéines de pois. L'analyse en composantes principales et l'analyse de regroupement ont été utilisées pour établir des variétés uniques basées sur le regroupement des propriétés rhéologiques des produits à base de protéines de pois. Les résultats de l'algorithme de réseau neuronal artificiel d'apprentissage automatique ont montré des variations dans le nombre optimal de neurones (100-200), les itérations (10 000-25 000) et les couches cachées (2-4) pour différentes propriétés, ainsi que des différences dans l'erreur absolue moyenne, le coefficient de détermination, l'erreur quadratique moyenne et le RMSE pour les ensembles de données d'entraînement et de test. Ces résultats fournissent des informations précieuses sur le potentiel des algorithmes d'apprentissage automatique pour prédire les propriétés des produits à base de protéines de pois. En conclusion, cette étude fournit des informations précieuses pour identifier les facteurs de composition clés qui affectent les propriétés techno-fonctionnelles, thermiques et rhéologiques des produits à base de protéines de pois. Elle aide également à la sélection du produit à base de protéines de pois le plus approprié pour le développement et la formulation de produits, et fournit des informations sur le comportement de transformation et le contrôle de la qualité des produits à base de protéines de pois.

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My foremost and deep appreciation to God, the source and Origin of my life, the one through whom knowledge and wisdom flows. My dream to study in one of the world top ranking Universities outside my country started in 2016. At this time, I was just hired as a Graduate Assistant in the department of Food Engineering in Ladoke Akintola University of Technology. My mentor as well as senior colleague then just got the opportunity to study in South Africa and he was really propelling me to study abroad by enlightening me on the opportunities this will avail me. Later in 2017, I began to reach out to potential supervisors one of which was my amiable supervisor, Dr Ngadi, I contacted him for an MSc position in his laboratory. His response was "Go ahead and apply for admission put me as your potential Supervisor as I will be glad to supervise you". Dr. Ngadi's fatherly attributes, patience, strong will, empathy, kindness and an academic achievement is very rare coupled with his listening ear at all times. Thank you very much, Dr. Ngadi, for giving me the opportunity to study at one of the world's leading institutions of learning and funding my research. Your belief in me made my dreams come true. I also appreciate Dr. Christopher Kucha for providing guidance and revising the manuscript several times throughout the research.

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CONTRIBUTION OF AUTHORS

Tolulope Oyinboola Abodunrin, the MSc candidate, is the primary author of all the manuscripts in the thesis. He was responsible for designing the experiments, setting up the laboratory, conducting analytical work, analyzing data, and preparing the manuscripts. All experiments were carried out in the Food and Bioprocess Engineering Laboratory located in the Department of Bioresource Engineering at McGill University's Macdonald Campus. The thesis was supervised by Dr. Michael O. Ngadi, James McGill Professor in the Department of Bioresources Engineering at McGill University's Macdonald Campus, who provided scientific advice, research funding, and technical supervision throughout the entire program.

Signed: Tolulope Oyinboola Abodunrin

Date: August 2023

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NOMENCLATURE

Abbreviation/acronyms	Full form
PPC	Pea protein concentrates
PPI	Pea protein isolate
PPP	pea protein products
ANNs	Artificial neural networks
ML	Machine learning
RP	Rice protein
LF	Lentil flour
PI	Isoelectric point
PS	Protein solubility
PPF	Pea protein flour
PCA	Principal component analysis
WSI	Water solubility index
WAI	Water absorption index
WAC	Water absorption capacity
ESI	Emulsifying stability index
EAI	Emulsifying activity index
OAI	Oil absorption index
OAC	Oil absorption capacity
EC	Emulsion capacity
FS	Foaming stability
FC	Foaming capacity
CA	Cluster analysis
DSC	Differential scanning calorimeter
BSA	Bovine serum albumin
ΔHg	Gelatinization enthalpy

RMSE	Root mean square error
MLP	Multi-layer perceptron
MAE	Mean absolute error
MSE	Mean squared error
LBFGS	Limited-memory broyder-fletcher-goldfarb-shanno
T ₀	Onset temperature
T _p	Peak or denaturation temperature
T _c	Enthalpy or conclusion temperature

THESIS FORMAT

This thesis is submitted in the format of papers suitable for journal publication. This thesis format has been approved by the Faculty of Graduate and Postdoctoral Studies, McGill University, and follows the conditions outlined in the Guidelines: Concerning Thesis Preparation, which are as follows:

"As an alternative to the traditional thesis format, the dissertation can consist of a collection of papers of which the student is an author or co-author. These papers must have a cohesive, unitary character making them a report of a single program of research". The structure for the manuscript-based thesis must conform to the following:

1. Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis).

2. The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges between the different papers are mandatory.

3. The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts. The thesis must include the following:

(a) A table of contents;

(b) An abstract in English and French;

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(c) An introduction which clearly states the rational and objectives of the research;

(d) A comprehensive review of the literature (in addition to that covered in the introduction to each paper);

(e) A final conclusion and summary;

4. As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

5. In general, when co-authored papers are included in a thesis, the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers".

THESIS ORGANIZATION

Chapter one: General introduction to the research work, including scope and research objectives.

Chapter two: Review of the relevant literature pertaining to the techno-functional, thermal,

rheological properties and machine learning techniques.

Chapter three: Techno-functional and thermal properties of pea protein products.

Chapter four: Rheological properties of pea protein products.

Chapter five: Machine learning-based prediction of techno-functional, thermal and rheological

properties for pea protein product.

Chapter six: General summary, conclusion and future research direction.

Chapter seven: Bibliography.

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1 Background

There is a growing demand for protein-based foods, and it is expected to increase rapidly in the coming years due to the increasing world population and societal prosperity (Lang, 2020). Thus, there is a need to create a more sustainable food system. Peas are generally identified as green or yellow annual cotyledon diversities that belong to the leguminous family called Fabaceae. Peas have garnered substantial interest due to their associated nutritional significance and promising healthcare benefits, including reducing LDL-cholesterol, weight control, prevention of deficiency-related diseases of selenium and folate, minimizing the occurrence of type-II diabetes mellitus and colon cancer (Abeysekara et al., 2012; Dahl et al., 2012). Peas are also relatively non-allergenic. Peas are rich in carbohydrates, low in fat, and consist of nine key essential amino acids (Oliete et al., 2018; Qamar et al., 2019). Peas are high in antioxidants (Zhang et al., 2013; Qamar et al., 2010; Jiang et al., 2010).

Proteins derived from peas are typically processed into isolates, concentrates, and flour, collectively referred to as pea protein products. In food production, these products have been utilized in the creation of novel food items, such as food paste, meat analogues, sausages, and soups, due to their emulsification capacity, water and fat binding capacity, gelation, foaming, and solubility (Pelgrom et al., 2013). The meat and sausage industry often substitutes pea protein concentrate (PPC) for meat due to its unique water and fat binding capacity, protein solubility, gelation, emulsification, and foaming capacity profiles. Pea protein isolate (PPI), on the other hand, is typically used to enhance the nutritional and functional qualities of products, such as pasta (Pelgrom et al., 2013). Ferawati et al. (2021) produced meat analogues using yellow pea and faba bean protein isolates and concentrate. However, the global food industry faces an ongoing challenge of discovering unique protein-based ingredients from various plant varieties that will significantly influence the desired techno-functional, thermal and rheological properties to meet the evolving preferences of consumers.

Generally, the types of ingredients, variety, extraction technique, processing conditions, and composition of foods significantly influence peas techno-functional properties (Pazmiño et al., 2018; Arteaga et al., 2021). Various essential techno-functional properties, such as protein solubility, water absorption capacity, water solubility, water absorption index, oil absorption index, emulsifying capacity, forming property, and forming stability of protein products, play a crucial role in determining the characteristics of final food products (Barać et al., 2015). In order to achieve optimal performance during food processing, it is important for proteins to exhibit good solubility, which influences other properties such as emulsification, foaming, and gelation (Barac et al., 2010). Typically, pea protein products exhibit high protein solubility owing to their abundant protein content, surface composition, and hydrophobicity of the protein molecules (Lam et al., 2018; Bogahawaththa et al., 2019). During the preparation or extraction process, pea protein products are subjected to heat treatment, necessitating the study of their thermal properties, which can be assessed using onset temperature, peak or denaturation temperature, and gelatinization enthalpy (Branch & Maria, 2017). The rheological property is a key parameter that is commonly used to evaluate the quality of raw materials and products and to anticipate their performance during storage and processing (Dasa & Binh, 2019; Zhang et al., 2020; Mir et al., 2021). It also aids in predicting the way proteins, starch, and fats behave in certain food systems under external forces by enabling predictions of their flow and deformation (Wang et al., 2019).

Peas are known to have different varieties with distinct components such as protein, oil and starch content which could influence their functional properties such as techno-functional, rheological, and thermal properties. Machine learning presents the chance to examine data and possesses an edge over calculations performed by humans since machine learning algorithms are better equipped to recognize unconventional patterns within extensive data sets (Kim et al., 2018). At present, one of the prevalent techniques in machine learning for recognizing risk factors that can forecast the development of complications is polynomial regression. On the other hand, artificial neural networks (ANNs) are a different type of machine learning that is nonlinear and highly adaptable, unlike polynomial regression (Sanusi & Akinoso, 2021). This characteristic could enable the detection of nonlinear patterns, leading to more accurate predictions (Sanusi & Akinoso, 2022). Therefore, it is important to predict the techno-functional, thermal and rheological properties of pea protein products with artificial neural network machine learning because these properties have a significant impact on the performance of the final food

product during processing, storage, and consumption. Techno-functional properties such as protein solubility, water absorption capacity, and emulsifying capacity play a crucial role in determining the characteristics of the final food product, such as texture, appearance, and stability. Thermal properties such as onset temperature, peak temperature, and gelatinization enthalpy can indicate the suitability of pea protein products for various food processing applications. Rheological properties can aid in predicting how pea protein products will behave in certain food systems under external forces, such as during mixing, shearing, or pumping. By accurately predicting these properties with artificial neural network machine learning, manufacturers can optimize their processes to produce high-quality, consistent products that meet consumer expectations. Despite the tremendous works reported on the effect of composition on the different legume proteins, literature is spare on the evaluation of the effect of products. Therefore, based on the information available on the compositional effect, it would be worthy to study the effect of this composition, namely protein, oil, and starch content on the techno-functional, rheological and thermal of pea protein products.

1.2 Hypothesis

The composition of pea protein products (flour, concentrate and isolate), including protein content, starch content, and oil content, could significantly influences their techno-functional, thermal, and rheological properties. Limited understanding of these relationships is evident due to the scarcity of available literature. Conventional methods for evaluating these properties are resource-intensive, expensive, time-consuming, and inflexible. By utilizing modern data analytical tools such as pareto, principal component analysis, and artificial neural network machine learning algorithms, researchers can effectively interpret and predict results, identifying trends, patterns, and correlations. This approach could facilitate informed decision-making and meaningful conclusions. Through a comprehensive study of the influence of composition on the techno-functional, thermal, and rheological behavior of pea protein products, valuable insights can be obtained regarding their potential applications in the food industry.

1.3 Research Objectives

The overall objective of this research was to evaluate the techno-functional, thermal, and rheological properties of pea protein products using pareto, principal component analysis, and artificial neural network.

The specific objectives are to:

- 1. determine relationships between the composition of pea protein products and their techno-functional and thermal properties using pareto analysis,
- 2. determine relationships between the composition of pea protein products and their rheological properties using pareto analysis and,
- 3. evaluate the techno-functional, thermal and rheological properties for pea protein products using artificial neural network machine learning algorithm.

CHAPTER TWO

LITERATURE REVIEW

2.1 Pea protein products

Pea is one of the legume crops that has been an important part of the diet for thousands of years (Jiang et al., 2017). It is commonly regarded as the seed or the peapod of either field pea or garden pea (*Pisum sativum*). However, peas are sometimes quoted as a word describing further plant seeds such as pigeon pea, chicken pea, cowpea, and grass pea. Peas are often identified as the green or yellow annual cotyledon diversities that belong to the leguminous family called Fabacea. Pea protein are protein components extracted from pea seeds and are widely used as functional ingredients in various food systems due to their exclusive nutritional, technofunctional, rheological and thermal properties (Jiang, 2015). Pea proteins are typically processed into pea flour, pea protein concentrate and pea protein isolate, which are generally referred to as pea protein products as shown in Figure 2.1. Pea flours are typically made from dehusked and milled beans. Pea protein concentrations (PPC) are made by air-classification of the pea flours (attained through the milling beans), a mechanical drying technique or solvent-based process that separates protein from other non-protein components of the pea such as fibre and starch granules. Protein concentration in concentrates is about 50-80%, with the remaining components being carbohydrate, fats and minerals. Instead, a further processing of the protein concentrate through ultrafiltration or other separation methods by separating and concentrating the concentrates produces pea protein isolates (PPI). Alkaline or acidic conditions can also be used for protein isolate extraction (Sandberg, 2011). Protein isolate usually contains around 90% protein and has a lower content of carbohydrates, fats, and minerals compared to protein concentrate.

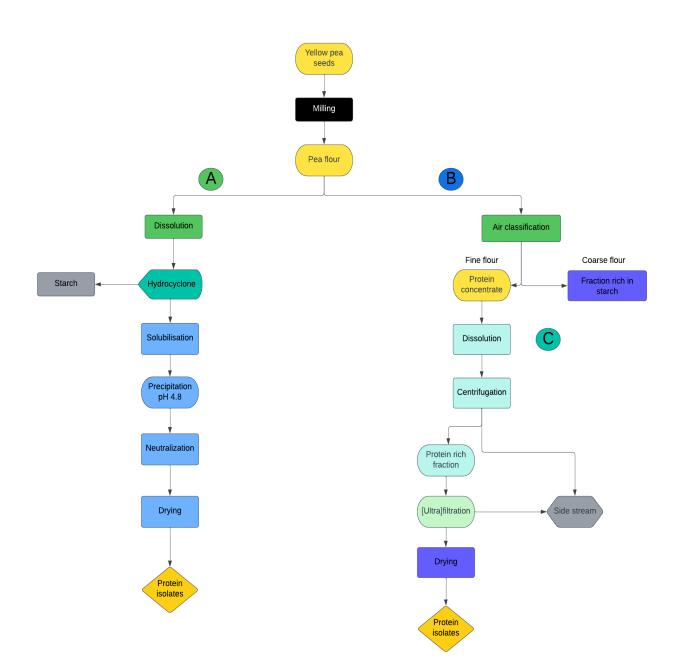


Figure 2.1. From pea seeds to pea protein ingredients. A. Wet extraction; B. Dry fractionation; C. Mild fractionation (Reinkensmeier et al., 2015).

The successful incorporation of pea proteins into the human diet primarily relies on consumers' preferences (Jiang et al., 2017). A few numbers of literature have focused on the potential utilization of pea protein products (isolate, concentrate and flour) in various food products, as they function as a supplement or substitute to grain flour (such as in pasta or bread) or to meat products (such as in patties, and hamburgers), as well as in texturized products, ready-to-eat snacks, and as milk substitutes (Baugreet et al., 2016; Chao et al., 2018). The inclusion of pea protein products chiefly influences product texture, mouthfeel, and cooking duration of the product. Intrinsically, alterations of the pea protein products are relatively required for the acceptability of the organoleptic characteristics of the food products processed from the pea products. For instance, pea protein concentrate (PPC) has a unique application in the development of non-fat-dry milk alternatives in the baking industry. A non-milk icy dessert was produced due to the high organoleptic properties of pea protein isolate. Pea protein isolates possess high water, and fat binding capability, protein solubility, foaming, and emulsifying capacity which helps to ensure high stability and texture of the targeted product (Sandberg, 2011). Pea protein has also a great application in fish and meat products, pastry and biscuits making, soups, desserts, sauces, and baby food, as shown in Table 2.1.

2.2 Techno-functional Properties of Pea Proteins

The techno-functional properties of foods are associated with its physical and functional properties specifically the behavior of food during processing, preparation, storage, and overall consumption (Pazmiño et al., 2018). In addition, the techno-functional characteristics of foods are essential parameters with ultimate significant influence during their cooking (Bishnoi and Khetarpaul, 1993). The techno-functional property of foods depends on the various functional properties such as organoleptic (color, odor, flavor), rheological/textural (chewiness, aggregation, elasticity, adhesiveness, cohesiveness, dough formation, network formation, extrudability, texturizability), kinesthetic (texture, mouthfeel, smoothness, grittiness), hydration (solubility, gelation, swelling, water absorption, gelling, syneresis, thickening, wettability, viscosity), and surface (emulsification, film formation, foaming) which are usually possessed by food proteins.

Products	Ingredients	References
Beef patties	Pea protein isolate (PPI), rice protein (RP), and lentil flour (LF)	Baugreet et al. (2016)
Salad dressing	Lentil, pea protein isolates, and chickpea	Ma et al. (2016)
Noodles	Yellow field pea protein isolate (PPI) and semolina	Chao et al. (2018)
Gluten-free muffins	Field pea, kidney bean, and amaranth protein isolate	Shevkani & Singh, (2014)
Pea protein-based yoghurt	Pea protein and yoghurt starter culture	Yang et al. (2021)
Grass pea flour	Ground grass pea	Romano et al. (2019)
Pea protein flour	Nutralys F85M (R1), Nutralys S85F (R2), PURIS Pea 870 (C1), Vitessence Pulse 1550 Protein (I1), and Pea Protein 85 (G1)	Burger et al. (2022)

Table 2.1: Pea protein-based products

Major functional properties of pea protein in terms of the techno-functional properties are protein solubility, emulsification, foaming capacity, foaming stability, water absorption capacity, water absorption index, water solubility index, swelling index, and amino acid sequence (Wijaya et al., 2015). The techno-functional requirements of pea protein products vary depending on how it will be used in particular food and food system applications. A protein's techno-functional properties are often influenced by various elements, which are classified into two categories, including the intrinsic and extrinsic influences. The amino acid sequence and composition, size, shape, hydrophobicity/hydrophilicity ratio, conformation, and reactivity are the intrinsic factors. The ionic strength, pH, temperature, conformation, the fraction of hydrophobicity to hydrophilicity, and the extraction method are extrinsic factors that might influence the techno-functional properties of pea protein is important for developing high-quality food products that meet consumer demand for sustainable plant-based ingredients with desirable sensory and nutritional qualities. Some major techno-functional properties of pea protein are well-defined and highlighted in Table 2.2.

2.2.1 The solubility properties of pea protein products

The protein solubility is a dependent factor on the ratio and organization of hydrophobic and hydrophilic groups on the surface of a molecule of protein and the protein solubility is consequently determined by several intrinsic factors. These include such as amino acid distribution and composition, charge, isoelectric point, molecular flexibility. The extrinsic factors include temperature, ionic strength, and pH) (Lam, 2016). In order to achieve optimal performance in food processing applications, it is important for proteins to exhibit good solubility. (Barac et al., 2010). The solubility of pea proteins affects other functional qualities like emulsification, foaming, and gelation (Burger & Zhang, 2019). The solubility of protein differs based on the quantity of polar and a-polar groups and how they are arranged within the molecule (Barac et al., 2012). Protein solubility changes with ionic strengths, and pH have a significant impact on their solubility properties (Boye et al., 2010b; Barac et al., 2014). The proportion of the major proteins may have an impact on the solubility of the protein product (Barac et al., 2010; Barac et al., 2012; Barac et al., 2014).

Property	Definition		References	
Protein Solubility	Describes the ability of pea protein to dissolve in water or other solvents under		Jiag et al. (2015);	
	specific conditions (temperature, pH, ionic strength, and protein concentration).	Wijaya	et	al.
	At neutral or slightly alkaline pH values, pea protein is highly soluble between	(2015)		
	80-90%, but can be reduced at low or high pH values, where the protein may			
	undergo denaturation or aggregation. Also, an important protein requirement for			
	industrial use as emulsion, foams, and gels.			
Foaming Capacity	Ability of pea protein to form stable foam as air is introduced into an aqueous	Wijaya	et	al.
	solution of the protein. It is also a protein adsorption mechanism enabling the	(2015)		
	creation of new interfaces due to a reduction in surface tension. Foaming			
	capacity is essential in several food applications including whipped toppings,			
	meringues, and aerated foods to create desirable textures and mouthfeel in the			
	products.			
Foaming Stability	Refers to the ability of pea protein to maintain the volume and structure of a	Wijaya	et	al.
	foam over time or the ability of protein to stabilize a two-phase system	(2015)		
	involving air cells, that is detached by a continuous thin layer of liquid, against			
	mechanical and gravitational stresses. This depends on strength of protein layer			
	and its gas permeability. It also depends on the temperature, protein			
	concentration, and pH.			
Water Absorption	Ability of pea protein to uphold its particular and extra water as it experiences	Wijaya	et	al.
Capacity	force, centrifugation, heat or pressing. Water absorption capacity is an essential	(2015)		
	functional property that can influence pea protein product processing, texture,		orio e	t al.
	and shelf life stability.	(2018)		
Water Solubility	The water solubility index (WSI) of pea protein describes a measure of the	Chandra	et	al.
Index	quantity of protein that can dissolve in water. Pea proteins have good water	(2015)		
	solubility, which is an important functional property for their use in a variety of			
	food applications where a smooth and creamy texture is desired.			

Table 2.2. Definition of Techno-functional Properties of Pea Proteins

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Oil Absorption	Describes the quantity of oil absorbed per unit gram of protein. It is an	Wang et al. (2020)
Capacity	important techno-functional property that influences products mouthfeel,	
	product yield, flavor, and texture. The hydrophobic and hydrophilic regions of	
	pea proteins allow them to interact with both water and oil which help to	
	stabilize emulsions and improve the overall stability of food products.	
Emulsifying	Measures the activities of pea proteins to form and stabilize an emulsion over	do Carmo et al.
Capacity	time. It describes its ability to interact with oil and water phases in an emulsion.	(2020) ; Pedrosa et
	This is largely influenced by product surface properties such as hydrophobicity	al. (2020)
	and charge.	

Major pea proteins are globulins, which are most soluble above and least soluble below the point of isoelectric (pH 4.5), respectively (Barac et al., 2010; Barac et al., 2014). However, in general, proteins tend to have higher solubility at pH values that are close to their isoelectric point (pI), where the net charge of the protein is zero. At pH values away from the pI, the protein carries a net charge, which can affect its solubility. As a result, natural pea proteins and the products made from them have a U-shaped pH-solubility dependence, as is also true of some legume proteins (Barac et al., 2014). According to Jiang et al. (2017), the protein solubility content of a commercially controlled pea protein isolate (PPI) was affirmed to be 2.07 mg/mL, and a solubility of 8.17% by pH Shifting method, which is relatively lower compared to most reported studies (Barac et al., 2010; Adebiyi and Aluko, 2011). The reason for the reduced solubility levels in commercially available pea protein products was attributed to the heat-induced alteration (Shand et al., 2007). The variation in values may be triggered by the various PPI method of treatment reported in the literature. Comparably, the salt-extraction method of protein was recorded to show a unique solubility to alkali or acid precipitation method of extraction (Jiang et al., 2017). Also, the solubility of four pea protein (PP) powder was analyzed. The solubility of R1, R2, G1, and C1 varied between 35.9 and 50.6 g kg-1 at pH 4.0, 110.7-199.5 g kg-1 at pH 2.0, and 89.5 and 124.5 g kg-1 at pH 9.0. The protein solubility changes as the pH are driven away from the point of isoelectric (pH of 4.0-5.0), which was comparatively small. Achieving an ideal emulsifying property in a food protein necessitates a greater degree of solubility, which facilitate the movement of proteins towards the oil/water interface (Barac et al., 2010; Karaca et al., 2011).

2.2.2 The emulsifying property

When creating food systems, proteins can be extensively exploited as emulsifying agents because they are surface-active and amphiphilic substances. Proteins' emulsifying abilities or activities and emulsion stability are typically used to describe their emulsifying capabilities. Emulsion activity measures the amount of oil that a protein can emulsify per unit of each protein, while emulsion stability measures how stable an emulsion is over a specific period (Boye et al., 2010a). Emulsion suitability of an ideal protein and protein isolate is a dependent factor on the degree of proteins diffusion into the interface, and on the deformability of its conformation under the effect of surface denaturation (interfacial tension). For a protein to function as an effective

emulsifying agent in an oil-in-water emulsion, it should possess certain characteristics such as low molecular weight, a balanced composition of charged amino acids, high water solubility, residues that are both polar and non-polar, a stable conformation, and well-established surface hydrophobicity (Barac et al., 2014).

Several methods have been employed in the literature to determine the emulsifying properties of foods, for instance, turbidimetric methods, droplet size measurements, and conductivity (Burgera & Zhanga, 2019). The two major turbidimetric methods of measuring emulsification are emulsifying stability index (ESI) and emulsifying activity index (EAI). Emulsifying activity index determines the protein's degree of adsorption toward the boundary, whereas emulsifying stability index is a determination of the layer absorption stability over a specified period (Boye et al., 2010; Stone et al., 2015a). The measure of the quantity of oil needed to reverse an emulsification state of oil-in-water emulsion into a water-in-oil emulsion is the emulsion capacity (EC) (Stone et al., 2015). Taherian et al. (2011) defined emulsion stability as the measure of the quantity of creaming per the time specified. Another common technique is the emulsion particle size or diameter of particle droplet, which is assessed using dynamic light scattering. Often, particle droplet of smaller sizes having a narrower size distribution is usually measured in water or buffer systems.

Emulsifying capacity is a critical functional attribute within the food industry. Proteins are responsible for forming colloidal systems known as oil-in-water emulsions, which could transport various substances such as functional lipids and nutritional supplements (Jiang et al., 2015). Pea protein exhibits excellent emulsifying properties, making it well-suited to produce oil-in-water emulsions (Lu et al., 2019). Pea protein isolate are identified to usually have greater emulsifying properties at pH 3.0 than at some pH values, although most proteins showed the weakest blending properties at pH 5.0 (Lam et al., 2018). Pea proteins' alkaline treatment alters the structural makeup of pea protein and improves their capacity to prevent emulsions oxidation (Jiang et al., 2015). Emulsions are immiscible liquid dispersion. Chemicals (binding agents) that form interface films and stop the disperse phases from mixing stabilize them.

2.2.3 Foaming properties of pea

In food systems, proteins serve as foam-forming and stabilizing components (including baked goods, and desserts sweets). In similarity to how proteins have various emulsifying qualities, different proteins have different capacities for forming and stabilizing foams. This is a result of the varying physicochemical characteristics of the proteins (Barac et al., 2010; Barac et al., 2014). Good solubility, strong surface hydrophobicity, low molecular weight, a small net charge (meal pH), and simple denaturability are the characteristics of the perfect foam foaming and stabilizing protein (Barac et al., 2012).

Foaming stability (FS) and foaming capacity (FC) are typically used for the description of the foaming capabilities of proteins. Lam et al. (2017) defined foaming stability (FS) as the ability of protein molecule to maintain a stable foam (a two-phase system involving air cells that is detached by a continuous thin layer of liquid), against mechanical and gravitational stresses, and often ruled by film thickness, cohesiveness, and flexible deformation from stress. Generally, pea protein isolates are usually a good source of foaming stability. Several writers investigated the pea protein isolates' foam characteristics (Barac et al., 2010). These studies show that the foaming characteristics of pea protein isolates vary in pH and concentration. The study by Lam et al. (2017) on pea isolates produced from three pea genotypes recorded an FS value of between 68.0 and 69.6%. Nevertheless, Shevkani et al. (2015) established a higher FS value (94.0 to 96.0% after 30 minutes) for pea protein isolates produced from four pea cultivars. Agreeing to the study of Wierenga and Gruppen (2010), foaming stability showed a significant correlation to foaming capacity at r=0.63 and P <0.001.

2.2.4 Gelling properties of pea

The denaturation of proteins influences the gelation temperature of pea protein. Gelation qualities also heavily depend on protein content. Firmer gels are typically produced by higher concentrations of protein. The gelling point, however, does not depend on concentration. The characteristics of pea protein that cause gelation are only slightly affected by heating and cooling rates (Sun & Arntfield, 2010). The gelling point was affected by the heating rate in such a way that slower gelling was caused by higher heating rates (higher gelling temperatures). The elasticity of the gel was weakened as a result of faster heating and cooling rates (Barac et al., 2014). Wani et al. (2010) evaluated the gel property of starch obtained from three kidney beans

genotypes (Phaseolus vulgaris L. cv. Master Bean, Contendor, French Yellow). French Yellow gels exhibited the highest hardness (201.4 g) while the gel from Contender starch displayed the lowest content (153.8 g). The gel hardness was established to have a relationship between the amylose content as well as the viscosity. Additionally, gel produced from starches of different kidney beans recorded some values of cohesiveness ranging between 0.28 and 0.4, gumminess (43.1 and 89.4), and springiness (0.5 and 0.94). The French Yellow was observed to have the maximum gumminess content (89.4) while Master Bean had the lowest value of springiness (0.5) and gumminess (43.1), as the other three genotypes displayed the same values of springiness. Furthermore, adhesiveness and chewiness of kidney bean starch gels were affirmed to vary between 183.1 and 273.8 g.s and 23.9 and 81.6 g, respectively (Wani et al., 2010). The gel from Master Bean starch was observed to have the minimum chewiness and maximum adhesiveness while French Yellow had the maximum value of chewiness, where the maximum adhesiveness was observed at Local Red gels. According to Biliaderis (1997), the difference in the textural characteristics of starch gels could be an influence of the rheological properties of the amylose matrix, the relationship between the gel's continuous and dispersed phase, as well as the phosphorus content. These factors are consequently recorded to depend on the amylopectin structure, and amylose content (Yamin et al., 1999). In addition, the techno-functional properties of the pea protein products were well summarized in Table 2.2. The table highlighted the various studies on the techno-functional properties of protein ingredients (flour, concentrate and isolate) from different varieties of pea and focused on the major discoveries or result from the studies.

2.3 Thermal Properties of Legume Protein

The thermal property of food reflects the behavior of a food material when it is heated in a programmed heating rate of a differential scanning calorimeter (DSC) to assess the thermal transition. It is associated with enthalpy involved during food processing. Thermal transition refers to the changes that occur in a material's properties as it is heated or cooled, which can be characterized by changes in temperature, energy, or other physical properties, such as viscosity or thermal conductivity. It can provide insights into the material's composition, structure, and behavior (Ahmed & Rahman, 2014). Most legumes such as peas exhibit distinct thermal transitions, including starch gelatinization, protein denaturation, and other thermal peaks related

Pea	Techno-	Major discoveries on the techno-functional	Additional comments	References		
	functional Properties	properties				
Pigeon	Protein	The solubility capability of Pigeon protein	The decreased solubility could be	Pazmiño et al.,		
pea	solubility (PS)	concentrates of mature seeds, and green seeds	an outcome of pigeon pea pH near	(2018)		
		(MPPPC and GPPPC) was presented to increase at	the isoelectric protein point.			
		higher pH of 12.0 with solubility 62.77±0.35%				
		and 59±2.12% for MPPPC and GPPPC,				
		respectively.				
		While a decrease was observed at pH 4.0 with				
		solubility of 6.98±0.25% and 3.29±0.39% for				
		GPPPC and MPPPC, respectively.				
	Foaming	Mature and green pigeon pea PC showed a	The variation in the values	Pazmiño et al.		
	capacity	significant difference in the functional properties	obtained are attributed to protein	(2018)		
	(FC)	as MPPPC was recorded to have FC value of	changes in at various phases of			
	(I°C)	68.50% while the GPPPC recorded a FC value of	development, including			
		27.30%.	composition, structure, charges,			
			hydrophobicity, and concentration.			

	Foaming stability (FS)	GPPPC and MPPPC showed a higher value of FS of between 27.88% - 71.15% and 66.02% - 96.03%, respectively.	Ĩ	Pazmiño et al. (2018)
Pigeon pea	Protein solubility	Pea protein isolate (PPI) treated using pH shifting and ultrasonication combination method where the solubility content pea protein s was 2.07 mg/mL and considered comparably low.	of the PPI applied in different	Jiang et al. (2017)
Field pea	Water Absorption Capacity (WAC)	The swift water uptake during preliminary soaking determines the water absorption capacity of peas, until it reaches a saturation level.	6 6	C
Kidney Beans	Water Absorption Capacity	The intermolecular rate of unification between complex starches due to force of association such as covalent and hydrogen bonding, influences the WAC.	(1.9–2.1 g/g) for different kidney	
	Pasting Characteristics	The starch granules of peas of larger sizes (for instance Sturt) showed to present pasting appearances of moderately greater viscosities and	had a substantial influence for all	0

		inferior temperatures.		
Grass Pea	Water holding	Using the method of D'Appollonia, WHC of pea	WHC of pea is significantly	Romano et al.
	capacity (WHC)	flour was presented to be 176%.	specific to product characteristics, including retrogradation of starch, product staling and moistness.	(2019)
Pea Protein	Protein composition	Major bands of pea protein were spotted which are the 8S globulin, legumin, vicilin, and convicilin subunits.	1 1	e

to amylose-lipid or protein-lipid complexes. The thermal transition is mostly associated with three temperatures: onset (T_o), peak (T_p), and enthalpy or conclusion (T_c), following the concept of glass transition. The midpoint of the transition is considered during the first scan due to the irreversible transition of legume starch/protein. Protein and starch undergo thermal transitions that are influenced by the available moisture content that fluctuates during processing (Ahmed et al., 2021).

2.3.1 Peak temperature/ Gelatinization

When protein samples are isolated in aqueous dispersions, they experience a phase transition and exhibit a sharp melting peak at a specific temperature, known as gelatinization. This process is irreversible and causes the native semicrystalline structure of the starch granule to transform into a polymer solution in the rubbery state. The gelatinization temperature is influenced by the glass transition of the amorphous fraction of the starch and the type of starch present. In the case of legumes, which possess a C-type structure comprising an inner B-polymorph surrounded by an A-type polymorph, the gelatinization of pulse starch starts from the central hilum region. The Btype polymorphs melt at a lower temperature than the A-type polymorphs due to their loose packing (Dong & Vasanthan, 2020). Table 2.3 provides an overview of the thermal transition temperatures of starch from various legume sources. The thermal properties such as enthalpy (ΔH_{gel}) of starch are influenced by factors such as legume source, isolation method, moisture content, and other factors. The gelatinization enthalpy is indicative of the order-disorder transition of starch granules, particularly the breakdown of amylopectin crystallites. ΔH_{gel} quantifies the loss of molecular order and crystallinity within the starch granule (Cooke & Gidley, 1992). The melting of amylopectin crystallites is determined by the crystal type and architecture within the grain, and the processing conditions, such as heat/pressure severity. The range of enthalpy for starch gelatinization is wide, typically between 4 to 18 Jg⁻¹ (Chávez-Murillo et al., 2018; Do et al., 2019). However, these values are not fixed and depend on several factors. A higher enthalpy value is associated with a more ordered structure that demands higher energy, indicating higher granular resistance to gelatinization (Barichello et al., 1990). Li et al. (2011) conducted an evaluation of the thermal properties of starches from ten mung bean cultivars and reported that T_o, T_p, and T_c values varied from 57.3°C to 62.4°C, 66.5°C to 66.8°C,

Legumes	T ₀ (C)	T _p (C)	T _c (C)	References
Pigeon pea	70.04	80.74	93.03	Olagunju et al. (2020)
Pea starch	54	62.6	69	Hoover & Manuel (1996)
Pea starch	57.3	65.3	72	Guo et al.(2020)
Chickpea	59.6	64.5	70.5	Do et al.(2019)
Chickpea flour	68.43	69.14	70.28	Chávez-Murillo et al.(2018)
Field pea starch	60	66.1	77.8	Dong & Vasanthan (2020)
Lima bean	68.4	76.5	85.2	Do et al. (2019)
Mung bean starch	59.6	71.6	75.4	Guo et al. (2020)
Faba bean starch	61.4	66.4	77.1	Dong and Vasanthan (2020)
Adzuki bean	58.8	66.7	75.1	Do et al. (2019)
Black bean starch	62.5	71	82	Hoover & Manuel(1996)
Red bean starch	60.4	69.4	78	Guo et al. (2020)
Black bean flour	67.36	70.26	72.41	Chávez-Murillo et al. (2018)
Lentil starch	56	62.4	69	Hoover & Manuel (1996)
Lentil starch	55.71	63.72	68.78	Ahmed et al. (2016)
Lentil starch	54.4	59.7	68.4	Do et al. (2019)

Table 2.3: An overview of the thermal properties of starch from various legume sources

 $\overline{T_o}$ is onset gelatinization temperature; T_p is peak gelatinization temperature; and $\overline{T_c}$ is enthalpy or conclusion gelatinization temperature.

and 73.3°C to 75.7°C, respectively. The lowest and highest enthalpy values were recorded from 8.2 to 16.4 J g⁻¹, respectively, with an average of 12.2 J g⁻¹.

2.3.2 Protein denaturation

Legume protein denaturation can be identified by a distinct thermal peak using DSC, and Table 2.4 summarizes the thermal denaturation temperatures of legume proteins. The denaturation temperature (T_d) indicates the thermal stability of the protein, with higher T_d values indicating higher thermal stability for globular proteins. For various bean proteins, T_d values ranged between 84°C and 91°C, with adzuki and kidney beans having the highest T_d values of 90.1°C and 90.2°C, respectively (Tang, 2008; Yousif et al., 2003). Legume protein isolates (PIs) showed only one major endothermic peak with denaturation attributed to 7S or 8S vicilin fractions. The thermal stability of legume proteins is believed to be associated with the disulfide bond within the subunits of vicilin. Rui et al. (2011) reported T_d values for a series of bean PIs and observed a single endothermic peak likely corresponding to the denaturation of 7S vicilin. Cranberry and light red kidney bean PIs have lower thermal stabilities than other bean types. For kidney bean PI, three distinct peaks were recorded, corresponding to peak I (57.12–62.16°C; enthalpy 3.36– 4.5 Jg⁻¹), peak II (88.78–101.46°C; enthalpy: 1.42–2.97 Jg⁻¹), and peak III (110–115.71°C; enthalpy: 300-500 Jg⁻¹) (Ahmed et al., 2018). The third peak was relatively broad with an abruptly higher enthalpy value, and these salt-soluble proteins are believed to be globulins, with T_d likely corresponding to the denaturation of vicilin (7S) and legumin (11S). A high T_d value indicates a more compact tertiary conformation of the polypeptides (Tang & Sun, 2011).

2.4 Thermal Properties of Pea Protein

The thermal properties of food ingredients are frequently altered by heat treatment. Stabilized emulsions of protein are frequently exposed to thermal treatment methods like sterilization and pasteurization (Cui et al., 2021). Usually, partial unfolding and subsequent protein aggregation are caused by heat processes exceeding the temperature of denaturation (Wang et al., 2012). Protein subunits in solutions of pea protein become dissociated when heated, and the temperature of heating affects how proteins assemble. Temperature is a key factor in the heat-related protein aggregation process (Cui et al., 2021). During the preparation or extraction of pea protein products, heat treatment is applied, which necessitates the examination of their thermal properties. These properties can include the onset temperature, peak temperature, and

Legumes	Moisture				
	Content	T_0 (°C)	Td(°C)	$T_c(^{\circ}C)$	References
	(%)				
Pea protein isolate	7.3	58.10	76.20	94.40	Philipp et al. (2017)
	9.8	34.70	51.50	68.30	
	12	32.0	40.30	48.70	
Kidney bean protein isolate		62.16	62.89	65.32	Ahmed et al. (2018)
		85.30	90.20		Tang (2008)
Pink bean		84.70	89.55	_	Rui et al. (2011)
White bean		85.33	90.58		Rui et al. (2011)
Black bean		84.18	88.97		Rui et al. (2011)
Red bean		79.50	87.10		Tang (2008)
Red kidney bean		85.30	90.20		Tang (2008)
Mung bean		77.30	84.60		Tang (2008)
Dark red kidney bean		85.77	91.14		Rui et al. (2011)
Light red kidney bean		77.13	82.14		Rui et al. (2011)

Table 2.4: Thermal denaturation temperatures of legume proteins

Note: where To is the onset temperature, T_d is the denaturation temperature, enthalpy or conclusion gelatinization temperature and – means not reported.

denaturation temperature, as well as gelatinization enthalpy. The onset temperature signifies the temperature at which protein start to lose their functional properties due to denaturation. This property can vary significantly depending on factors such as the type of protein used, treatment methods, and formulation. This was noted by Liang et al. (2013), who emphasized the importance of understanding onset temperature for an effective application of pea protein in different products. The denaturation temperature provides an indication of a protein's thermal stability, with a high denaturation temperature typically indicating high thermal stability in globular proteins. The gelatinization enthalpy measures the proportion of undenatured proteins and the level of protein structural organization. According to Shevkani et al. (2015), these properties need to be studied to assess the suitability of pea protein for different applications. Emkani et al. (2021) suggest that variations in thermal properties are influenced by the product's protein conformation, amino acid composition, and structure. For example, compared to pea isolates, kidney bean isolates exhibit a higher denaturation temperature value. Thus, understanding the thermal properties of pea protein is critical for the development of new products and the improvement of existing ones.

2.5 Rheological properties of plant proteins

Rheology is the study of material flow and distortion. The field of physics known as rheology focuses on how materials behave when forces or stresses are applied, leading to deformation or flow (Fischer et al., 2009). The study of the deformation and flow of raw materials, intermediate products, and end products of the food, is described as food rheology. In a food system, the rheological characteristics depend on the ingredient system and composition. Due to its intimate connection to the structure and behavior of food materials, it is a science that is very important to the food industry (Joyner, 2018). Rheological properties are attributes of a material that control how precisely these deformation or flow behaviors take place. Continuous deformation over time is a definition of flowing. Viscosity, flow, deformation, yield stress, relaxation times, and compliance are the widely known rheological characteristics. Viscosity is an important characteristic of rheology that describes the amount of fluid resistance required for the gradual deformation caused by tension or shear. Apparently, viscosity defines the flow resistivity of a fluid. (Fischer et al., 2009).

Rheological characteristics of food control how food behaves when they experience mechanical loading. The distinct shape of a solid structure undergoes stress and deformation when exposed to a mechanical load. On the other hand, in the case of liquid without a distinct shape, only experience a change in the atomic position and does not undergo stress and deformation (Álvarez-Castillo et al., 2021). The changes in the atomic position of liquid are referred to as creep. Several forms of mechanical stress are applicable to materials such as shear or torsional, and these stresses are withstood by substances based on stress variation (Sternet al., 2001). Structuring and understanding the rheology properties of food enable food researchers to control the textural characteristics of food through instrumental measurements. However, it is often difficult to evaluate these properties, as a result of complexity in the structure and composition of food, and due to poor establishment of causes of food changes that occur during processing (Yang et al., 2018). It is noteworthy to understand the challenges of rheological study (rheometry) as an indication of the textural properties of food. Principal rheological tests of food are restricted to some food materials that are isotropic and homogeneous. Food materials of larger particles possess an anisotropic structure such as in meat and mozzarella, and rapidly become separated which causes difficulty in the attainment of an accurate analysis (Oppen et al., 2022). Also, the rheological tests often demand several rheological analyses to obtain enough data for the prediction of textural behavior.

The tests are also not suitable for a measure of temporal changes, as they function best for firstbite textural attributes. Notwithstanding, the study of rheological behavior remains an auspicious tool to understand those parameters that have a significant impact on the textural behavior of food, since deformation, flows, fractures, and food breakdown are important elements of textural sensations (Joyner, 2018). Various devices have been employed for the measurement of rheological properties of food, including rheometers, dynamic oscillators, and farinographs (Mirsaeedghazi et al., 2008; Wang and Hirai, 2011). A rheometer and farinograph was used to evaluate the suitability of different rice doughs to make rice bread by studying their rheological characteristics (Sivaramakrishnan et al., 2004). Also, the dynamic rheological characteristics of an extruded pastes from flaxseed-maize was investigated by creep-recovery and dynamic oscillation tests (Wu et al., 2010). Substantial attention and efforts have been focused to evaluate the rheological behaviors, and properties in relation to sensory texture characteristics of foods, as summarized in Table 2.5.

Rheological	Products	Major characteristics	References
properties			
Linear viscoelastic	Dough	The test of frequency sweep was done within LVR to determine the mechanical	Álvarez-Castillo et al.
range (LVR)		spectra of the dough formulation. Addition of PPP (porcine plasma protein) was	(2021)
		observed to cause a steady rise in both storage moduli (G'), and loss moduli	
		(G'').	
Viscoelastic	Mayonnaise	Mayonnaises upholds a viscoelastic property which can be assessed by yield	Sternet al. (2001)
		value. Nonlinear relationships were revealed between the apparent viscosity and	
		yield. However, correlation was established between the sensory properties of	
		mayonnaise and apparent viscosity, where otherwise was established for yield	
		value.	
Viscosity	Chocolate	At a steady temperature of between 32 and 40°C, and pressure of between 3.5	Hao et al. (2010)
		and 7.0 Pa, the viscosities of chocolate were nearly remained constant. There is	
		difficulty in the shaping of chocolate at extreme temperatures, and pressures.	
Storage and loss	Dough	The flour, butter, and sucrose contents steadily and greatly increase the G' and	Yang et al. (2018)
moduli		G'' value	
Shear stress	Milk powder mixed	The yield stress of the paste after 3D printing was associated with the stability of	Lille et al. (2018)
	with starch and	paste shape. A constant value of plateau was observed to be continuous at small	
		value of stress by G' during stress sweep measurements. Also, the structure of	

Table 2.5: Rheological Properties of Foods

cellulose nanofibre the samples showed to be gel-like, and elasticity-dominated

Hardness	Gelatin	The gelatin with an exclusive of additives exhibits low self-support profiles where the samples possess higher fracturability and hardness, and result in sample flaws.	Kim et al. (2018)
Pseudoplastic characteristics	Dough	The dough was simply extruded due to the pseudoplastic characteristics as the shear rate increases with decrease in the viscosity.	Yang et al. (2018)
Apparent viscosity	Gum (carbohydrate)	The addition of gum as an additive increases the raw material apparent viscosity, which gives a hard structure to the extruded products.	Israr et al. (2018)
Plasticity and Extensibility	Expanded wheat flour mixed with dough	The plasticity of the dough increases with the flour while it decreases with the dough extensibility. This is suitable in forming of 3D printed objects of sharping geometric	Martimez et al. (2013)
Storage modulus	Pea protein isolate (PPI)	The storage modulus (G') value of PPI_n with an exclusive of the enzymatic crosslinking stayed constant throughout the heat treatment between 20°C and 71°C, where the (G') was observed to rise after 71°C	Shand et al. (2008)

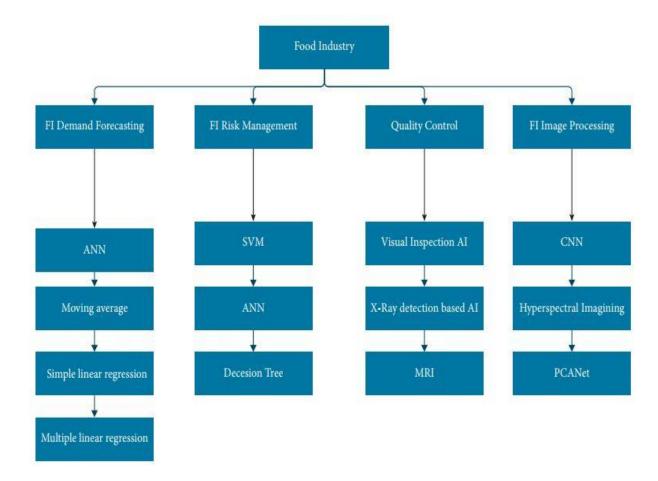
2.6 Machine Learning (ML)

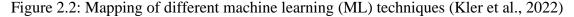
Machine learning uses artificial intelligence to improve the execution of tasks that are guided by existing data, without the use of explicit programming (Schleder et al., 2019). Due to the increased use of big data in numerous industrial and scientific fields, machine learning has grown in prominence in recent years (Jordan et al., 2015). Machine learning (ML) is currently used to create predictions and recommendations based on mathematically derived models as cutting-edge powerful technology (Ongsulee, 2017). Many efforts have been made to apply machine learning in other scientific domains outside of computer science. Particularly, it has been widely used in biomedical fields to detect disorders and enhance diagnostic accuracy (Nilashi et al., 2017: Vayena et al., 2018).

Furthermore, the prediction of industrial accidents, early earthquake warnings by discrimination of seismic waves, and inferring weather forecast uncertainty, ML applications are rooting for more multiplicity and complexity. Principal component regression (PC-R), artificial neural network, least square regression (OLS-R), partial least square regression (PLS-R), stepwise linear regression (SL-R), boosted logistic regression (BLR), k-nearest neighbors' regression (kNN-R), support vector regression (SVM-R), and random forest regression (RF-R) are the various techniques of ML. ML has a wide range of applications in food processing as it has assisted in minimizing the cost of food sensory evaluation, in the making of a decision, as well as improving the business tactics (Lu et al., 2020). Erban et al. (2019) employed ML technology to investigate food identity markers through metabolomics, while the analytical evaluation of food quality and authenticity by machine learning was recently reviewed (Jimenez-carvelo et al., 2019). In addition, an artificial recurrent neural network (such as long short-term memory (LSTM)) has been applied for pH detection during the fermentation process of cheese (Li et al., 2020). Furthermore, machine learning has been affirmed with the potentiality of sales trend prediction in the food industry (Tsoumakas, 2019). ML was also utilized to forecast the generation of food waste and present an intuition to the production system (Garre et al., 2020). Also, in the UK, machine learning has proven to be helpful as it was used for the prediction of food insecurity (Parnell et al., 2020).

2.7 Machine Learning Techniques

To achieve accurate predictions of future data or trends, machine-learning techniques or approaches rely on training samples to discover patterns (Alpaydin, 2020). However, as data analysis becomes more complex and the scale and difficulty of data increases, traditional machine learning methods have become inadequate. The different machine-learning approaches can be seen in Figure 2.2. In the following subsections, traditional machine learning methods will be introduced separately, along with their algorithms and their applications in food processing.





2.7.1 Traditional Machine Learning Methods

In traditional machine learning, small sample sets are manually analyzed to extract features. This approach balances the validity of the learning results with the interpretability of the learning model and provides a framework for solving learning problems when limited samples are

available. Traditional machine learning is categorized into supervised learning, unsupervised learning, and reinforcement learning based on the training method and whether the training data have labels or not. The theoretical foundation of traditional machine learning is statistics, and its primary analysis includes pattern classification, regression analysis, probability density estimation, and other similar analyses. Some common algorithms used in traditional machine learning include Support Vector Machine, Logistic Regression, K-nearest neighbors, K-means, Bayesian Network, Fuzzy C-means, and Decision Tree.

Support Vector Machine (SVM): SVM is a supervised learning method that classifies data by identifying a hyperplane that meets the classification requirements and maximizes the interval between the hyperplane and the samples in the training set, as explained by Cortes et al. (1995). To achieve this, a convex quadratic programming problem is formulated and solved by minimizing a regularized hinge loss function. However, the data may not always be linearly separable, making it difficult to find a hyperplane that satisfies the condition. In such cases, a kernel function is used to map the input from the low-dimensional space to the high-dimensional space. In the high-dimensional space, the optimal separating hyperplane is created to classify the nonlinear data.

Logistic Regression (LR): Logistic Regression is a machine learning technique utilized to solve binary classification problems (i.e., 0 or 1) and estimate the probability of a particular outcome, as explained by Kleinbaum et al. (2002). However, the output of logistic regression is not a probability value in the mathematical sense and cannot be directly used as such. Instead, the output is the odds ratio between the probability of the predicted category occurring and not occurring. This enables the classification of the category, which is useful in tasks that utilize probability for decision-making. For instance, it can be applied to predict whether a specific customer will purchase a particular product or not.

K-Nearest Neighbors (KNN): KNN is a classification method that operates by measuring the distance between different feature values. The basic concept is that if the majority of the K nearest neighbors in the feature space belong to a specific category, then the sample being evaluated also belongs to that category. Generally, K is a positive integer no greater than 20, as described by Altman (1992). In the KNN algorithm, the chosen neighbors are all the objects that have been correctly classified. This technique makes a category determination based solely on

the category of the nearest sample or samples in the classification decision. The distance between objects is calculated as a non-similarity index to avoid the matching problem between objects. Typically, the distance metric used is the Euclidean or Manhattan distance.

K-means: K-means is a widely used unsupervised learning technique for clustering. The purpose of clustering is to automatically group a set of unlabeled data into several categories, ensuring that the data within the same category share similar characteristics. However, this method can only be applied to continuous data and requires the user to manually specify the number of categories before clustering. The similarity between data can be evaluated using Euclidean distance, which is a crucial assumption of the K-means algorithm (Likas et al., 2003).

Bayesian Network (BN): A Bayesian network is a model that uses the Bayesian method to reason causally under uncertainty. In mathematical terms, it is represented by a directed acyclic graph (DAG) G = (I, E), where I is the set of nodes in the graph and E is the set of directed edges connecting them. Each node i in the DAG represents a random variable Xi (Friedman et al., 1997).

Decision Tree: A decision tree is a hierarchical structure, in which each non-leaf node indicates a test on a specific attribute, each edge represents the outcome of that test, and each leaf node denotes a final classification or decision (Safavian et al., 1991).

Random Forest: Random Forest (RF) is an ensemble learning technique that utilizes multiple decision trees, each of which works independently of the others. In a classification task, when a new sample is introduced, each decision tree in the forest independently generates a decision. The classification result that appears most frequently among all the trees is then considered the final result. This method of combining multiple decision trees with no correlation between them is known as Random Forests (Breiman, 2001).

Fuzzy C-means (FCM): In contrast to the K-means algorithm that assigns a label to each sample and clusters them into a certain class, Fuzzy C-means (FCM) assigns a probability vector to each sample, indicating the likelihood of it belonging to each category, rather than just one (Ghosh et al., 2013). FCM is an unsupervised fuzzy clustering method, and its goal is to optimize the objective function.

Dimensionality reduction methods: When the number of features in practical applications exceeds a certain threshold, the performance of the classifier often decreases, which is commonly referred to as the "curse of dimensionality." The curse of dimensionality makes pattern recognition for high-dimensional data difficult. Therefore, it is necessary to reduce the dimensionality of the feature vector first. Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA) are popular methods for feature selection and dimensionality reduction. Although PCA and LDA have many similarities, they have different mapping goals. Both methods reduce the dimensionality of the sample space, but PCA maximizes the divergence of the mapped sample, while LDA maximizes the classification performance of the mapped samples. As a result, PCA is an unsupervised dimensionality reduction method, whereas LDA is a supervised dimensionality reduction method (Van Der Maaten et al., 2009).

2.8 Application of Machine Learning in Food Processing

Table 2.6 shows some of the applications of machine learning in food industries. Food processing is the conversion of raw materials into new edible materials with improved properties (Fellows, 2009). Food processing is categorized as primary and deep processing, depending on the degree of processing (Zhu et al., 2021). Primary processing involves simple procedures to keep the original nutrients of the product and meet transportation, storage, and reprocessing requirements, while deep processing involves elaborate procedures to further improve the characteristics of the products. Examples of primary processing include drying, shelling, milling of grains, slaughtering of live animals and poultry, and freezing processing of meat, eggs, and fis (Fellows, 2009; Zhu et al., 2021). Deep processing involves processing grains into different foods like bread, noodles, biscuits, vermicelli, or soy sauce, which is an important way to increase the economic value of agricultural products (Fellows, 2009). Traditional food processing methods are labor-intensive and do not allow for the optimization of resources, leading to high labor costs and waste of raw materials. With the increase in the human population and diversification of consumer demands, there is pressure on the food industry to reduce costs and increase efficiency while maintaining high-quality standards. Although traditional methods still play an important role in food processing, industry practitioners and researchers are working on innovative and emerging techniques to enhance the quality of food, reduce costs, and improve processing efficiency (Van Der Goot et al., 2016).

Application	ML methods	Important result	References	
Apple	Linear discriminant analysis, adaptive boosting	Accurate classification of apples at 100% rate using the collected acoustic emission signals	Li et al. (2018)	
Artichoke		Ionic patterns properties which were prepared for induvial enzyme having above 95% prediction rate	Sabater et al. (2019)	
Beer	Artificial Neural Network (ANN)	The chemical composition of beer was classified using ANN model having 95% total accuracy		
Biscuits	Convolutional neural network	The developed model was able to classify and evaluate the quality of different types of biscuits with an accuracy up to 99%		
Cheese	Long Short-Term Memory (LSTM)	The application of LSTM and mechanistic modeling techniques aid in the description of the change in the lactose, and lactic acid content, as well as biomass	Li et al. (2020)	
		A developed model of ML has also help to predict the pH of cheese during fermentation		
Citrus limetta (Mosambi peel)	SVM-ANN, SVM-Gaussian process regression (SVM- GPR)	-Prediction and classification of results obtained for lime powder taste which has been prepared using salt having 1.0 accuracy	Younis et al. (2019)	
		- ML tool helps to optimize to maintain the taste and uphold the lime polyphenol content		
Fruits (Arbutus	RF, SVM, ANN	Determine the stability of the fruit extracts in	Astray et al. (2020)	

Table 2.6: Application of machine learning in food industries

unedo I	2.	the Arbutus unedo L. in aqueous and powder	
fruits)		systems by the ML methods having total	
		coefficient ranging from 0.9128 to 0.9912 for	
		the top models selected	
Milk	Support Vector Regression	Evaluation of the presence and level of	Gutiérrez et al.
	(SVM)	antibiotics cow milk by SVM classifiers having	(2020)
		more than 83% accuracy rate and bigger	
		sensitivity compared to the typical metrics	
Wine	SVM, RF, MLP	Evaluation of wine quality by comparing three	Shaw et al. (2020)
		algorithms, where the best result was gotten	
		using the RF method having a mean accuracy	
		of 81.96%, while others showed a low	
		accuracy value.	
Lamb meat	SVM	Accuracy classification of fat from lamb meat	Alaiz-Rodriguez
		increased from 89.70 to 93.89%	and Parnell (2020)
Mangoes	Naive Bayes, SVM	Maturity detection of mangoes on the basis of	Pise and Upadhye
Attributes		their quality	(2018)

Advanced technology can be implemented in various stages of the food supply chain, ranging from farms to factories to consumers. The use of modern technology, such as Machine Learning (ML), can be integrated into food processing procedures, including freezing, drying, and canning, to enhance the preservation period of food. Additionally, ML can be utilized in packaging and foreign object detection to increase the shelf-life of food (Zhu et al., 2021). Furthermore, the use of ML procedures is necessary to identify high-quality and sub-standard foods during the processing monitoring and packaging, and foreign object detection. Image processing techniques are desirable for acquiring information about the appearance, texture, and components of food, and can be used to determine the next steps in food processing. Therefore, the use of ML in food processing is justified, with a focus on its application in food safety and quality evaluation, food process monitoring and packaging, and foreign object detection.

2.8.1 Machine learning (ML) in food safety and quality evaluation

Leiva and Valenzuela (2013) introduced MVSs as a means of detecting defects in blueberries automatically. The process involved utilizing a pattern recognition algorithm to distinguish between the stem and calyx while also identifying blueberries afflicted with diseases and their orientation. The authors then tested different models including LDA, Mahalanobis distance (a measure of data covariance distance), KNN (with a fixed K value of 5), and SVM to determine the best classifier. Nandi (2016) utilized Multi-attribute Decision Making (MADM) to grade mangoes and successfully predicted the optimal time for shipping the harvested fruits to the market by utilizing Support Vector Regression (SVR). Furthermore, the authors employed a fuzzy incremental learning algorithm to assess the mangoes' grade based on SVR and MADM. MADM refers to decision-making in a limited (infinite) set of solutions that conflict with each other and cannot be shared. Also, Amatya (2015) developed an MVS-based application for automated cherry harvesting that predicted partially covered branches using a Bayesian classifier, which achieved an accuracy of 89.6% in branch pixel classification.

Keresztes (2016) presented a system for detecting early apple bruises that consisted of a SWIR illumination unit and a line scan camera. The study utilized thirty Jonagold apples to showcase the effectiveness of the detection system in identifying fresh bruises. The image pixels were

divided into three subcategories: bruised, unbruised, and glare, revealing significant differences in reflectance values between the classes. The most effective classifier for image pre-processing was the partial least squares discriminant analysis (PLS-DA) classifier, which can alleviate the impact of multicollinearity between variables. The post-processing method used was physically based rendering (PBR), which can achieve physical reality throughout the entire rendering process, with mean centering as the reluctance calibration. The prediction of bruised apples achieved 98% accuracy, and the processing time for the three increasing speeds of 0.1, 0.2, and 0.3 m/s was 400, 300, and 200 milliseconds (ms), respectively, rapid enough for real-time conveyor apple sorting.

Zhang (2014) proposed a system for detecting defects on apples using automatic lightness correction and a weighted relevance vector machine (RVM), which achieved an overall prediction accuracy of 95.63%. Extreme Learning Machines (ELM) use random numbers and the law of large numbers to solve problems. Iraji (2018) developed a novel grading system for tomatoes, named Multi-layer Architecture of a SUB-Adaptive Neuro-Fuzzy Inference System, which combined multiple input features, neural networks, regression, and ELM, as well as Deep Stacked Sparse Auto-Encoders (DSSAEs). The authors used the full tomato images to grade tomatoes, achieving an accuracy of 95.5%. Noordam (2000) used an MVS that included a 3-CCD line-scan camera and mirrors to capture a full view of potatoes. The author applied a method that combined LDA and a Mahalanobis distance classifier to segment pixels in the images, to detect the appearance defects of potatoes. The study also used a Fourier-based shape classification method to detect misshapen potatoes. The proposed MVS effectively graded and inspected the quality of either red or yellow skin-colored potatoes, with a processing capacity of about 50 potatoes per second. Su (2018) demonstrated another grading technique based on depth images, using a depth camera to obtain 3D features of potatoes such as bumps, divots, and bent shapes. The information was used to calculate the volume and mass of the potatoes and finally grade them. The system integrated 2D and 3D surface data to detect the irregular shape, hump, and hollow defect of potatoes, achieving an appearance grading accuracy of 88%. The acquired information was also used to rebuild a virtual reality potato model, which could be useful for food packaging research.

2.8.2 Machine learning in process monitoring

Aghbashlo (2014) suggested the use of intelligent systems for the purpose of monitoring and regulating the speed of food drying, to enhance the quality of the resulting dried food. The proposed system involved examining the food's texture, colour, size, and shape by applying techniques such as co-occurrence, Fourier transform, wavelet transform, thresholding, and masking to the input images. This was followed by the utilization of PCA and FCM to manage the moisture levels during the drying process, resulting in an optimal degree of dryness. On the other hand, Liu (2016) developed a new approach to address the issue of local uniform fragmentation or patches in complex grain images. One approach involved utilizing Gaussian Derivative Filtering (GDF) at multiple scales and orientations, along with a Sparse Multikernel-Least Squares Support Vector Machine (SMK-LSSVM) classifier. The authors demonstrated that their method was suitable for use in an assembly line. Another method, developed by Zareiforoush (2016), employed machine vision and fuzzy logic techniques to manage the rice whitening process. The system analyzed the degree of milling and the percentage of broken kernels to regulate the process, with the fuzzy logic algorithm producing the whitening pressure level as the output for process monitoring. The authors found that the system was 31.3% more effective than human labor and had an 89.2% accuracy rate. Meanwhile, Zhu et al. (2021), reported a segmentation technique to calculate the ratio of the browned parts to the entire cookie, which was useful for monitoring the baking process and assessing the quality of baked goods. This method also had potential applications in the evaluation of potato chip safety, as the browning ratio of potato chips had a strong linear correlation ($R^2 > 0.88$) with acrylamide concentration.

2.8.3 Machine learning in foreign object detection

Lorente (2015) utilized a laser-light backscattering imaging system to identify citrus fruit decay caused by fungi, with a maximum overall classification accuracy of 93.4% achieved through the use of a Gaussian-Lorentzian cross-product distribution function. Meanwhile, Coelho employed a binary decision tree and transillumination technique to examine and categorize clam images, allowing the detection of parasites and flattening of clam thickness. Parasites were classified, located, and their shapes determined via a self-generated spatial reference system. Einarsdottir and colleagues proposed a grating-based X-ray imaging method that offers three modalities to

detect objects with textures that cannot be discerned using the classic X-ray method, successfully evaluating images of seven different food products containing foreign objects. Einarsson introduced the Sparse Linear Discriminant Analysis (SDA) for foreign object detection, which is a sparse version of the LDA. Finally, Dutta suggested a non-destructive technique to identify acrylamide in potato chips using statistical features extracted from images in the spatial domain and an SVM classifier, resulting in a 94% accuracy and 96% sensitivity.

CONNECTING TEXT TO CHAPTER THREE

Chapter two of the thesis is a literature review that provides an overview of recent research on the techno-functional properties, thermal, and rheological aspects of legumes, with a specific focus on pea protein. The literature review examines the effects of various processing conditions, such as ionic strength, pH, temperature, conformation, fraction of hydrophobicity to hydrophilicity, and extraction method on the techno-functional and thermal properties of pea protein.

The chapter highlights that there has been extensive research conducted on the impact of processing conditions on the techno-functional and thermal properties of pea protein. However, there is limited information available on the influence of composition (starch, oil, and protein) on the techno-functional properties and thermal properties of pea protein products. Therefore, chapter three of the thesis aims to fill this gap by focusing on the impact of composition on the techno-functional properties and thermal properties of pea protein.

The literature review presented in chapter two has been prepared in manuscript format and is ready for publication. All the sources cited in this chapter are listed in the reference section of the thesis. Overall, the chapter serves as a valuable resource for researchers and industry professionals interested in the techno-functional and thermal properties of legumes, specifically pea protein.

CHAPTER THREE

3. TECHNO-FUNCTIONAL AND THERMAL PROPERTIES OF PEA PROTEIN PRODUCTS

Abstract

Techno-functional and thermal properties of pea protein products (PPP), namely pea flour, pea protein concentrate, and pea protein isolate, from different varieties of peas, were studied. The effect of the composition (protein, starch, and oil contents) of the PPP on the techno-functional properties (water absorption capacity, water solubility index, water absorption index, oil absorption index, protein solubility, emulsifying capacity, forming capacity, forming stability) and thermal properties (onset temperature, peak temperature, and gelatinization enthalpies) was evaluated. Principal component analysis (PCA) and cluster analysis (CA) were also used to analyze the techno-functional and thermal properties of pea protein products. The technofunctional properties ranged from 40.32 to 87.15% for protein solubility (PS), 49.71 to 69.90% for water absorption capacity (WAC), 48.70 to 75.33% for water solubility index (WSI), 0.12 to 2.57% for water absorption index (WAI), 65.07 to 86.02% for oil absorption index (OAI), 23.07 to 49.11% for emulsifying capacity (EC), 2.50 to 15.00% for forming capacity (FC), and 1.00 to 5.00% for forming stability (FS). The onset temperature of the pea protein products ranged from 93.3 to 166.19°C, with peak temperature ranging from 128.97 to 180.74°C and gelatinization enthalpy ranging from 89.04 to 280.42 J/g. The biplot of principal components 1 and 2 explained 54.09%, 52.37%, and 64.42% of the total variability of techno-functional and thermal properties of pea flours, pea protein concentrates, and pea protein isolates. The most unique variety based on the cluster of the techno-functional and thermal properties of the pea protein products was established. The protein content of the pea protein products was noticed to have the most significant influence on the WAC, OAI, FC, peak temperature, and enthalpy. The oil content showed the most significant influence on FS and onset temperature while the interaction of oil and protein contents influenced the WSI, WAI, and PS the most. The results of this study can be valuable in identifying the key composition factors that affect the techno-functional and thermal

properties of pea protein products. Additionally, it can assist in the selection of the most suitable pea protein product for use in product development and formulation.

3.1 Introduction

Demand for novel, healthy food ingredients and products has been accelerating in the last decade. In 2020, the global market for plant-based protein was estimated to be US\$10.3 billion, and it is expected to rise to US\$14.5 billion in 2025 at an annual increment of 7.1% (Mondor and Hernández-Álvarez, 2022). The nutritional advantages and functional properties of plant-based protein products such as flour (with 20-25% protein), concentrate (with 50-85% protein), and isolate (with over 90% protein) are causing them to be more frequently incorporated into a diverse selection of commercial food items. Pea (Pisum sativum) proteins are evolving as potential alternatives to conventional proteins (derived from soy and animal) in numerous food applications. They are characterized by their elevated protein content with unique functionality, availability, sustainability, less allergenicity and affordability. They also contain all essential amino acids except for methionine, interesting functional attributes (e.g., solubility, foaming and emulsification capacity) and high antioxidant and bioactive compounds (de Oliveira et al., 2020; Boukid et al., 2021). Pea protein products (flour, concentrates, and isolates) are employed as novel food ingredients with unique processing characteristics. Pea flour has been used as a base material for extruded products (Luo and Koksel, 2020). Pea protein concentrate (PPC) is utilized in the meat and sausage industry as a substitute due to its favorable water and fat binding capacity, protein solubility, gelation, emulsification, and foaming capacity profiles. While pea protein isolate (PPI) is used to enhance the nutritional and functional characteristics of products, such as pasta (Pelgrom et al., 2013). The global food industry is continually challenged with the task of finding unique protein-based ingredients from different plant varieties that will impact desired techno-functional and thermal properties of products to meet evolving consumers' preferences.

In general, the techno-functional properties of foods are influenced by their ingredients, variety, extraction technique, processing conditions, and structure (Pazmiño et al., 2018; Arteaga et al., 2021). Important techno-functional properties such as protein solubility, water absorption capacity, water solubility, water absorption index, oil absorption index, emulsifying capacity, forming property, and forming stability of ingredients are key determinants of the characteristics

of the resulting final food products (Barać et al., 2015). Pea protein products with good protein solubility are desirable in most food processing applications. Other properties, such as oil absorption, foaming, and emulsification, depend on protein solubility (Boukid et al., 2021). Pea protein products undergo heat treatment during preparation or extraction processes, resulting in a need to study their thermal properties, which could be assessed by onset temperature, peak or denaturation temperature, and gelatinization enthalpy. The denaturation temperature is an estimate of the thermal stability of a protein. A high denaturation temperature value is mostly related to a globular protein with high thermal stability. The gelatinization enthalpy describes the percentage of undenatured proteins and the degree of protein structural organization (Shevkani et al., 2015). According to Emkani et al. (2021), variations in thermal properties are largely dependent on the product's protein conformation and structures as well as its amino acid composition. For instance, kidney bean isolates showed higher denaturation temperature values than corresponding pea isolates.

Plant protein contents are significantly influenced by the genotypic variation that determines quality characteristics of plant proteins (Barac et al., 2010). High-quality protein products with suitable techno-functional and thermal properties are targeted for industrial applications (Chimphepo et al., 2021). Several studies have been reported on functional properties of different plant proteins. Barac et al. (2010) examined the techno-functional properties of pea (*Pisum sativum*) isolates from six different genotypes. The genotypic variation significantly influences the functionalities of the pea isolates. Karaman et al. (2021) comparatively studied the techno-functional properties of common bean concentrates, and the variation in the techno-functional was attributed to the influence of genotype characteristics, processing conditions, and environmental conditions. Similarly, Dasa and Binh (2019) investigated the functional properties of flour from different varieties. The study reported that the flour from the Tesema variety had the highest water absorption capacity, protein solubility, and water adsorption index.

Various techniques such as principal component analysis (PCA) and cluster analysis (CA) have been employed to evaluate the variations with a sample group (El-Hashash et al., 2016; Manish and Pandit, 2018). PCA can be used to extract the most important features or variables that contribute to the variance in the dataset. In contrast, cluster analysis enables the grouping of individuals into clusters to maximize the similarity within a group and display the dissimilarity among other groups (El-Hashash et al., 2016). Despite the various advantages of developing exclusive plant protein-based ingredients from different varieties, information on comparable techno-functional and thermal properties of pea flour, pea concentrate, and pea isolate produced from different pea varieties is sparse. Furthermore, the use of principal component analysis and cluster analysis to assess the effect of intrinsic parameters (such as varieties and composition) on techno-functional and thermal properties of pea protein products is scant and inadequate. However, this information would aid in the identification and selection of the most suitable product for processing formulations based on their techno-functional and thermal properties. Therefore, this study aimed to evaluate the effect of proximate compositions (starch, oil content, and protein content) of PPP on techno-functional and thermal properties, namely protein solubility, water absorption capacity, forming capacity, forming stability, onset temperature, peak temperature, and gelatinization enthalpy.

3.2. Materials and Methods

Thirty-six (36) varieties of pea products were selected and used for this study to elicit samples of different proximate compositions. The samples include 12 varieties of pea protein flour (labelled: NRC_CT_001, Homecraft Pulse 1135, Belle Pulse Pea, NRC_CT_002, Fiesta Pea Flour, SC 111-22, SC 107-22, SC 108-22, SC 109-22, SC 110-22, SC 112-22 and SC 113-2); 12 varieties of pea protein concentrate (Labelled: NRC_CT_003, NRC_CT_014, Prestige Pea Protein Concentrate, SC108-F, SC109-F1, SC109-F2, SC110-F1, SC110-F2, SC111-F1, SC111-F2, SC112-F1 and SC112-F2); and 12 varieties of pea isolate (labelled: Homecraft Pulse 1135, PISANE B9, PISANE M9, PISANE C9, Nutralys F85F, Nutralys F85M, Nutralys S85F, Nutralys B85F, Pea 870H, 870MV, Vitessence Pulse 1803 Pea Protein, and Isolate Vitessence Pulse 1853 Pea Protein). These varieties were selected based on their different compositions and obtained from the National Research Council of Canada through a material transfer agreement.

3.3 Techno-Functional Properties

The response surface design in Minitab software version 20 was used to study the effect of compositions (starch, oil content, and protein content) of PPP on their techno-functional and thermal properties. The low and high values of the experimental factors namely starch, oil content and protein content (labelled C, B, and A, respectively) of samples used in the study

were 0.05 and 64.65, 0.60 and 3.36, 11.46 and 81.55, respectively. Pareto chart was used to determine the significant composition while contour plots were used to study the influence and interactions of the compositions on the techno-thermal properties of PPP.

3.3.1 Water absorption capacity (WAC)

WAC was determined following the procedure of Deriu et al. (2022). Two (2) grams of each sample were dispersed in 20 mL distilled water inside a 50 ml centrifuge tube. The dispersions were kept at room temperature (25° C) for 30 min, vortexed for 30 s, and allowed to rest for 10 mins. Afterward, the tube was centrifuged at 6000 rpm for 25 min. The sediment was weighed after the removal of the supernatant. The water absorption capacity was expressed as the amount of water absorbed by the material relative to its dry weight. The results were expressed as g/H₂0 retained/g of sample dry matter.

3.3.2 Water solubility index

The water solubility index was determined using the approach described by Abebe et al. (2015). Two (2) grams of each sample were weighed into a centrifuge tube (W_o) and dispersed in 30 mL of distilled water. The dispersions were cooked for 15 min in a 90°C water bath and the formed gel was cooled for 1 h at room temperature. The gel was centrifugated at 6000 rpm for 10 min using a centrifuge. The solid content of the resulting supernatant was determined by evaporation at 110°C. The water solubility index (WSI) was calculated by dividing the amount of dissolved substance by the original amount of substance and multiplying by 100.

3.3.3 Oil absorption capacity

Two (2) gram of each sample was added to 20 mL of oil in a 50 mL centrifuge tube. The dispersions were vortexed for 30 s, rotated every 5 mins for 30 mins, and allowed to relax for 10 mins. After this procedure, the tube was centrifuged at 2000 rpm for 15 mins. The weight of the sediment was determined after removing the supernatant. Oil absorption capacity was determined by subtracting the weight of the original substance from the weight of the mixture, and then dividing it by the weight of the original substance (Wang et al., 2020). The results were expressed as the content of oil absorbed per gram of sample.

3.3.4 Emulsifying Capacity

The emulsifying capacity was determined using the method described by Arteaga et al. (2020). Two (2) g of each sample was weighed and dispersed in distilled water of 30 mL in a centrifuge tube. Twenty (20) mL of olive oil was added to the mixture in a blender and blended for 120 s at 1600 rpm to form an emulsion. The emulsion was poured into a calibrated centrifuge tube where the height of the liquid (HT) was taken. The emulsion was heated inside a water bath at 80°C for 30 mins and cooled for 15 mins under running water. The cooled sample was centrifuged at 2000 rpm for 15 min in a centrifuge. The height of the emulsifier layer (HI) was recorded. Emulsifying capacity (EC) was determined by dividing the height the of emulsifier layer by height of the liquid.

3.3.5 Foaming capacity (FC)

Foaming stability was determined by using the approach described by Abebe et al. (2015). Two gram (2 g) of each sample was mixed with 40 mL of distilled water using a blender at 30°C in a 100 mL measuring cylinder. The suspension was stirred at 600 rpm for 15 min to produce foam. The volume of foam was measured after 0 min (FV₀) and 60 min (FV₆₀). The foaming capacity was established directly as FV₀.

3.3.6 Foaming stability (FS)

Foaming stability (FS) was determined as described by Abebe et al. (2015). This was evaluated as the percent ratio of the volume of the foam after 60 min (FV60?) and the volume of the foam before 60 min (FV0??).

3.3.7 Protein Solubility

The dispersion for each sample was stirred for 30 mins and adjusted to either pH 4.5 or 7.0 using either 0.1M HCl or NaOH solution. The suspension was shaken for 1 h and centrifugated at 10000 rpm for 10 mins using the approach described by Morr and German (1985). The soluble protein content was analyzed photometrically at 550 nm using bovine serum albumin (BSA) as the calibration standard in the Biuret method of AACC Method 46-15.01. The soluble protein content was expressed as the %protein content present in the sample.

3.4 Thermal Measurement

Ho and Aziah (2013) modified approach for thermal measurement was adopted for this study. Differential scanning calorimeter (DSC, TAQ 100, TA Instruments, Delaware, USA) was calibrated with pure indium as a standard. Each sample was mixed with distilled water in a ratio of 1:2 at 25°C. Two (2) mg of each ingredient was placed in T₀ aluminium pans and 10 μ L of deionized water was added to the sample in the DSC using a micro-syringe, and hermetically sealed. The sealed pans were allowed to equilibrate in desiccators for 1 h before being subjected to analyses. An empty T₀ pan was used as a reference. Pans were heated from 30°C to 150°C at a rate of 2°C/min with a liquified nitrogen gas flow of 50 ml/min. The values for the onset temperature (T₀), denaturation temperature (T_{dn}), and gelatinization enthalpy (Δ H_g), of the samples, were obtained directly from the analysis by the Nanoanalyze software; TA Instruments Advantage Software Universal Analysis 2000 version 2.6.362.

3.5 Statistical analysis

Data were collected in duplicate and analyzed statistically at a 95% confidence level using the Duncan multiple range test of ANOVA in the Statistical package for social science (IBM SPSS Statistics 2019 Version 26). The principal component analysis (PCA) and cluster analysis were carried out using Origin Pro, 2022, Version 9.

3.6. Results and Discussion

3.7 Techno-functional Properties of Pea Protein Products

3.7.1 Water absorption capacity (WAC)

WAC of pea protein products ranged from 49.7 to 69.9% for all the samples used in the study. There was significant effect (at p<0.05) of composition (protein, oil and starch contents) on the WAC of the different PPP. Comparably, Ge et al. (2020) reported lower WAC of 2.7 g/g from pea protein isolate while Zhao et al. (2020) reported WAC of 3.38 and 5.16 g/g from commercially produced pea protein and soy protein products, respectively. Du et al. (2014) reported variations of 11.2 to 18.9% in the WAC of different legume flours. The disparities in WAC of the pea protein products compared to the literature may be due to several factors such as the type of pea protein, particle size, chemical composition, and preparation conditions

(Gharsallaoui et al., 2010; Shanthakumar et al., 2022). Pareto analysis indicated that oil and protein contents had the most significant influence on WAC of the pea protein products (Figure 3.1).

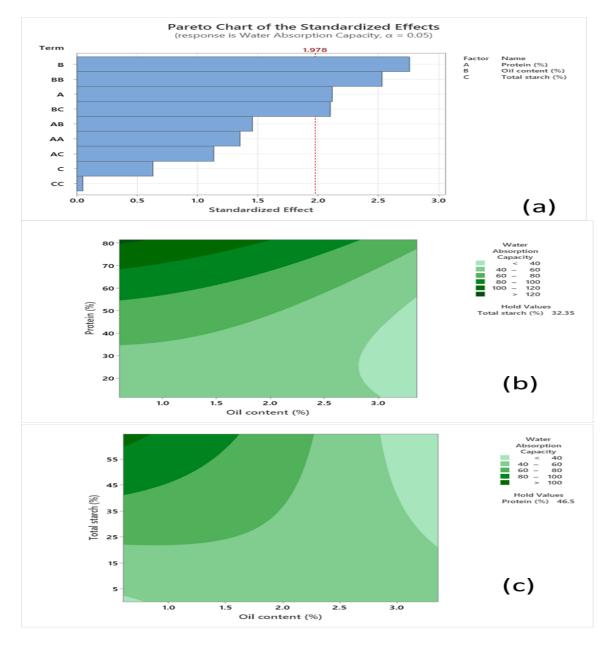


Figure 3.1. Effect of protein, total starch, and oil contents on the WAC properties of pea protein products. Standardized effects are shown in (a); interaction effects of protein and oil in (b); interaction effects of total starch and oil in (c).

Products with higher oil content tended to have lower WAC whereas increasing protein content increased WAC of the products. This is attributed to oil interference with water and protein interaction, which decreased the quantity of water absorbed and held by the protein (Chou et al., 1979). The structural arrangement of the protein matrix such as the pore size could also significantly influence the relationship between protein and WAC. This can be further explained as the WAC occurs through a combination of ion-dipole, dipole-dipole, dipole-induced dipole, and hydrophobic interactions of protein (Lam et al., 2018). In addition, oil could cover the surface of protein particles making them less soluble (Damodaran, 2005). Lam et al. (2018) found that, the protein profile (e.g., amino acid composition) plays a key role the in influencing the WAC. Highly charged proteins tend to have a stronger electrostatic attraction to water. Hall et al. (1996) also reported a positive linear correlation between the water absorption and protein. Furthermore, the negative influence of oil content on the WAC could be due to the ability of oil to binds to the hydrophobic groups of the protein chains, thereby reducing protein's ability to bind and hold large water molecules (Mandliya et al., 2022). Starch content had positive linear influence on WAC of the pea protein products (Figure 1c). This aligns with Cruz-Solorio et al. (2014) who reported a linear correlation between carbohydrate content and WAC in legumes flours. Pea protein products with higher WAC have greater suitability as novel ingredients in several food applications, including bakery products, plant-based meat analogues, protein bars, and dairy alternatives.

3.7.2 Water Solubility Index (WSI)

The WSI of pea protein products ranged from 48.7 to 75.3%. The interactions of oil and protein contents showed the most significant influence on the WSI of the pea protein products (Figure 3.2). Apparently, increasing protein content or total starch content tended to lower WSI for products with lower oil contents. However, reversed effects of protein and starch were observed at higher oil content as indicated by the Pareto analysis.

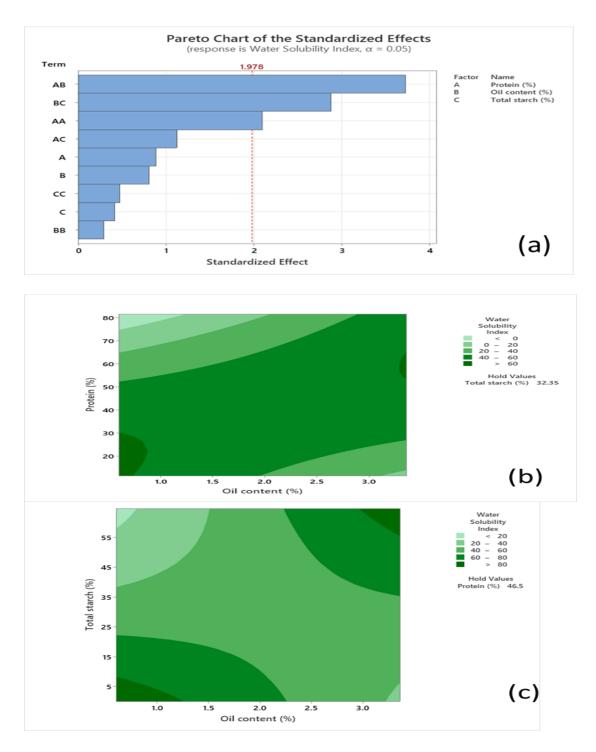


Figure 3.2. Effect of protein, total starch, and oil contents on the WSI properties of pea protein products. (a) Pareto chart of WSI, (b) Influence of protein and oil contents on WSI, (c) Influence of total starch and oil contents on WSI

The decrease in WSI with increasing starch content could be due to high solubility properties of starch (Akalu et al., 1998). For instance, some sugars, such as glucose, have a high water solubility index but a low water absorption capacity due to presence of a number of polar hydroxyl groups which can form hydrogen-bond with water molecules. This means that they dissolve easily in water but do not absorb much of it (Zakrzewska et al., 2010). Pea protein products with increased protein content have a higher solubility due to their low proportion of non-protein components, such as fats, fiber, and starch, which interfere less with non-protein components (Jiang et al., 2017). The oil content of pea protein products significantly affects their WSI by hindering the water and protein interaction, resulting in a lower quantity of absorbed water by the protein (Lam et al., 2018). The relationship between oil content and pea products can be influenced by the chemical and physical characteristics of the oils present (Krause et al., 2022). Pea protein products with a lower oil content tend to have a higher WSI, and the same trend is observed with total starch content, as an increase in starch content reduces the WSI due to the formation of a semi-crystalline structure and disruption of the starch granules (Saeid et al., 2015). The complexity of protein-starch and amylose-lipid interactions during heat treatment may also contribute to the variation in WSI of pea protein products (Aditi and Arivuchudar, 2018).

3.7.3 Oil Absorption Capacity (OAC)

The OAC of the PPP ranged from 65.1 to 86%. There was significant effect (at p<0.05) of composition on the OAC of the pea protein products. The oil absorption capacity of the pea protein increased under the influenced under the influence of compositions (protein, oil, and starch contents). These OAC values was lower than the OAC of 130 to 156% reported by Chandra et al. (2015) from different composite flours while Ika et al. (2020) obtained a lower OAC of 0.27 to 2.86% from cowpea concentrates. The OAC value of commercial pea protein isolate and soybean isolates were reported to be 1.59 and 1.23 g/g, respectively (Fuhrmeister and Meuser, 2003). The high OAC observed in the PPP could be the consequence of the high degree of oil entrapment by the pea protein products due to the hydrophobicity of the pea protein products (Wang et al., 2020). Additionally, the structure of the protein matrix, the form of lipids, as well as the organization and stability of the lipids could influence the OAC (Zhao et al., 2020).

Pareto analysis displayed that the protein content showed the most dominant influence on the OAC of pea protein products (Figure 3.3).

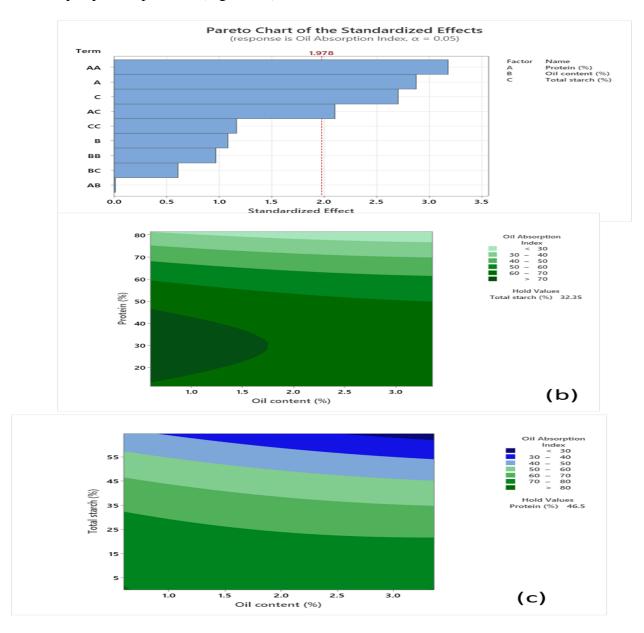


Figure 3.3. Effect of protein, total starch, and oil contents on the OAC properties of pea protein products. (a) Pareto chart of OAC, (b) Influence of protein and oil contents on OAC, (c) Influence of starch and oil contents on OAC.

This may be due to the hydrophilic and hydrophobic regions of proteins that enable them to interact with oil and water. Thus, pea protein products with high OAC indicate that there are larger protein molecules available in the pea product to interact with oil, thus increasing the OAC (Withana-Gamage et al., 2011). Products with higher protein and oil content showed higher OAC of the pea products. The effect of oil content with OAC of pea products could be due to the interaction of proteins with oils interact through the binding of the aliphatic chains of lipid to the nonpolar side chains of amino acids. Thus, proteins with the higher protein hydrophobic regions are likely to have a greater capability to hold oils. through the release of oil-protein complexes (Sanjeewa, 2008; Withana-Gamage et al., 2011). In addition, the OAC of pea protein products can also be influenced by arrangement of proteins within a matrix, the specific lipid variety that is present, and the way in which lipids are distributed and maintained (Lam et al., 2018). The pea protein ingredient with high OAC could be employed in food applications where better palatability, shelf stability, and flavor retention are required, such as in the bakery and meat industry.

3.7.4 Emulsifying Capacity (EC)

The pea protein products have a varied range of EC between 23.7 and 49.1%. The effect of composition on the EC of pea protein products were observed to be significantly at p<0.05. Pareto analysis showed that the interaction oil content and total starch content had the most significant effect on the EC of pea protein product. The starch content of pea protein does form complexes with proteins, which affects the structure and functionality of proteins due to their interaction. This leads to a change in the protein's surface properties and the ability of the protein to interact with other components in the emulsion (Ouyang et al., 2022). Pea protein products that contained more oil had a tendency to exhibit a lower EC, while higher levels of starch tended to result in an increased EC (Figure 3.4). The presence of oils can diminish the degree of interaction between water and the surface of a protein, achieved by reducing the interfacial tension that exists between the oil and water phases, which allows proteins to absorb onto the interface (Alzagtat and Alli, 2002). In addition, a higher oil content can result in the formation of larger oil droplets, making it hard for the pea protein product to stabilize the emulsion.

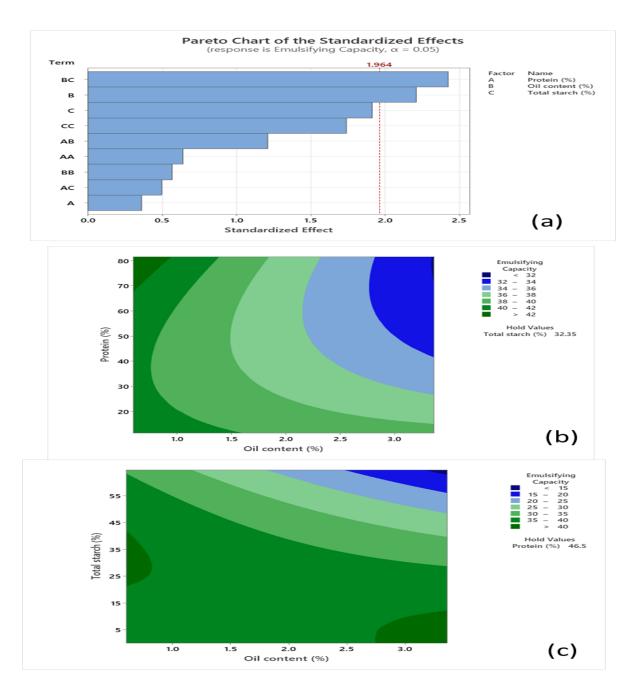


Figure 3.4. Effect of protein, total starch, and oil contents on the EC properties of pea protein products. (a) Pareto chart of EC, (b) Influence of protein and oil contents on EC, (c) Influence of starch and oil contents on EC

However, an increase in protein content result into an increased EC of the pea protein products. Asides the effect of composition, EC can be largely influenced by protein concentration, protein structure, and protein-oil-water contact time (McCarthy et al., 2016). This aligns with the conclusion of Chen et al. (2019) that an increase in protein concentration increases the EC of pea proteins. According to Sahin and Sumnu (2006), a high quantity of hydrophobic surface area leads to more flexibility in protein structure, which improves the emulsion stability through interfacial action. Scientists have reported that the preparation method and extraction method of pea protein products could also significantly influence the emulsion capacity (Ivanova et al., 2014; Adenekan et al., 2017; Cruz-solorio et al., 2018). In comparison to the EC in the study, Du et al. (2014) reported EC values ranging from 61.14% to 92.20% for ten legume flours. Ivanova et al. (2014) reported EC between 45.85% and 60.88% for the protein isolate of different Sunflower meals. The disparity in the EC values to the literature is owned to different protein types, compositions, treatment method and extraction technique (Barac et al., 2010; Karamanet al., 2021; Mir et al., 2021). Pea protein products with high emulsion capacity exhibit high emulsion stability and great fat-binding properties.

3.7.5 Foaming Capacity (FC)

Pea protein products are known to have good foaming capacity, which makes them unique ingredients in food products such as bakery goods, whipped toppings, and meringues (Zhao et al., 2020). The FC of pea protein products ranged from 2.5 to 15%. The compositions (protein, oil, and starch contents) showed significant effect (at p<0.05) on the FC of pea protein products. Adenekan et al. (2017) reported a higher FC of 25.30% from pigeon pea flour and varied FC of 33.23–35.37% in pigeon pea isolates, while Ika et al. (2020) reported varied FC of soybean concentrates between 18 and 45.33%. Cruz-Solorio et al. (2018) reported that P. tuber-region sclerotia flours had higher FC than their concentrates. The protein content had the most significant effect on the FC of pea protein products, as shown in Pareto analysis. The starch content also had a similarly significant influence on the FC of pea protein products. Sreerama et al. (2012) stated that other non-protein components such as carbohydrates also influence the foaming capacity of pea proteins. Apparently, the FC of the pea products tend to increase with increase in protein and starch contents (Figure 3.5).

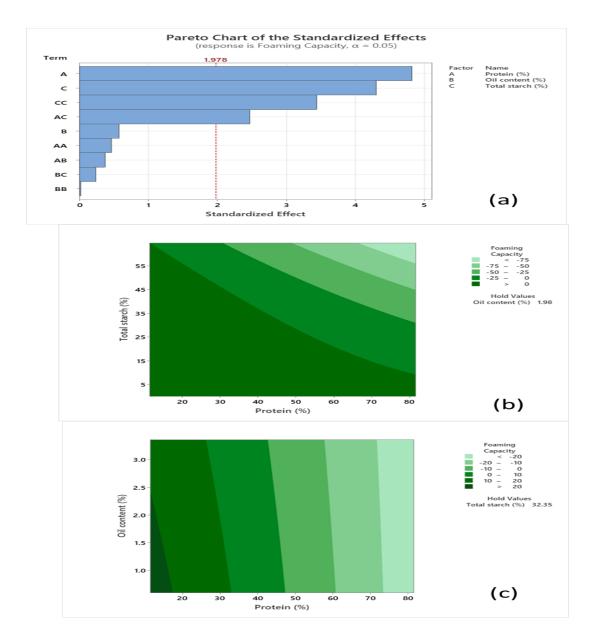


Figure 3.5. Effect of protein, total starch, and oil contents on the FC properties of pea protein products. (a) Pareto chart of FC, (b) Influence of protein and oil contents on FC, (c) Influence of starch and oil contents on FC

The increased FC of the pea products with protein content could be due to the surface-active property of protein molecules that allow them to stabilize air bubbles in a foam. Thus, a higher concentration of protein indicates that larger protein molecules are available to form a steady interface between liquid and air (Dickinson, 1999). In addition, Toews and Wang (2013) conveyed that products with high protein content tend to increase the foaming capacity, by supporting the interaction between protein-protein to create foam collapses. The positive influence of starch content on the FC of pea protein products could be due to the type of starch and quantity of starch used (Awuchi et al., 2019).For instance, literature has reported that the addition of a specific type of starch, e.g., modified tapioca starch, can enhance the FC of pea protein product through the formation of a network structure that helps to stabilize the foam structure (Awuchi et al., 2019). The differences in the FC of pea protein products indicate that the ability of the protein to dissolve in water and absorb water may differ depending on the particular type and variation of pea products being considered. However, the FC of pea products can be partly modified by other factors such as the protein concentration and treatment conditions (Barać et al., 2011).

3.7.6 Foaming Stability (FS)

The foaming stability (FS) of the pea protein products ranged between 1 and 5% (Figure 3.6). To achieve good foaming stability, the pea proteins should possess a high solubility in the water phase, which allow the proteins to decrease the interfacial tension and form strong elastic films containing the dispersed air bubbles (Murray, 2007). Zhao et al. (2020) reported an estimated FS of 68.03, 82.44, 89.74 and 50.00% for wheat, soybean, pea, and rice proteins, respectively. It is important to understand the differences in the FS values obtained compared to the literature may be attributed to the variations in protein products, their compositions, and processing methods used in different studies as reported by researchers (Barac et al., 2010; Karaman et al., 2021; Mir et al., 2021). From Pareto analysis, the oil content has the most impactful influence on the FS of pea protein products. Lipids could hinder the formed network of protein at the air-liquid interface and result in destabilized foams (Lam et al., 2014). The FS of the pea protein products tend to decrease at high oil and starch contents whereas an increase in protein content favors the FS of the pea products. The increased FS with increasing protein concentration indicates a longer foam half-life time and longer gravity drainage time (Bao, 2022).

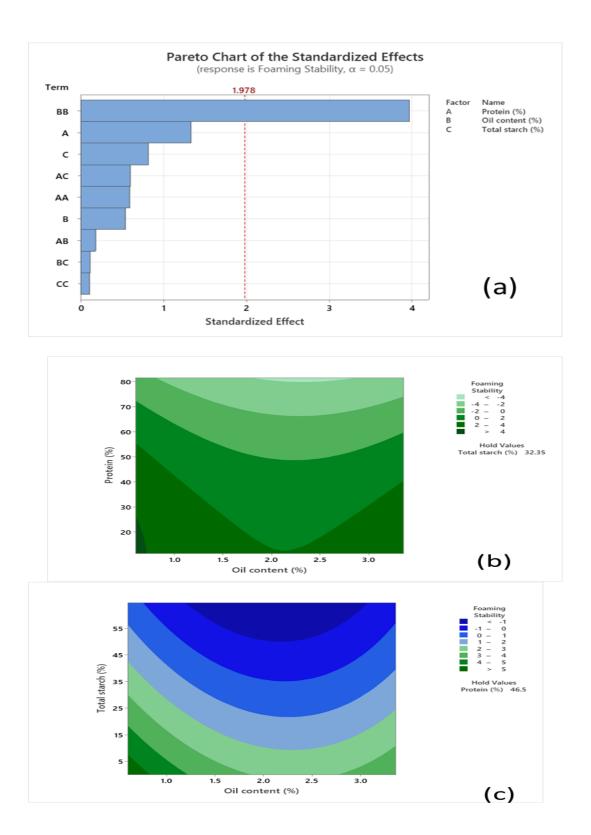


Figure 3.6. Effect of protein, total starch, and oil contents on the FS properties of pea protein products. (a) Pareto chart of FS, (b) Influence of protein and oil contents on FS, (c) Influence of starch and oil contents on FS.

The decrease in foaming stability with the oil content may be because the oils can compete with proteins for absorption into the air-liquid interface during whipping resulting in a decrease in the amount of protein molecules available to stabilize the foam (Branch and Maria, 2017). Branch and Maria (2017) reported a higher foaming stability value of 50.40% and 53.66% from mung beans and soybean protein products. The variation was stated to be due to protein denaturation. This is similarly reported by Fidantsi and Doxastakis (2001). In addition, the ability of foam to remain stable is primarily dependent on how well the protein film functions and its capacity to allow gases to pass through it. The reason behind the high foaming stability reported, could be attributed to the production of a cohesive film with high elasticity that is viscous and gel-like in nature, formed by proteins. Also, proteins that exhibit optimal intermolecular interactions and create a cohesive, unbroken network have the potential to generate stable foams at the interface between air and liquid, as per research by Nakai and Modler (1996). Contrarily, Zhao et al. (2020) reported that the fat contents in four proteins (wheat, soybean, pea, and rice) showed little influence on the forming stability due to low their fat contents. However, it is important to note that the effect of oil on FS can be influenced by the type of oil and quantity of oil used. For instance, the use of a small quantity of a specific oil type, e.g., soybean oil or sunflower oil, can enhance the FS of pea protein products through a reduction in the degree of liquid drainage and enhancing the coalescence resistance of the foam bubbles (Nesterenko et al., 2013). The differences observed in the FS of pea protein products could be attributed to protein denaturation, as stated by Branch and Maria (2017). Specifically, the ability of a protein film to effectively maintain foam stability and allow gases to permeate depends on the protein's ability to form a cohesive, viscous, and gel-like film with high elasticity. Additionally, proteins that demonstrate ideal intermolecular interactions and produce a cohesive, continuous network have the potential to create stable foams at the air-liquid interface, as noted by Nakai and Modler (1996).

3.7.7 Protein Solubility (PS)

The PS of the PPP ranged between 40.32 and 87.15%. The protein solubility of PPP can be significantly affected (at p<0.05) by the composition, namely; protein, oil, and starch contents. Pareto analysis displayed that the interaction of protein and oil contents were observed to have the most significant influence on the protein solubility of pea protein products (Figure 3.7).

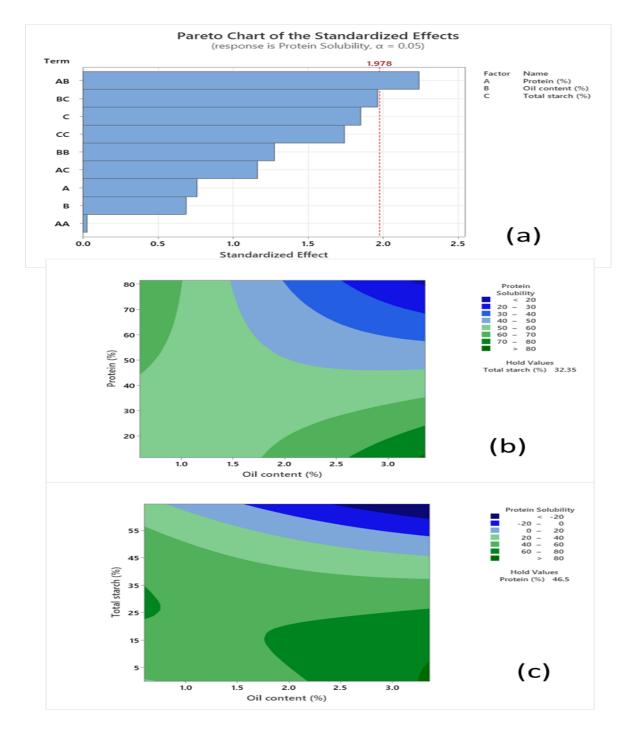


Figure 3.7. Effect of protein, total starch, and oil contents on the PS properties of pea protein products. (a) Pareto chart of PS, (b) Influence of protein and oil contents on PS, (c) Influence of starch and oil contents on PS.

Generally, PPP are usually high in protein solubility due to their great protein content (Lam et al., 2018; Bogahawaththa et al., 2019). The increased PS of pea products could be due to the hydrophobicity and surface composition of protein molecules of the different pea products. Kouakou et al. (2013) and Shanthakumar et al. (2022) reported a similar phenomenon of the dependency of protein solubility on the hydrophobic stability of protein molecules, protein treatment, conformation, and surface composition of polar or non-polar amino acids. These factors influence the interaction of protein-to-protein and protein-to-solvent thermodynamics during the extraction process. Additionally, it is important to understand that protein products with high protein solubility can be associated to good protein products with good emulsifying and foaming properties (Branch and Maria, 2017; Ma et al., 2022). The contour analysis above showed that the PS of the pea protein products tend to increase with an increase in the protein and oil contents. This supports the observation by Karaca et al. (2011) that assessed the PS from faba bean and soybean protein products and discovered that the protein solubility of the pea products was high. Also, Adenekan et al. (2017) observed a similar phenomenon and reported a protein solubility (PS) of 12.1% from pigeon pea flour and varied PS from 90.7% to 97.13% from pigeon pea isolates.

3.8 Thermal Properties of Pea Protein Products

3.8.1 Onset Temperature (T₀)

The thermogram showed the mean values of the onset temperature (T_0) of the PPP that ranged from 93.3 to 166.19°C. The T_0 of pea protein products describes the temperature at which the PPP starts to denature and lose its functional properties. Significant effect (p<0.05) of composition were observed on the T_0 of the pea protein products. Wong et al. (2021) reported variations in T_0 attained from three flour starches with values that ranged from 59.8 - 81.7°C. Similarly, Branch and Maria reported an onset temperature of 155.54°C, which supports the values of this study. Other than that, findings retrieved from Kornet et al. (2021) established a low T_0 between 59.3 and 81.8°C from pea fractions, while Guimarães et al. (2012) reported a lower T_0 of 30°C from defatted Baru flour. In contrast, Chakravartula et al. (2019) reported higher values of onset temperature from edible films ranging from 175 to 225°C. The dissimilarities observed between the obtained T_0 values with literature could be attributed to the diversity in the thermal stability of proteins, which are stabilized by polar bonds (such as hydrogen bonds) and nonpolar interactions (such as hydrophobic bonds) (Branch and Maria, 2017).

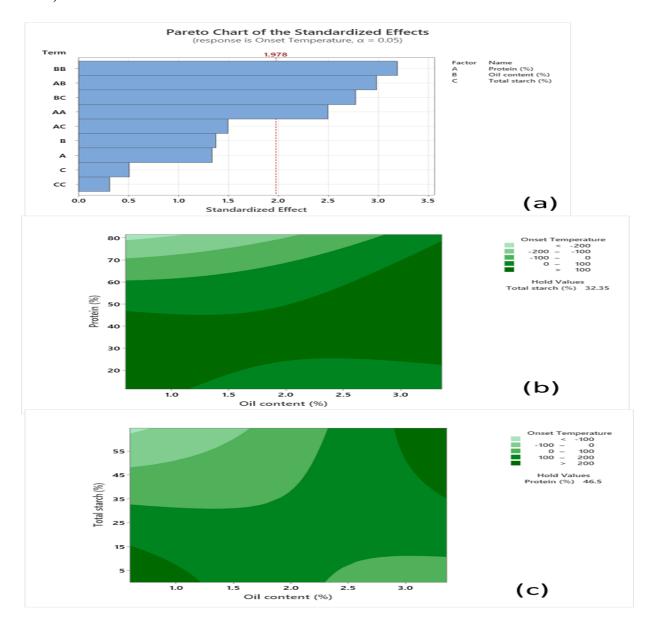


Figure 3.8. Effect of protein, total starch, and oil contents on the T_0 of pea protein products. (a) Pareto chart of T_0 , (b) Influence of protein and oil contents on T_0 , (c) Influence of starch and oil contents on T_0

The oil content in PPP had the most significant influence on their T_0 (Figure 3.8). Additionally, Figure 3.8b shows that the T_0 of the PPP increases as the protein content increases. This is because the increase in protein content has a positive correlation with the high hydrophobicity of protein. Whereas pea protein products with high oil content exhibited lower T_0 . The lower T_0 observed of the PPP could be due to the lower boiling point of oil than water, which is a major component of the PPP. Consequently, the presence of oil can reduce the energy required to initiate boiling, thereby lowering the T_0 of the PPP (De Angelis et al., 2020). However, the effect of oil content may vary among different PPP depending on the type and concentration oil present in the pea products. For instance, a typical pea protein concentrate contains fat content that ranged from 5-8% while pea protein products explains the high purity of products with fewer nonprotein components (such as fiber, starch, and oils) which can contribute to rapid protein denaturation (Osen et al., 2014; Geerts et al., 2017). In addition, Liang et al. (2013) reported that T_0 of pea products can be influenced by factors such as the type of pea protein, treatment methods, and formulation.

3.8.2 Peak Temperature (T_{dn}) of Pea Protein Products

Peak temperature (T_{dn}) defines the thermal stability of protein and is the temperature at which the three-dimensional form of protein undergoes an irreversible change (da Silva et al., 2021). The T_{dn} is an important property for specific food applications, including the production of meat alternatives, as the protein gel can deliver a meat-like texture. The T_{dn} obtained varied from 128.97 to 180.74°C. Branch and Maria (2017) reported a close T_{dn} value of 158.38°C. He further stated that the T_{dn} indicates the higher thermal stability of the protein products which could be due to the disulfide bonds within the protein molecules. The Pareto analysis showed that protein content of the pea protein products has the most dominant factor influencing the T_{dn} . A positive linear effect was observed between protein content and T_{dn} of the PPP, implying that the T_{dn} of the pea products increased with protein content (Figure 3.9b).

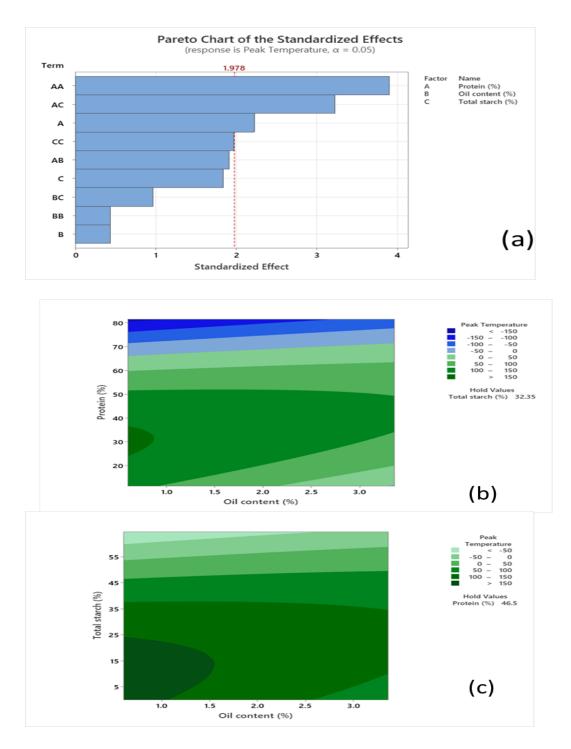


Figure 3.9. Effect of protein, total starch, and oil contents on the T_{dn} of pea protein products. (a) Pareto chart of T_{dn} , (b) Influence of protein and oil contents on T_{dn} , (c) Influence of starch and oil contents on T_{dn}

These results explain that the T_{dn} of pea protein products is favored by the induced denaturation temperature of processing that led to the isolation/extraction of protein products. This is because the pea products have tightly packed protein molecules that are difficult for them to unfold and denature. This aligns with the findings by da Silva et al. (2021). The high thermal denaturation temperature (T_{dn}) observed in pea products can be attributed to the presence of a greater number of globular proteins in the pea protein products. These proteins are soluble in water and have a highly folded three-dimensional structure, contributing to their increased thermal stability. Furthermore, Proteins with largely ordered structures tend to show a good thermal stability (higher T_{dn}), as described by Shevkani et al. (2015). However, from a technological standpoint, pea protein products with lower denaturation temperatures tend to consume less energy needed to change the protein conformation to form a gel, and consequently have a cheaper processing cost (de Silva et al., 2021).

3.9 Gelatinisation Enthalpy (ΔH_g) of Pea Protein Products

The gelatinization enthalpy (ΔH_g) of PPP ranges from 89.04 to 280.42 J/g. In PPP, the gelatinization enthalpy indicates the quantity of energy needed to collapse the crystalline structure of the pea products, allowing them to form a gel (Shevkani et al., 2015; Asen and Aluko, 2022). The protein content had the most significant influence on the ΔH_g of the pea protein products, as shown in Figure 3.10. Also, linear correlation was observed between protein, starch contents and ΔH_g , where the increase in protein and starch contents increase the ΔH_g of the pea protein products (Figure 3.10b and c).

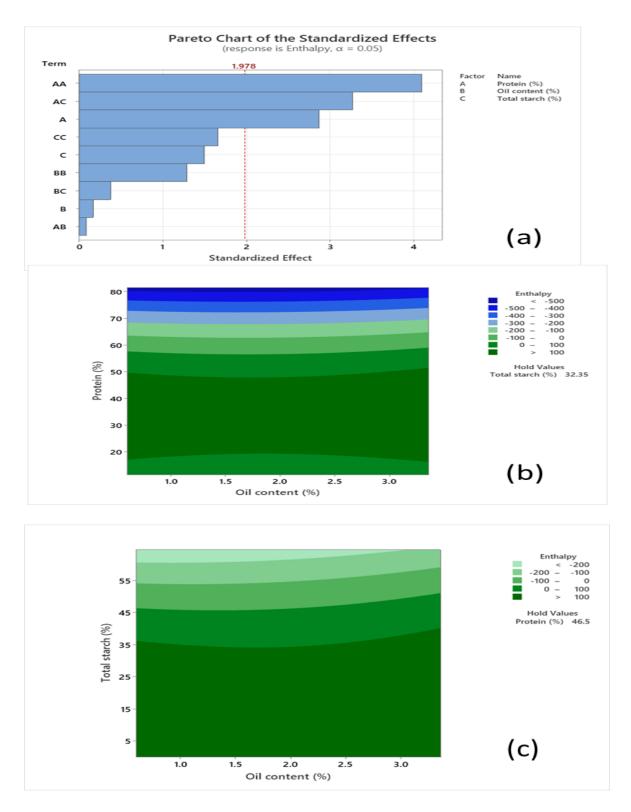


Figure 3.10. Effect of protein, total starch, and oil contents on the ΔH_g of pea protein products. (a) Pareto chart of ΔH_g , (b) Influence of protein and oil contents on ΔH_g , (c) Influence of starch and oil contents on ΔH_g .

The high ΔH_g values achieved in the different pea protein products explains the high starch content of pea products, which enables them to withstand higher temperatures before the change in the starch granules. This also indicates the well-ordered protein structure of the pea protein products and their tendency to form a stable gel during food applications, such as the preparation of meat alternatives. This result supports the observations by Emkani et al. (2021). The high ΔH_g also indicates the presence of hydrophobic interactions in a large network of protein (Asen and Aluko, 2022). Branch and Maria (2017) reported a lower ΔH_g of 41.64 Jg⁻¹ and 50.20 Jg⁻¹ from mung beans and soybean protein isolates respectively. The low ΔH_g in proteins products signifies that the proportion of proteins in the product was thermally held together by the noncovalent hydrophobic bonds, and these results corroborate with the findings of Guimarães et al. (2012).

3.10 Principal Component Analysis of the Composition

The principal component analysis (PCA) was performed to identify and evaluate the variation in the composition of pea protein products. The PCA of the composition showed that there are two principal components at eigenvalues ≥ 1 . The eigenvalues of the composition of the pea protein products produced two components with values of at least 1. These components account for 76.36% of the total variation in principal component 1 while the second component represents 22.1%. Among the pea protein products (in Figure 3.11), protein and oil were the dominant variables with the optimum positive value on PC1 and PC2. Total starch was loaded positively on the PC2. The PC1 and PC2 are considered to describe the variation in data when multidimensional data is predictable in the form a single-dimensional data (Lee et al., 2017).

3.11 Principal Component Analysis of the techno-functional and thermal properties of pea protein products

The principal component analysis (PCA) was performed to identify and evaluate the variation in the techno-functional properties of PPP. The PCA of the PPP showed that there are four principal components at eigenvalues ≥ 1 . The eigenvalues of the pea protein products produced four components with values of at least 1. These components account for 79.24% of the total variation for pea flours, with PC1, PC2, PC3, and PC4 representing 32.45%, 21.64%, 13.11%, and 12.04%, respectively.

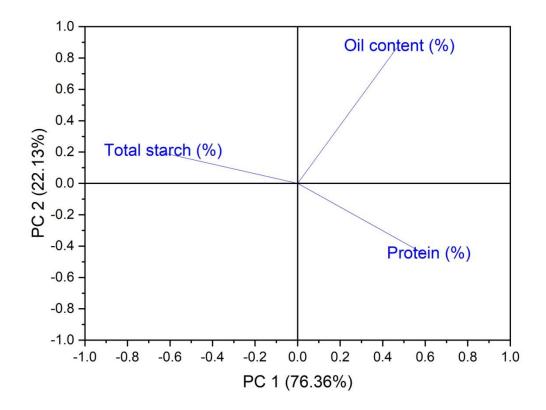


Figure 3.11. PCA analysis based on the composition of pea protein products

For pea protein concentrates, the four components explain 79.02% of the total variation, with PC1, PC2, PC3, and PC4 accounting for 29.50%, 22.87%, 15.20%, and 11.45%, respectively. The first four components of the pea isolates (with eigenvalues ≥ 1) explain 89.09% of the total variation, with PC1, PC2, PC3, and PC4 representing 36.17%, 28.25%, 15.23%, and 9.44%, respectively. The relationship between the pea protein products, techno-functional, and thermal properties are presented in the biplots of Figures 8. The biplots signify the variety and properties that have the largest contributor to the total variation on every dimension (Sharma et al., 1998). In addition, the curves that are nearer to each other on the biplotar are positively correlated, whereas those in reverse directions are negatively correlated (Singh et al., 2008). Among the techno-functional and thermal properties (in Figure 3.12), peak temperature, onset temperature, foaming stability, and enthalpy were the dominant variables with the optimum positive value on PC1 and PC2. WAI and WAC were loaded negatively on PC1 but positively on PC2, while WSI

has a negative score on PC1 and PC2. PS, EC, and FC all had a positive score on PC1 but a negative on PC2. The positive sample score along the PC1 is high for pea flour samples from Belle Pulse pea flour, Home=craft pulse 1101, SS 111-22, NRC_CT_002, and SC 110-22.

They were observed to have a positive value along PC1 and PC2. Flour 28, SC 109-22, and SC 110-22 were loaded positively on PC2 but negatively on PC1. SC 112-22 and SC 108-22 were both in the negative direction of PC1 and PC2. The biplot in Figure 8, based on PC1 and PC2, shows the genetic variation, techno-functional, and thermal properties of the twelve pea protein concentrates. The dispersion in genotypes shown in the biplot indicates an acceptable amount of genetic variation among the pea concentrates. PC1 and PC2 accounted for 29.50% and 22.87% of the total variability, respectively. The most significant variables of pea protein concentrates are emulsifying capacity, foaming stability, oil absorption capacity, water solubility index, and water absorption index, which have positive values on PC1 and PC2. Water absorption capacity, foaming capacity, denaturation temperature, and peak temperature are negatively loaded on PC1 but positively on PC2, while protein solubility is positively loaded on PC1 but negatively on PC2.However, enthalpy had a negative value along PC1 and PC2. Among the pea concentrate samples, samples 34, 31, 32, 35, and 38 were positively correlated with PC1 and PC2, while samples 19 and 18 were negatively correlated.

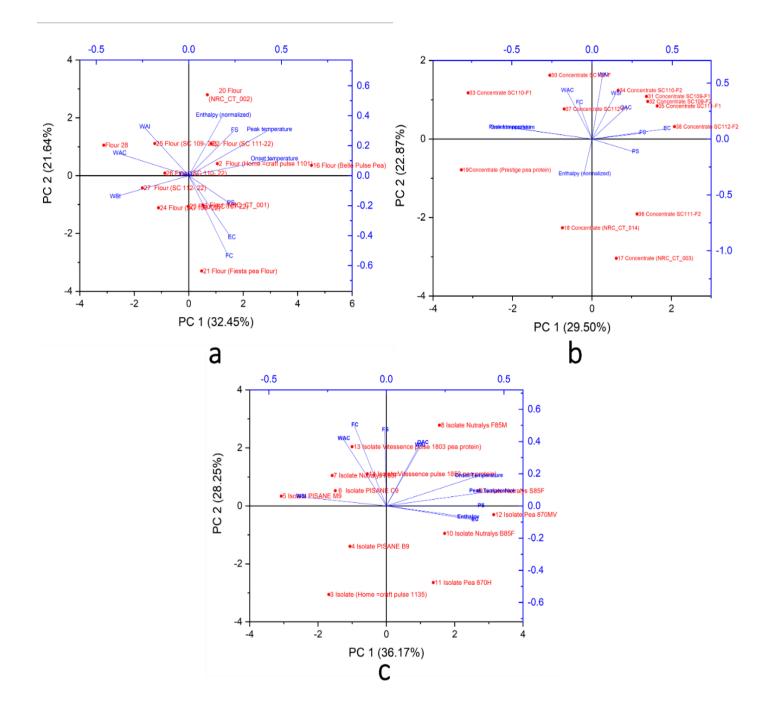


Figure 3.12. PCA analysis based on the techno-functional and thermal properties of pea protein products from 12 varieties for each of the protein products. Biplot for pea flour (a), Biplot for pea protein concentrate (b) Biplot for pea protein isolates (c).

where is the protein solubility (PS); water absorption capacity (WAC); water solubility index (WSI); water absorption index (WAI); oil absorption index (OAI); emulsifying capacity (EC); forming capacity (FC); and forming stability (FS), onset temperature (T_0), peak/denaturation temperature (Tdn), and gelatinization enthalpy (Δ Hg)

The biplot of pea protein isolates based on PC1 and PC2 is presented in Figure 8c. The biplot, based on PC1 and PC2, shows the genetic variation, techno-functional, and thermal properties of the twelve pea protein isolates. The dispersion in genotypes shown in the biplot indicates an acceptable amount of genetic variation among the pea protein isolates. PC1 and PC2 accounted for 36.17% and 28.25% of the total variability, respectively. The dominant properties in PC1 and PC2 of pea protein isolates are denaturation/peak temperature, onset temperature, forming capacity, oil absorption capacity, water absorption index, and water solubility index. The PC1 and PC2 were primarily correlated with positive scores for pea protein isolate genotypes: samples 8 and 12 due to the closeness of their coefficients to unity. According to Poudel et al. (2017), the variables (genotype and quality parameters) with the largest absolute or coefficient values closer to 1 within the first and second principal components have the most influence on the clustering (eigenvectors) more than those with lower absolute values closer to zero, as the cluster is complemented with PCA.

3.12 Cluster Analysis of Pea Protein Products

To compare and assess the relationships between different genotype varieties of the pea protein products, a hierarchical cluster analysis was performed based on techno-functional and thermal properties. As shown in Figure 3.13, the dendrogram consisted of two main groups for each of the pea protein products and was further divided into multiple clusters. In Figure 9, the two major clusters of groups of pea flour further divide into five clusters. The first cluster contains the largest genotypes, consisting of six genotypes that are distributed between four sub-clusters which are NRC_CT_001, SC 110-22, Homecraft 1101, Fiesta pea flour, SC 108-22, SC 112-22 of pea flour. The second contains one genotype (Flour 28), the third contains one genotype (NRC_CT_002), the fourth cluster contains three genotypes with two sub-clusters (SC 111-22, SC 109-22, SC 107-22), and the fifth cluster has one genotype (Belle Pulse Pea). However, the most represented cluster observation was in 1 Flour (NRC_CT_001) while the least was in 20 Flour (NRC_CT_002). The dendrogram of the pea protein concentrates in Figure 9 presented three clusters. The first cluster has three genotypes which include NRC_CT_003, SC 111-F2, and SC 112-F2.

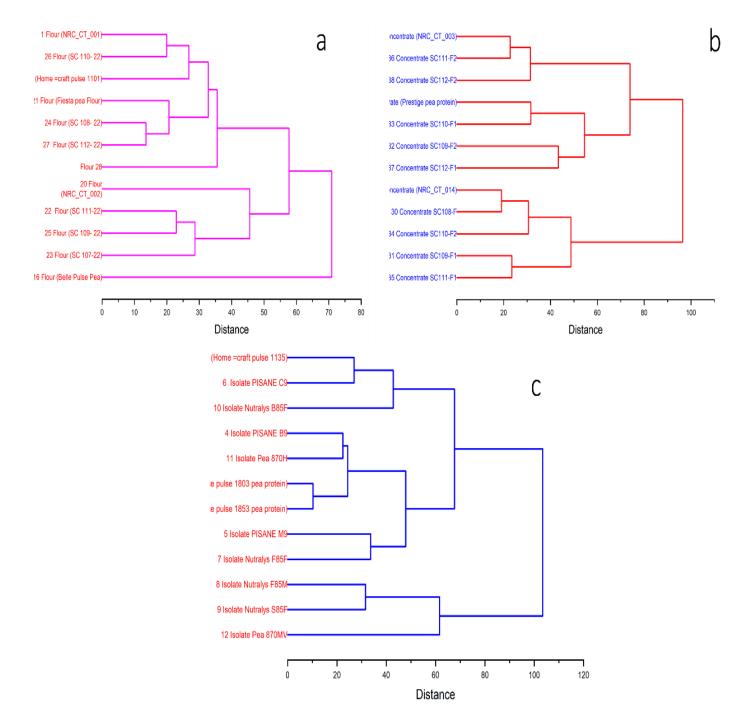


Figure 3.13. Dendrogram of twelve pea protein products Barley accessions using the Hierarchical Ward's clustering method based on eleven measured traits. Dendrogram for pea flour (a), Dendrogram for pea protein concentrate (b) Dendrogram for pea protein isolates (c).

The second cluster contains four genotypes, which include Prestige pea protein, SC110-F1, SC 109-F2, and SC 112-F1 while the third cluster contains the highest genotypes comprising five genotypes distributed between three sub-clusters which are NRC_CT_104, SC 108-F, SC110-F2, SC 109-F1, and SC 111-F1. However, the most represented cluster observation was in sample 30 (Concentrate SC108-F) while the least was in sample 31 (Concentrate SC109-F1). Similarly, the dendrogram chart for pea protein isolates has four clusters (Figure 5C). The first cluster contains three genotypes (Homecraft pulse 1135, PISANE C9, Nutralys B85F). The second cluster has the largest, comprising six genotypes, which are PISANE B9, Pea 870H, Vitessence pulse 1803 pea protein, Vitessence pulse 1853 pea protein, PISANE M9, and Nutralys F85F. The third cluster contains Nutralys F85M, Nutralys S85F, and Pea 870MV. The most represented cluster observation was in sample 11 (Isolate Pea 870H) while the least was in sample 12 (Isolate Pea 870MV). These results describe that the pea flour, pea concentrates, and isolates from different genotypes showed variations in the techno-functional and thermal properties, which align with the results of the principal component analysis.

3.13. Conclusions

This study has presented valuable information on the effect of composition (protein, starch, and oil contents) on the techno-functional and thermal properties of pea protein products (pea flours, concentrates, and isolates) from different varieties. There was a significant difference at p<0.05 in the techno-functional (water absorption capacity, water solubility index, water absorption index, oil absorption index, protein solubility, emulsifying capacity, forming capacity, forming stability) and thermal properties (onset temperature, peak temperature, and gelatinization enthalpies) of the pea protein products under the influence of composition. The onset temperature of the pea protein products ranged from 93.3°C to 166.19°C, with peak temperature ranging from 128.97°C to 180.74°C and gelatinization enthalpy ranging from 89.04 J/g to 280.42 J/g. The biplot of principal components 1 and 2 explained 54.09%, 52.37%, and 64.42% of the total variability of techno-functional and thermal properties of pea flours, pea protein concentrates, and pea protein isolates. The most unique variety based on the cluster of the variety of the pea protein solates. The most unique variety based on the cluster of the variety of the pea protein products was noticed to have the most significant influence on the water absorption index, oil absorption index, foaming capacity, peak temperature, and enthalpy.

The oil content showed the most significant influence on FS and onset temperature while the interaction of oil and protein contents influenced the water solubility index, water absorption index, and protein solubility the most. The outcome of this study would be useful in selecting the best pea flour, pea concentrate, and pea isolate based on desired techno-functional and thermal properties of the varieties under consideration.

CONNECTING TEXT TO CHAPTER FOUR

The preceding chapter of the thesis investigated the influence of the composition of pea protein products, specifically the protein, oil, and total starch content, on their techno-functional and thermal properties. These properties are important indicators of the quality and potential applications of the products. However, there is still a need to investigate how these composition factors affect some rheological properties of the pea protein products, specifically their storage modulus, loss modulus, loss factor, and complex viscosity.

Rheological properties refer to how materials behave under stress and strain, and they are important for understanding the flow behavior of food products during processing and storage. The rheological properties of the pea protein products, specifically the storage modulus, loss modulus, loss factor, and complex viscosity, are important indicators of the products' texture, mouthfeel, and stability.

The third chapter of the thesis has been drafted based on the format of the Industrial Crops and Products journal, which is a peer-reviewed scientific journal that publishes original research articles. The chapter is currently at the submission stage, which means that it has been written and is being prepared for submission to a scientific journal for peer review and publication. The next chapter presents the results of the investigation of the rheological properties of pea protein products and their relationship with the composition factors.

CHAPTER FOUR

RHEOLOGICAL PROPERTIES OF PEA PROTEIN PRODUCTS

Abstract

In efforts to develop protein-based products with optimal functional and textural characteristics, the investigation of the effect of composition on the rheological properties of pea protein products (PPP) is required. Therefore, this study aimed to explore the diversity in effect of composition (protein, oil, and starch) on the rheological properties (storage modulus (G'), loss modulus (G"), loss factor (tan δ) and complex viscosity (tan δ)) of PPP. The relationship between rheological properties and varieties of PPP was analyzed by principal component analysis (PCA) and cluster analysis (CA). The rheological properties of PPP are significantly influenced by their composition. Increasing the protein concentration led to a significant rise in storage modulus, loss modulus, and loss factor, while the complex viscosity decreased. Conversely, an increase in oil caused a decrease in storage modulus, loss factor, and complex viscosity. Furthermore, a rise in starch content led to a significant increase in the complex viscosity of pea protein products. The first two principal components, PC1 and PC2 of the biplots explained 98.68%, 87.94%, and 77.77% of the total variability of rheological properties of pea protein products. The unique varieties based on the cluster analysis among the pea protein products were established. This study outcome would be valuable in predicting the processing behavior of PPP and in controlling the quality of its final products.

4.1 Introduction

The utilization of protein-enriched ingredients in food applications has become progressively crucial in the last few years. This results from food insecurity, malnutrition, increasing population, the elevated cost of animal-based foods, limitations caused by allergies, and dietary preferences. These necessitate the utilization and incorporation of novel protein products to augment conventional food formulations and meet diverse consumer choices (Pazmiño et al., 2018; Boukid et al., 2021). Food proteins' ability to form heat-induced gels offers significant rheological properties to foods and is essential in product development. Pea (Pisum sativum) proteins are recognized as auspicious and novel raw materials owned to their unique and high

protein compositions, non-allergenicity, low cost, and potential health benefits (Arteaga et al., 2020). They also exhibit unique nutritional and functional properties, such as solubility, foaming, emulsifying, and gelling characteristics, which govern their performance and behaviour during processing (Lamsal et al., 2007; Boukid et al., 2021). Pea protein products such as flour, concentrate, and isolate can be employed to develop numerous functional foods. These include high-protein meat substitutes, gluten-free products (snacks, bread, pasta), ready-to-eat cereals, and heat-induced gels that exhibit unique and acceptable attributes (Pedrosa et al., 2020). However, it is worthwhile to understand their rheological properties to efficiently utilize pea protein products as functional raw materials in the food matrix.

Rheological property is an essential physicochemical characteristic of food. The rheological property indicates the complex interaction between the composition, molecular conformation, and structure of the food components (Dasa & Binh, 2019). It is a central index often employed for assessing raw materials and product quality and predicting its behaviour during processing and storage (Dasa & Binh, 2019; Zhang et al., 2020; Mir et al., 2021). It also assists in predicting the flow and deformation of protein, starch, and fats in specific food systems under external forces (Wang et al., 2019). Furthermore, the rheological properties of protein pastes or gels are a function of the frequency. Rheology is often measured through oscillatory mechanical spectroscopy (Sonawane et al., 2020). Rheological or viscoelastic properties such as storage modulus (G'), loss modulus (G''), loss factor, and complex/apparent viscosity control the physicochemical characteristic of protein products and products by indicating their elastic and viscous behaviour. Generally, the G' value defines the strength and stability of a gel network, whereas the G" value defines the elastic nature of the gel (Wang et al., 2019). The rheological property of paste protein products largely depends on their composition, protein-to-starch gelatinization, degradation degree, and swelling power. Also, the nature of the continuous phase, the interaction between the dispersed and continuous phase, and the volume occupied in the dispersed phase of gel similarly influence its rheological properties (Guha & Ali, 2011).

The pea is known to have different varieties with distinct genetic makeup, which can consequently influence the gelation properties of its protein products during food application (Maharjan et al., 2019). Understanding the genotypic variation in the rheological properties of pea protein products is significant in optimization and textural assessment and maximizes flow

processes. It is also crucial in measuring processing and storage stability and evaluating the change in the conformation of pea protein products (Dasa & Binh, 2019). It can also be employed to develop highly suitable pea protein products in food applications such as 3D printing (Zhang et al., 2020). According to Maharjan et al. (2019), the protein composition of paste, its average size of starch granules, and the dynamic rheological properties are significantly influenced by the variation in variety. Romero and Zhang (2019) investigated the rheological behaviour of four varieties of bean flour to develop gluten-free pasta. The rheological properties of the studied bean flour differ among different flour varieties. Also, the genetic variation in different grass pea flour examined by Bala et al. (2020) showed that the compositional effect (such as protein and starch) significantly influence the rheological properties of the dough samples prepared from the flour samples. Also, the G' and G'' of the different dough samples increase with the frequency, which reflects a robust elastic response or gels; however, G' had a higher value than G'' in the frequency sweep owned to the different particle sizes of the different grass pea flour.

Similarly, Katyal et al. (2019) studied the difference in rheological properties of composite flour from different varieties of wheat flour. The wheat flour varieties demonstrated different rheological behaviour due to variations in their protein content, which are influenced by the different varieties. Flours with higher protein content exhibited improved rheological properties and vice versa. In efforts to compare and evaluate the relationships of different varieties of the pea protein products, cluster analysis is often applied to amplify the similarity or discrepancy within the cluster groups based on the rheological properties of the pea protein products (El-Hashash et al., 2016). The literature has mainly focused on the rheological properties of flour from different varieties of beans (Katyal et al., 2019; Romero & Zhang, 2019; Bala et al., 2020; Zhang & Zhai, 2020; Zhang et al., 2022). However, there is sparse information on effect of composition on the rheological properties of pastes of pea protein products from different pea varieties.

Furthermore, the use of cluster analysis to assess the effect of variation in varieties on the comparable rheological properties of pea protein products is lacking. Thus, this information would aid in identifying and selecting the most suitable varieties of pea protein products based on their rheological properties. The studied rheological properties are storage modulus, loss

modulus, loss factor, and complex viscosity. Therefore, this study aimed to evaluate the effect of composition (protein, oil, and starch) on the rheological properties of pea protein products.

4.2. Materials and Methods

Thirty-six (36) varieties of pea products were selected and used for this study to elicit samples of different proximate compositions. The samples include 12 varieties of pea protein flour (labelled: NRC_CT_001, Homecraft Pulse 1135, Belle Pulse Pea, NRC_CT_002, Fiesta Pea Flour, SC 111-22, SC 107-22, SC 108-22, SC 109-22, SC 110-22, SC 112-22 and SC 113-2); 12 varieties of pea protein concentrate (Labelled: NRC_CT_003, NRC_CT_014, Prestige Pea Protein Concentrate, SC108-F, SC109-F1, SC109-F2, SC110-F1, SC110-F2, SC111-F1, SC111-F2, SC112-F1 and SC112-F2); and 12 varieties of pea isolate (labelled: Homecraft Pulse 1135, PISANE B9, PISANE M9, PISANE C9, Nutralys F85F, Nutralys F85M, Nutralys S85F, Nutralys B85F, Pea 870H, 870MV, Vitessence Pulse 1803 Pea Protein, and Isolate Vitessence Pulse 1853 Pea Protein). These varieties were selected based on their different compositions and obtained from the National Research Council of Canada through a material transfer agreement.

4.3 Dynamic Rheological Properties of Pea Protein Products

The response surface design in Minitab software version 20 was used to study the effect of compositions (starch, oil content, and protein content) of pea products on their rheological properties. The low and high values of the experimental factors namely protein content, oil content and starch content (labelled A, B, and C, respectively) of samples used in the study were 0.05 and 64.65, 0.60 and 3.36, 11.46 and 81.55, respectively. Pareto chart was used to determine the significant composition while contour plots were used to study the influence and interactions of the compositions on the rheological properties of pea protein products.

4.3.1 Rheological measurements

A modified approach by Arntfield et al. (1989) was adopted to determine the rheological characteristics of the pea flour, pea protein concentrate, and pea protein isolate pastes. 4 g pea protein isolate samples were dispersed in 20 ml of water. The dispersions were stirred/mixed at 900 rev/min for 2 hours. After then, stored in the refrigerator for 4°C. The samples were loaded on the rheometer plate of 40mm diameter (Model: MCR 302 Antin Paar). The rheometer gap was adjusted to 1000mm or 2000mm depending on the viscosity of the samples. The samples

were allowed to stabilize ad equilibrate for 2 minutes in the rheometer. For steady shear, the shear rate was adjusted to $1-100^{s-1}$ at 25°C. For the frequency sweep test, the temperature was left at 25°C with an angular frequency that ranged between 0.1-100rad/s. For the temperature sweep test, the temperature was set to 25-95°C at a speed of 5°C/min. After this procedure, the samples were held and heated at 95°C for 2 minutes. The samples were cooled at 25°C at 5°C /min. The G' and G'' and loss modulus were evaluated and recorded. Also, the loss factor and complex viscosity were also determined.

4.4 Statistical analysis

The data were duplicated and analysed statistically at a 95% confidence level using the OriginPro version 9, 2022 for the analysis of variance and multivariate analysis in terms of principal component analysis and hierarchical analysis.

4.5 Results and Discussion

4.5.1 Storage Modulus (G') of Pea Protein Products

The G' of the PPP showed less frequency-dependent properties and a clear difference in G' among the different PPP was observed during the frequency sweep. According to the Doublier (1995), if the storage modulus (G') of protein is independent of frequency, this suggests the presence of a strong paste network with sturdy junction zones that won't break during the experiment. Conversely, if G' shows a strong dependence on frequency, this suggests weaker molecular interaction. Storage modulus describes the energy stored in the elastic structure of a food, which will be released after mechanical stress or deformation (Amiri et al., 2018; Sonawane et al., 2020). This energy results from the conformational modification of the protein chains (Zhang et al., 2022). Significant influence (p<0.05) of composition was observed on all the PPP. Pareto analysis indicates that the fat contents have the most significant influence on the G' of the PPP. The interaction of protein and starch had an equal significant effect on the G' (Figure 4.1a). The contour plots show that the storage modulus of the PPP tend to increase with an increase in protein content and decrease in oil and starch contents (Figure 4.1b and c).

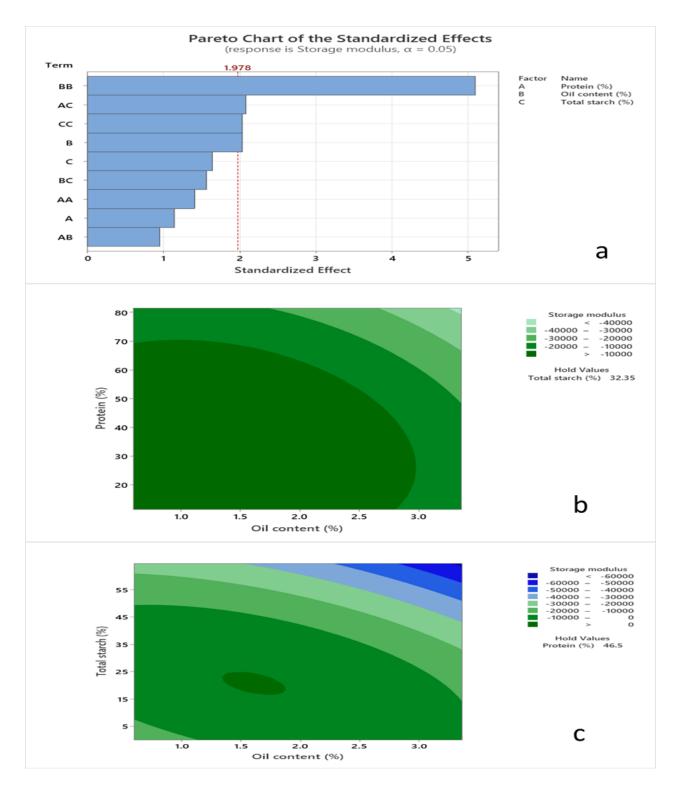


Figure 4.1. Effect of protein, total starch, and oil contents on the G' properties of pea protein products.(a) Pareto chart of G', (b) Influence of protein and oil contents on G', (c) Influence of starch and oil contents on G'

These observations align with the results of Saha et al. (2011) who examined the effect of composition on rheological characteristics of biscuit dough. The difference in the rheological properties of the dough was reported to be due to oil content along with protein and starch contents. Sudha et al. (2007) described that fat covers the surface of the flour particles which impeded the creation of the gluten proteins. Also, Menjivar and Faridi (1994) reported that the free fat can disrupt the gluten network of the developed dough causing softer cracker and cookie doughs. Higher storage modulus of the pea protein product specifies a greater ability of the paste to recover its shape after disruption, i.e., exhibit high elastic or strong solid characteristics. Pea protein product with high G' could be recommended to food manufacturers owned to their high G' as the basic ingredient in different food applications such as sausage, comminuted meats, and 3D printed pea proteins.

4.5.2 Loss Modulus (G") of Pea Protein Products

The G" of the PPP depends on the angular frequency and are significant difference among the different pea protein products. The increase in frequency with G" enhances the distribution of viscous components to the viscoelastic properties of the pea protein pastes. These results follow a similar pattern to Tangsrianugul et al. (2019) that the loss modulus properties of rice gels increased with angular frequency among the different varieties. The large G" observed in pea protein products reflects the ability of the pea pastes to resist flow with high viscous properties (Zhang et al., 2022). In contrast, a low G" value in pea protein paste indicates the stiffness and rigidity of the paste (Kulamarva et al., 2009). As presented in Pareto analysis, the fat content has the most significant influence on the G" of the PPP. This is equally influenced by the interaction of protein and starch contents (4.2a). This could be ascribed to an intertwined network between macromolecules such as protein, fat, and starch. This is equally stated by Zhang et al. (2022) from the rheological properties of starches isolated from common bean (Phaseolus vulgaris L.) varieties, where the G" was reported to differ in the common bean varieties. The increase in protein content of the pea protein paste tends to increase their loss modulus (Figure 4.2b). But the increase in oil and starch contents had a negative correlation with loss modulus (Figure 4.2c). This can be affirmed in the contour plots.

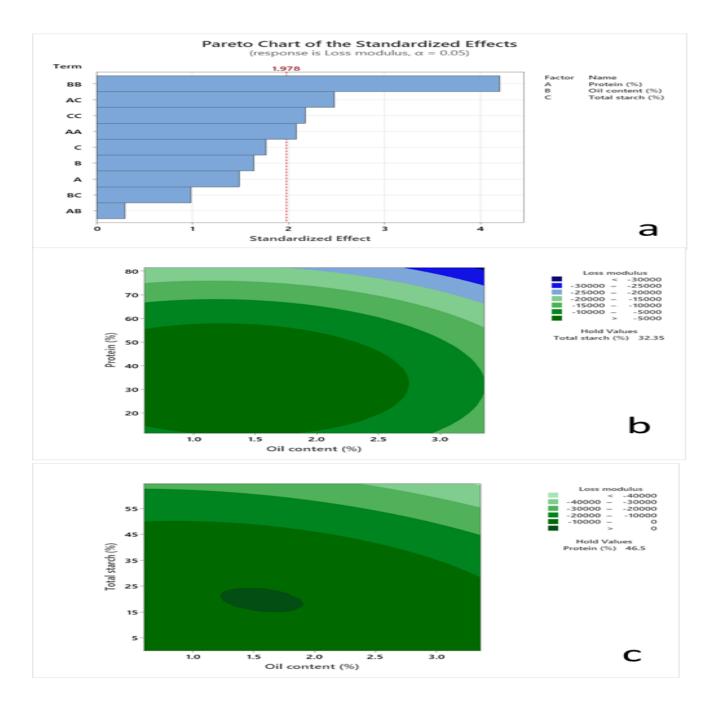


Figure 4.2. Effect of protein, total starch, and oil contents on the G" properties of pea protein products.(a) Pareto chart of G", (b) Influence of protein and oil contents on G", (c) Influence of starch and oil contents on G"

The effect of protein content on the G" of pea protein doughs was established by Ziobro et al. (2013) and Klost and Drusch (2019). Significant changes were observed in the rheological properties of the bread dough with increase in protein content (Ziobro et al., 2013). For instance, addition of lupine protein resulted into a significant increase in the loss modulus. This is because proteins are important structural components of the material and contribute to the viscoelastic properties of the paste (Ziobro et al., 2013). Contrary to this, a decrease in the slope of G" of pea gel was observed which was correlated to the protein content of the fermented pea gels (Klost and Drusch, 2019). This dissimarities in the literature can be justified that, the relationship between protein content and loss modulus is not always linear and can depend on other factors. These include protein concentration, starch composition, paste structure, binding energy, and the degree of protein chains' interactions in the paste sample (Ahmed et al., 2016; Zhu et al., 2016; Amiri et al., 2018).

Furthermore, the decrease in loss modulus with starch content might be due to the relatively rigid component of starch, and as the starch content increases, the paste becomes more rigid and less able to dissipate energy, thereby lowering the loss modulus. For instance, amylose content was described to have a negative relationship with loss modulus, as pastes with higher G" exhibit low amylose content. Similarly, the flow of starch paste is influenced by external mechanical forces, which consequently change the paste conformation. This corroborates Zhang and Zhai (2020) on the rheological properties of varieties of red adzuki bean starch.

4.5.3 Loss Factor (tan δ) of Pea Protein Products

Loss factor, otherwise known as loss angle or phase angle, describes the amount of energy dissipation when a food material is vibrated to the maximum potential energy stored in the food material (Franck & Germany, 1993). It indicates the degree of elastic or viscous components of the viscoelastic characteristics of the food (Zhang et al., 2022). The loss factor for all pea protein products was less than 1 (unity). This supports the study of Atudorei et al. (2021) on the rheological properties of wheat-bean composite flour, where all the analyzed samples had loss factor values of less than 1. Pastes from pea protein products with a high loss factor demonstrate the high viscous behaviour of the pea pastes, while the low loss factor demonstrates elastic-dominant pastes. The variation in the loss factor among the pea protein products could be attributed to difference in varieties of pea protein products. This support Haddarah et al. (2014)

that the loss factor (phase angle) of bean gum depends on the varieties of the carob bean varieties. Furthermore, the tan δ of the pea protein products is largely influenced by the pea protein composition where the protein content had the most significant influence on the tan δ . Also the starch content also affect the degree of elastic and viscous components of the viscoelastic characteristics of the pea pastes. This can be seen in the Pareto analysis (4.3a). In addition, the protein content had a positive linear relationship with the tan δ , where the tan δ of the pea protein pastes increased with an increase protein content (4.3c). This increase can be influenced by the high thermal treatment of the pea products resulting to highly purified and processed products. This tends to lower the loss factors and results in a higher concentration of protein and a lower amount of non-protein components. The tan δ of the pea protein products is also influenced by the starch content and type of starch. For instance, high amylose content in a paste could result in a lower loss factor, exhibiting a negative correlation with amylose content (Tangsrianugu et al., 2019). However, this may differ among different types of starch present in the pea products.

4.5.4 Complex viscosity ($|\eta^*|$) of Pea Protein Products

Complex viscosity ($|\eta^*|$), also known as apparent viscosity, describes the changes in the intermolecular forces of food protein at a steady shear test (Wang et al., 2019). Non-newtonian fluids such as pastes are fluids with viscosity that remains constant regardless of the applied shear rate at constant temperature (Yahia et al., 2016). The pea protein products, therefore, displayed non-newtonian fluid behavior since their viscosities did not vary linearly with the shear rate. All the pea products exhibited a shear-thinning phenomenon, decreasing in viscosity as the shear rate increased. This phenomenon is consistent with the flow curve of pea protein dispersions reported by Chen et al. (2021) and Xia et al. (2022) and is attributable to Newtonian fluids. Pareto analysis showed that, the oil content had the most dominating influence on the complex viscosity of the pea protein products (Figure 4.4a). Similarly, contour plot indicated that the η^* of the pea protein protein tends to increase with decrease in oil content (Figure 4.4b).

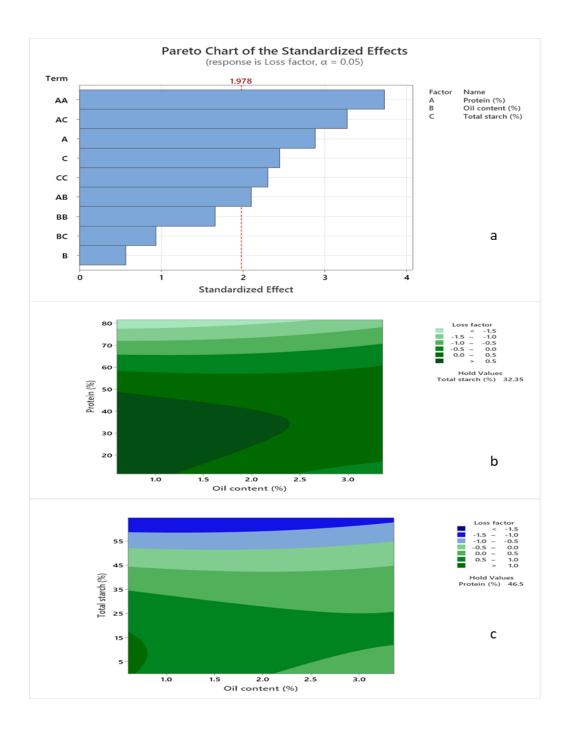


Figure 4.3. Effect of protein, total starch, and oil contents on the tan δ properties of pea protein products. (a) Pareto chart of tan δ , (b) Influence of protein and oil contents on tan δ , (c) Influence of starch and oil contents on tan δ .

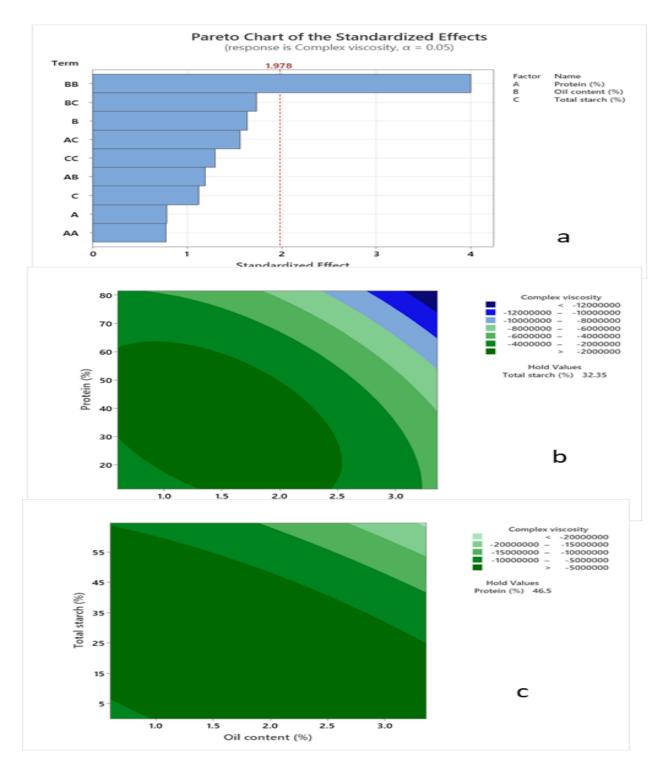


Figure 4.4 Effect of protein, total starch, and oil contents on the tan δ properties of pea protein products.(a) Pareto chart of η^* , (b) Influence of protein and oil contents on η^* , (c) Influence of starch and oil contents on η^*

This occurrence increase in the complex viscosity with decrease in oil content is referred to the resistance of a fluid to flow under deformation. This is due to the ability of oil to act as a lubricant, reducing the interactions between the protein molecules and leading to a more fluid-like consistency. This is could equally be due to the intermolecular (repulsive and attractive interaction) between the oil and proteins of the pea protein products, which could affect their complex viscosity (Krentz et al., 2022). The increase in starch content and decrease in protein content resulted into pea protein products with high η^* (Figure 4.4b). Bala et al. (2020) reported a similar increase in η^* with lower amount of protein and high amount of starch. Higher complex viscosity value specifies an increase in the molecular interactions and strengthening of dough structure. The increased complex viscosity observed explains an intensification in the molecular interaction and firmness of the paste structure. This phenomenon was also reported by Bala et al. (2020) on the rheological properties of grass pea (*Lathyrus sativus L.*) flour, where high η^* describes the increase in the strength and molecular interaction of the dough.

4.6 Principal Component Analysis of Pea Protein Products

In an effort to simplify the complexity of the rheological properties among the different varieties of pea protein products, the principal component analysis (PCA) was employed. At eigenvalues ≥ 1 , the PCA of the pea protein products exhibits three principal components. For pea protein flour, the principal component (PC) amounts to 98.68% of the total variation, whereas the PC1, PC2, and PC3 account for 75.30%, 23.08%, and 1.19%, respectively. In the pea protein concentrates, the PC presents 87.94 of the total variation (at eigenvalues \geq 1) with 57.36%, 30.58%, and 12.06% for PC1, PC2, and PC3, respectively. Likewise, the first three components (eigenvalues \geq 1) of the pea isolates explain 99.84% of the variation, with 52.58%, 35.19%, and 12.08% for PC1, PC2, and PC3, respectively. PC1 is considered to describe the most variation. In contrast, PC2 describes the second most variation in data when multidimensional data is predictable in the form of single-dimensional data, as described by Lee et al. (2017). The PC1 and PC2 of the pea protein flour accounted for 75.30% and 23.38% of the total variability in the 12 varieties of pea protein flour. The association between the different varieties of pea protein products and rheological properties are presented in Figures 4.5a - c of the biplots. The biplots display the pea variety and properties having the largest contributor to the total variation on every dimension (Sharma, 1998).

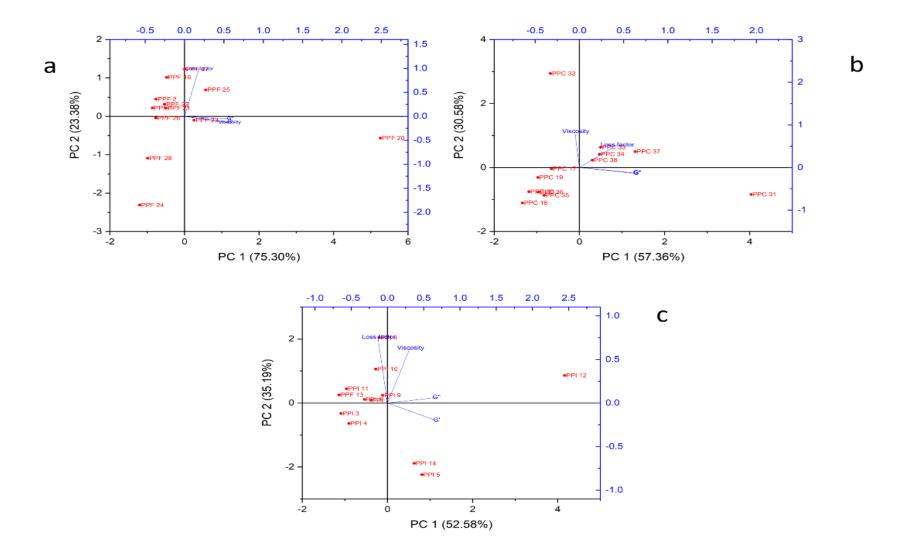


Figure 4.5. PCA analysis based on the rheological properties of pea protein products from 12 varieties for each of the protein ingredients. Biplot for pea flour (a), Biplot for pea protein concentrate (b) Biplot for pea protein isolates (c).

Singh et al. (2008) describes that the curves closer to each other on the bipolar are positively correlated, whereas those in reverse directions are negatively correlated. Thus, the loss factor had the leading variable with the positive peak value on the PC1 and PC2 (Figure 4.5a). The storage modulus, loss modulus, and complex viscosity were on the positive value of PC1 but negatively loaded on PC2. Similarly, PPF 20 had the dominant varieties among the pea protein flour, "PPC 31" for pea protein concentrate and "PPI 12" for pea protein isolate, along the PC1 and PC2.

4.7 Cluster Analysis of Pea Protein Products

The hierarchical cluster on the rheological properties of pea protein products was done to differentiate and evaluate the relationships of different genotypes of the pea protein products. This is presented in Figures 4.6a - c of the dendrograms. The dendrogram comprised two main groups each for the pea flour, pea protein concentrates, and pea protein isolate, which was further distributed into multiple clusters. As apparent in Figure 6a, the two major clusters for pea flour are further divided into four clusters. The primary cluster contains the largest genotypes, comprising nine genotypes that are distributed between four sub-cluster, which are PPF1, PPF 2, PPF 24, PPF 21, PPF 27, PPF 16, PPF 22, PPF 28, and PPF 26 of pea flour, the second contains one genotype (PPF 23), the third contains one genotype (PPF 25), and the fourth cluster also has one genotype (PPF 20). However, the most represented cluster observation was in PPF 16, while the least represented was in PPF 20. Figure 4.6b represents the dendrogram of the pea protein concentrates, which demonstrated four clusters. The first cluster has the highest genotypes and contains six genotypes distributed between two sub-cluster types: PPC 17, PPC 30, PPC 36, PPC 18, PPC 19, and PPC 32. The second cluster has three genotypes distributed between two subcluster: PPC 34, PPC 35, and PPC 38. The third cluster contains PPC 33 and PPC 37. However, the most represented cluster observation was in PPC 35, while the least was in PPC 31. Likewise, the dendrogram chart for pea protein isolates has four clusters (Figure 4.6c). The first cluster contains the highest genotypes consisting of four distributed between two sub-cluster, which include PPI 3, PPI 4, PPI 5, and PPI 13. The second cluster has three genotypes distributed between two sub-cluster, PPI 8, PPI 14, and PPI 9, whereas the third cluster contains PPI 7 and PPI 11. The fourth cluster has three genotypes distributed between two sub-cluster, which are PPI 6, PPI 10, and PPI 12. The most represented cluster observation was in PPI 8, while the least was in PPI 12.

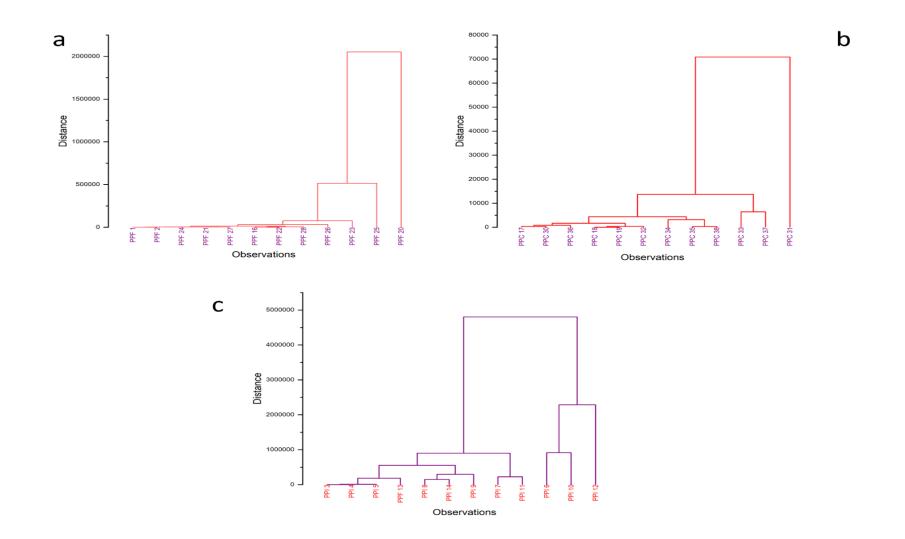


Figure 4.6. Dendrogram of twelve pea protein products Barley accessions using the Hierarchical Ward's clustering method based on eleven measured traits. Dendrogram for pea flour (a), Dendrogram for pea protein concentrate (b), Dendrogram for pea protein isolates (c).

These results explain the variability of the rheological properties of pea protein products among the different pea varieties. Similarly, this also showed the linearity in hierarchical cluster and principal component analysis results.

4.8 Conclusion

Rheological assessments, namely storage modulus, loss modulus, loss factor, and complex viscosity, are rheological indices for examining the rheological behaviour of pea protein products from different varieties. The composition of pea protein products, including their protein, oil, and starch contents, showed significant influence (at p<0.05) on their rheological properties. A higher protein content leads to higher storage modulus, loss modulus, and loss factor, while the addition of oil can decrease the storage modulus, loss factor and complex viscosity. The presence of starch tends to favour complex viscosity of the pea protein products. The most and least dominant rheological properties among the pea protein products was recognized. The pea protein products with utmost cluster were also established. Understanding the impact of composition on rheological properties is critical in designing pea protein products with desired texture, stability, and functionality for various applications in the food industry. Thus, the outcome of this study would be useful in the characterization of the rheological properties of pea protein products to control and optimize product properties and qualities.

CONNECTING TEXT TO CHAPTER FIVE

The preceding chapter investigated how different compositions of total starch, protein, and oil affected the rheological properties of pea products. Rheology is the study of how materials deform and flow, and in this case, it refers to the properties of how the pea products behave under different conditions. The study involved measuring the storage modulus, loss modulus, loss factor and complex viscosity of the pea products with different compositions.

In chapter five, a different approach was taken to predict the properties of protein products. Instead of relying on traditional experimental methods, an artificial neural network machine learning algorithm was used. This type of algorithm is modeled after the way the human brain works and can learn patterns from data, allowing it to make predictions about new data.

The machine learning algorithm was trained using data on the techno-functional, thermal, and rheological properties of protein products. The algorithm learned to recognize patterns in the data and make predictions about the properties of new protein products based on their composition.

Overall, the study in chapter five used artificial intelligence to predict the properties of protein products, which could be useful for food manufacturers looking to optimize their products for specific uses or applications. This approach could also reduce the need for time-consuming and expensive experimental testing, making it a more efficient and cost-effective way to develop new products.

CHAPTER FIVE

MACHINE LEARNING-BASED PREDICTION OF TECHNO-FUNCTIONAL, THERMAL AND RHEOLOGICAL PROPERTIES FOR PEA PROTEIN PRODUCTS

Abstract

This study aimed to predict the techno-functional, thermal, and rheological properties of pea protein products using a machine learning artificial neural network algorithm. The research revealed variations in the optimal number of neurons, iterations, and hidden layers for different properties such as water absorption capacity, water solubility index, oil absorption index, foaming capacity, protein solubility, emulsifying capacity, foaming stability, onset temperature, peak temperature, enthalpy, complex viscosity, storage modulus, loss modulus, and loss factor. Additionally, the study found differences in the Mean Absolute Error, coefficient of determination, Mean-Squared Error, and RMSE for the properties in both training and test datasets. These findings provide insights into the potential of machine learning algorithms for predicting the properties of pea protein products.

5.1 Introduction

Techno-functional, thermal and rheological properties of pea protein products are important for both product quality and process efficiency. Techno-functional properties of pea protein products describes those properties of the products except the nutritional ones, which affects their utilization in food systems. These include properties associated with protein hydration (such as protein solubility, water and oil absorption capacities) and protein surface characteristics (foaming capacity and stability, and emulsifying properties) (Zhao et al., 2020). The thermal properties of pea protein products, namely onset temperature, peak temperature, gelatinization enthalpy deals with their physical and chemical behaviors in response to temperature changes (Lu et al., 2019). These properties play significant role in the processing and utilization of pea protein-based foods, as they can affect the texture, stability, and overall quality of the final product (Ahmed et al., 2021). The rheological property on the other hand, is concerned with how

pea protein products behave and deform in response to an external force, which are important for ensuring product quality, sensory appeal, and formulation stability (Tarafdara et al., 2020).

These functional properties such as techno-functional, rheological, and thermal properties enable a wide utilization of pea protein products in various food-related products including extruded foods, edible films and encapsulation for bioactive ingredients. They also help to develop and produce dairy analogue drinks, curd, and fermented products (Shanthakumar et al., 2020). However, peas have different varieties that contain distinct components like protein, oil, and starch, which can affect their functional properties. The interactions between the various components in pea products can be complex and non-linear, making it challenging to predict their functional properties accurately during food applications. In addition, there is a large amount of data that needs to be collected and analyzed to develop accurate predictive models to predict their functional properties. Thus, machine-learning techniques may be required to develop accurate predictive models.

Machine learning offers an opportunity to analyze data and has an advanced advantage in performing "intelligence" tasks over calculations performed by humans since machine learning algorithms are better equipped to identify unconventional patterns in large data sets (Kim et al., 2018). Polynomial regression is currently one of the prevalent machine learning techniques used to identify risk factors that can predict the development of complications. On the other hand, artificial neural networks (ANNs) are a different type of machine learning that is non-linear and highly adaptable, unlike polynomial regression (Sanusi and Akinoso, 2021).

The artificial neural network (ANN) distinguishes itself from conventional programs due to its non-linear, multi-parameter coupling problems and ability to learn about a system without prior knowledge of the processing variables involved (Wang et al., 2019; Muhammed et al., 2022). Unlike traditional methods, a properly trained neural network can produce multiple outputs simultaneously. They can also be utilized to mimic human's ability for pattern recognition (Naik et al., 2022). These networks, if appropriately trained, can be efficiently used to process data and establish correlations using multiple iterations without utilizing any prior association data. These networks can be used to simplify the overall pattern recognition of computational data and have been used to predict different functional properties of foods (Naik et al., 2019). Additionally, ANNs can be utilized in situations where an exact mathematical description of the process is not

available (Bhattacharya and Bhavesh, 2007). ANNs are made up of neurons, which are the basic units of the network. The ANN is composed of three types of layers: the input layer, which receives the input parameters, the hidden layers, which contain neurons that apply transfer functions between the inputs and outputs, and the output layer. The structure and parameters of the ANN, such as the number of neurons, weights, and biases, are crucial for the network's performance. To optimize these parameters, the network is trained using various algorithms to minimize the error. One such algorithm is Levenberg-Marquardt, a curve fitting algorithm that has proven effective in solving non-linear least-squares problems (Gowida et al., 2019). As a result, they gained significant recognition and are intriguing techniques for estimating, predicting, and controlling bioprocesses (Ajasa et al., 2014).

In recent years, the use of ANNs in the field of food processing has a broad and comprehensive range of possibilities. It could transform electrophoretic focusing patterns as well as chromatographic and spectrum data into significant information that can be used to forecast different functional, physical, chemical, sensory, and rheological characteristics of various food products (Muhammed et al., 2022). They have also proven to be successful modeling tool in various food-processing applications include sensory analysis, quality control, classifications, microbiology, and drying applications (Kumar et al., 2019). Muhammed et al. (2022) used ANNs to forecast the quality characteristics of dates stored in cold conditions by analyzing their electrical properties; Wang et al. (2019) employed ANN to estimate the thermal conductivity of different nanofluids comprising ethylene glycol, while Rostami et al. (2020) utilized ANN to predict the thermal conductivity of a nano-fluid. In addition, the thermos-physical characteristics of deep-fat fried plantain chips (ipekere) was forecasted using artificial neural networks (Adeyanju et al., 2021), while Sanusi and Akinoso (2022) studied the energy consumption behavior in rice processing using Taguchi and artificial neural network methodologies. Abraham et al. (2020) evaluated the ability of ANNs to forecast time series data on Brazilian soybean production. Although there are numerous potentials to benefit from this method, particularly in complex systems, however, the application of artificial neural network for predicting the functional (techno-functional, rheological, and thermal) properties of pea protein products remains scarce up to the present date. Therefore, based on the information available in the literature, it would be worthy to adopt artificial neural network to accurately predict these functional properties, which would enable efficient optimization of food processes to produce

high-quality, consistent products that meet consumer expectations. Thus, this study aimed to predict the techno-functional, thermal and rheological properties of pea protein products using artificial neural network machine learning.

5.2. Methodology

Artificial Neural Network (ANN) is a machine learning technique that can be used to predict and model complex relationships between inputs and outputs. In this study, the Scikit-Learn Multi-Layer Perceptron (MLP) Regressor in Python version 3.11.3 was used to build an ANN model to predict the techno-functional (water absorption capacity, water solubility index, oil absorption index, foaming capacity, protein solubility, emulsifying capacity, and foaming stability), thermal (onset temperature, peak temperature, and enthalpy) and rheological (complex viscosity, storage modulus, loss modulus, and loss factor) properties of pea protein products based on its protein, oil and total starch contents as shown in schematic diagram 5.1, 5.2 and 5.3. The data obtained from the techno-functional, thermal, and rheological properties were split into training and test sets, and the performance of the model was evaluated using mean absolute error, mean squared error, root-mean squared error, and coefficient of determination (\mathbb{R}^2) values.

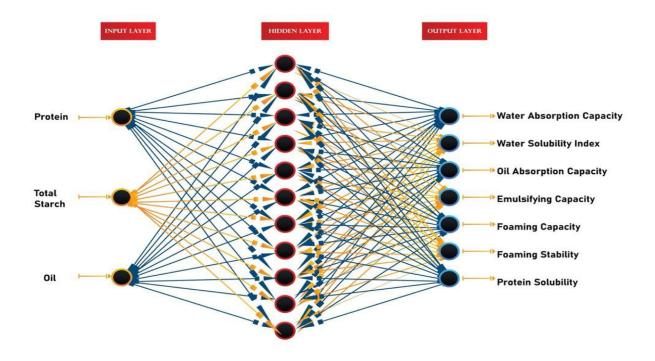


Figure 5.1. Schematic diagram of Artificial neural network (ANN) for prediction of technofunctional properties of pea protein products

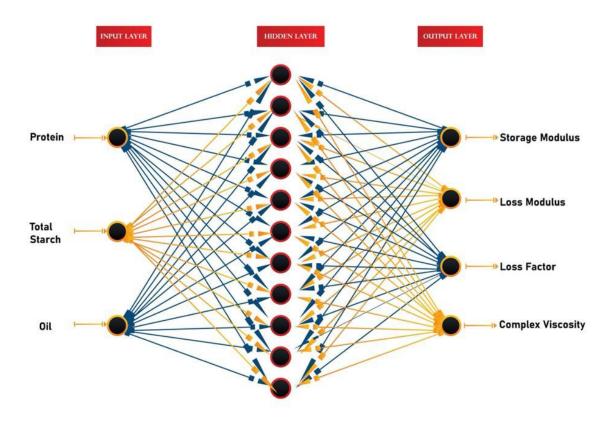


Figure 5.2. Schematic diagram of Artificial neural network (ANN) for prediction of thermal properties of pea protein products

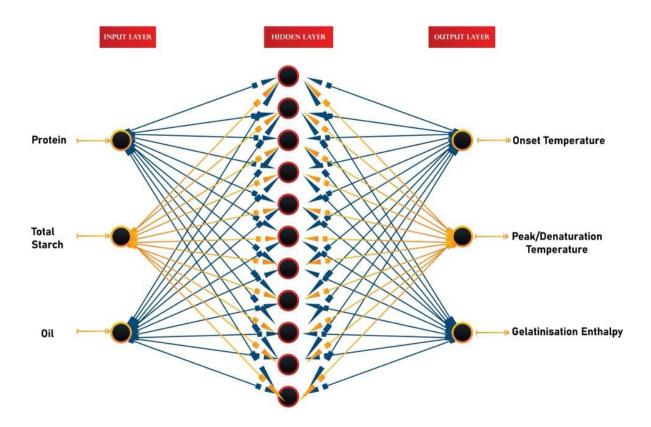


Figure 5.3. Schematic diagram of Artificial neural network (ANN) for prediction of rheological properties of pea protein products

5.3 Model Development Stages

The following steps were taken in developing the model:

Step 1: Importing the required libraries and data

The first step was to import the required libraries and load the data into the Python environment. The Pandas library was used to read the data from a CSV file and convert it into a Pandas DataFrame. The Scikit-Learn library was used to split the data into training and test sets and was also used to build and evaluate the MLP regressor model.

import pandas as pd

from sklearn.neural_network import MLPRegressor

from sklearn.model_selection import train_test_split

data = pd.read_csv('technofunctional thermal rheological_properties.csv')

Step 2: Data preprocessing

Before building the ANN model, the data were preprocessed to ensure that it is suitable for analysis. This involves removing any missing values, scaling the data, and splitting it into input and output features as shown in schematic diagrams 1, 2, and 3.

Scale the input features

from sklearn.preprocessing import StandardScaler scaler = StandardScaler()

X = scaler.fit_transform (data[['Protein', 'Oil content', 'Total starch']])

Split the data into input and output features

y = data [['Water Absorption Capacity', 'Water Solubility Index', 'Oil Absorption Index', 'Foaming Capacity', 'Foaming stability', 'Protein solubility', 'Emulsifying capacity']]

y = data [['Onset temperature', 'Peak temperature', 'Enthalpy']]

y = data [['Complex Viscosity', 'Storage Modulus', 'Loss modulus', 'Loss factor']]

Step 3: Splitting the data into training and test sets

The data set wassplit into training and test sets in the ratio 80:20. This was allowed to train the model on a subset of the data and test its performance on the remaining data.

Split the data into training and test sets

X_train, X_test, y_train, y_test = train_test_split (X, y, test_size=0.2)

Step 4: Building the MLP regressor model

Based on this, the MLP regressor model was built using the Scikit-Learn library. The default parameters were used for the MLP regressor, but different numbers of layers and neurons was also experimented to find the best configuration.

Build the MLP regressor model

mlp = MLPRegressor (*hidden_layer_size*, *max_iter*)

Train the model on the training set

mlp.fit(X_train, y_train)

Step 5: Evaluating the performance of the model

After training the model, the performance on the test set was evaluated using various metrics such as mean absolute error, mean squared error, root-mean squared error, and coefficient of determination (R^2) values described in Equations 5.1 to 5.4.

Mean Absolute Error (MAE): Mean Absolute Error is a commonly used metric for evaluating the performance of regression models. It measures the average absolute difference between the actual and predicted values.

$$MAE = \frac{\sum_{l=1}^{n} |y_i - x_i|}{n}$$
5.1

where: n is number of observations y_i is actual value of the ith observation xi is predicted value of the ith observation.

Mean Squared Error (MSE): Mean Squared Error is another commonly used metric for evaluating the performance of regression models. It measures the average squared difference between the actual and predicted values.

$$MSE = \frac{\sum_{i=1}^{n} (y_i - \dot{Y}_i)^2}{n}$$
 5.2

where n is number of observations, y_i is the actual value of the ith observation, \hat{y}_i is predicted value of the ith observation.

Root Mean Squared Error (RMSE): Root Mean Squared Error is the square root of the Mean Squared Error. It is also a commonly used metric for evaluating the performance of regression models. It measures the average absolute difference between the actual and predicted values but penalizes larger differences more heavily than MAE.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \dot{Y}_i)^2}{n}}$$
 5.3

where: n = number of observations $y_i =$ actual value of the ith observation $\hat{y}_i =$ predicted value of the ith observation

R-Squared (R^2) Value: R-Squared, also known as the coefficient of determination, measures the proportion of the variation in the dependent variable that is explained by the independent variables in the regression model.

$$R^2 = 1 - \frac{RSS}{TSS}$$
 5.4

where: RSS = sum of squared residuals (i.e., the sum of squared differences between the actual and predicted values) TSS = total sum of squares (i.e., the sum of squared differences between the actual values and the mean value of the dependent variable). R^2 takes values between 0 and 1, where 0 indicates that none of the variation in the dependent variable is explained by the model, and 1 indicates that all of the variation in the dependent variable is explained by the model.

Evaluate the performance of the model on the test set

from sklearn.metrics import mean_absolute_error, mean_squared_error, r2_score

mae = mean_absolute_error(y_test, y_pred) mse = mean_squared_error(y_test, y_pred) rmse =
np.sqrt(mse) r2 = r2_score(y_test, y_pred)

print("Mean Absolute Error:", mae) print("Mean Squared Error:", mse) print("Root Mean Squared Error:", rmse) print("R-Squared:",

Predict the target features

The trained MLP regressor was used to predict the target features (Water Absorption Capacity, Water Solubility Index, Oil Absorption Index, Foaming Capacity, Foaming stability, Protein solubility and Emulsifying capacity, Onset temperature, Peak temperature, Enthalpy, Complex Viscosity, Storage Modulus, Loss modulus, and Loss factor) for the test set.

Visualize the results and save the model

Matplotlib was used to plot the predicted values against the actual values for each of the targeted features. This was to allow the visual inspection to determine the accuracy of the predictions. Save the model: Finally, we save the trained MLP regressor using Scikit-Learn's "joblib.dump" function, which allows the load of the model later for prediction on new data.

5.3. Results and Discussion

5.4 ANN for Regression analysis of Techno-functional properties of Pea protein products

Table 5.1 shows the optimum ANN-machine learning parameters for the techno-functional properties of pea protein products.

5.4.1 Water absorption capacity

The model for water absorption capacity (WAC) has three hidden layers, each with 200 neurons. The model was trained using the Limited-memory Broyden-Fletcher-Goldfarb-Shanno algorithm (LBFGS) solver for 25000 iterations. The scores obtained for the training and test datasets are 0.983 and 0.895, respectively. These scores indicate that the model has performed well on the training data, but slightly worse on the test data, suggesting that the model may have overfit the training data. However, the test score of 0.895 is still relatively high, indicating that the model is useful for predicting water absorption capacity. The Mean Absolute Error (MAE) of the model on the training dataset is 0.426, indicating that the average difference between the predicted and actual water absorption capacity values on the training set is around 0.426. The R² value, which measures the goodness of fit of the model, is 0.983, indicating that the model explains 98.3% of the variance in the target variable. The Mean Squared Error (MSE) of the model on the training dataset is 0.612, which is the average of the squared differences between the predicted and actual values. The Root Mean Squared Error (RMSE) is 0.653, which is the square root of the MSE and represents the average deviation of the predicted values from the actual values. Finally, a plot of the predicted versus actual WAC values was generated for the training and testing dataset, which shows that the model's predictions are generally close to the actual values, although there are some outliers (Figure 5.4). Overall, these metrics indicate that the MLP neural network model has performed well in predicting the water absorption capacity.

Pea Protein	Neuron	Iteration	Hidde	R^2_t	MSE	MAE _t	RMSE	R ² _{tr}	MSE _{tr}	MAE _t	RMSE _{tr}
Properties	S	S	n layers		t		t			r	
WAC	200	25000	3	0.9 8	0.61	0.43	0.65	0.8 9	5.04	1.36	2.25
WSI	200	10000	2	0.7 8	8.45	1.69	2.91	0.7 5	6.16	1.60	2.48
OAC	200	10000	2	0.8 6	3.41	1.33	1.85	0.8 0	3.30	1.41	1.82
FS	100	10000	3	0.8 9	0.14	0.21	0.37	0.4 3	0.54	0.50	0.73
FC	200	10000	2	0.9 5	0.33	0.32	0.58	0.8 1	1.11	0.72	1.05
PS	100	50000	4	0.9 5	7.26	1.89	1.38	0.8 1	28.63	4.46	5.35
EC	100	50000	4	0.3 3	5.13	1.71	1.31	0.3 1	5.96	1.86	2.44

Table 5.1: Optimum ANN-machine learning parameters for techno-functional properties

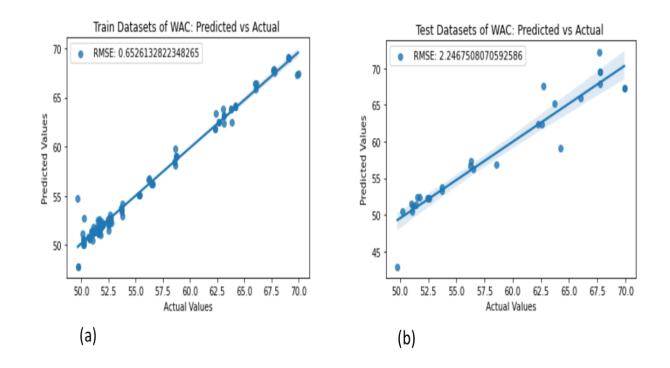


Figure 5.4. (a) Predicted values of WAC against actual values for training datasets (b) Predicted values of WAC against actual values for test datasets

5.4.2 Water solubility Index

The model for water solubility index (WSI) has two hidden layers, each containing 200 neurons, and was trained using the 'LBFGS' solver for a maximum of 10,000 iterations. The model's performance was evaluated using two metrics, the R-squared value and the mean absolute error (MAE). The R² value measures the proportion of the variance in the target variable (WSI) that is explained by the model. A value of 0.78 indicates that the model explains 78% of the variance in the target variable, which is a good result. The MAE is the average difference between the predicted values and the actual values, and a value of 1.69 suggests that the model's predictions are on average 1.69 units away from the actual values. Additionally, the mean squared error (MSE) was calculated, which is another measure of the model's accuracy, and its value was 8.45. The model's performance was also evaluated on a separate test dataset, and it achieved an R² value of 0.75. This indicates that the model is not overfitting to the training data and is able to generalize well to new data. Finally, a plot of the predicted versus actual WSI values was generated for the training and test dataset, which shows that the model's predictions are generally close to the actual values, although there are some outliers (Figure 5.5). The RMSE for the training and testing were 2.91 and 2.48. Therefore, the MLP neural network model shows good

performance for predicting the water solubility index and has the potential to be useful in various applications where this property is important.

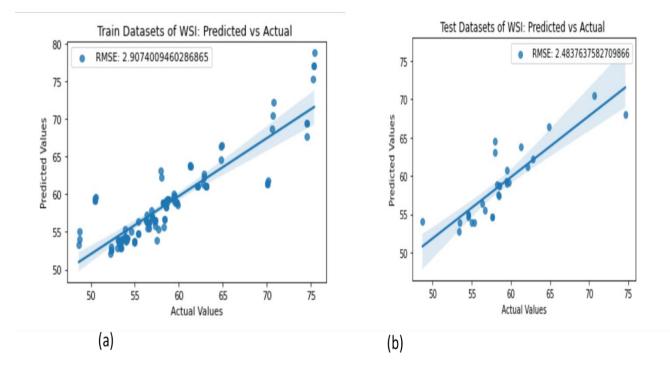


Figure 5.5. (a) Predicted values of WSI against actual values for training datasets (b) Predicted values of WSI against actual values for test datasets

5.4.3 Oil Absorption Capacity

The result indicates the performance of a multi-layer perceptron (MLP) regressor model in predicting the oil absorption capacity of a material. The model for oil absorption capacity (OAC) was trained using 2 hidden layer architecture with 200 neurons and the solver used was 'LBFGS' with a maximum iteration of 10,000. The scores of the model on the training and testing datasets were 0.86 and 0.80, respectively. The scores indicate that the model has good performance in predicting the oil absorption capacity of the material, with slightly better performance on the training dataset compared to the testing dataset. The Mean Absolute Error (MAE) of the model was 1.32, which means that the average difference between the predicted and actual values of oil absorption capacity is 1.32 units. The R² value of the model was 0.86, indicating that the model can explain 86.3% of the variance in the data. This suggests that the MLP regressor model is a good fit for the data and can accurately predict the oil absorption capacity of pea protein products. The Mean-Squared Error (MSE) of the model was 3.41, indicating that the model has a

relatively low error rate in predicting the oil absorption capacity of pea protein products. The Root Mean Squared Error (RMSE) of the model was 1.85, which represents the standard deviation of the residuals between predicted and actual values for the training while 1.81 for the testing datasets (Figure 5.6). Overall, the result suggests that the MLP regressor model is an effective tool for predicting the oil absorption capacity of pea protein products.

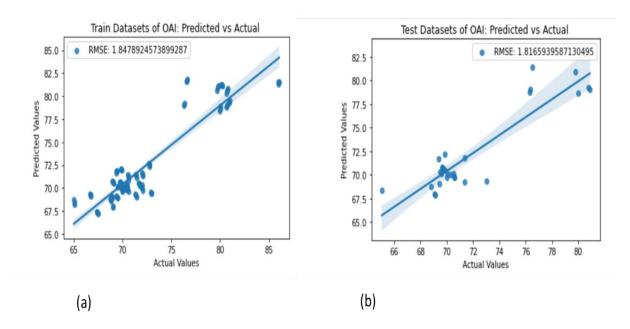


Figure 5.6. (a) Predicted values of OAI against actual values for training datasets (b) Predicted values of OAC against actual values for test datasets

5.4.5 Foaming capacity

The foaming capacity (FC) results suggested that the MLP Regressor model with 2 hidden layers of 200 neurons each and solver set to 'LBFGS' performed well. The R² value of 0.95 for the training data indicates that the model explains 95% of the variability in the data. The test score of 0.81 is also reasonably high, suggesting that the model has good generalization performance. Additionally, the mean absolute error of 0.32 and root mean squared error of 0.58 indicate that the model's predictions are on average off by 0.32 units and the error standard deviation is 0.58 units. These values are reasonably low, indicating that the model is making accurate predictions. Furthermore, the plot in Figure 5.7 (Train Datasets and Test Datasets of foaming capacity), predicted against actual is essential for understanding the model's performance. It shows the predicted foaming capacity values plotted against the actual values for the training dataset. If the

points on the plot are close to the line of best fit, it suggests that the model's predictions are accurate (Sanusi and Akinoso, 2022). It is worth noting that while the model has performed well.

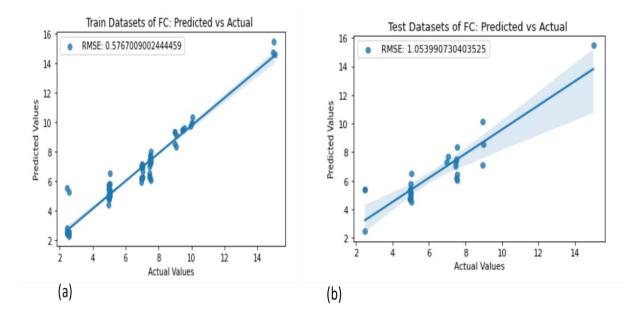


Figure 5.7. (a) Predicted values of FC against actual values for training datasets (b) Predicted values of FC against actual values for test datasets

5.4.5. Foaming stability

The result of foaming stability (FS) indicates that a multi-layer perceptron (MLP) regression model with three hidden layers, each with 100 neurons, was trained using the 'LBFGS' solver with 10,000 maximum iterations to predict foaming stability. The model achieved a high R² of 0.898 for the training dataset, indicating that 89.8% of the variability in the data was explained by the model. However, the R² value for the test dataset was low at 0.434, indicating that the model's generalization ability is poor, and it is overfitting to the training data. The mean absolute error (MAE) of the model was 0.212, indicating that the average difference between the predicted and actual values was 0.212, which is relatively low. The mean-squared error (MSE) was 0.138, indicating that the model's predictions were quite close to the actual values. The root mean squared error (RMSE) was 0.371, indicating that the model's predictions had a standard deviation of 0.371 from the actual values (Figure 5.8). Therefore, the model achieved good performance on the training data but poor generalization performance on the test data, suggesting that it may be overfitting to the training data. The MAE, MSE, and RMSE values indicate that the model's predictions were quite accurate.

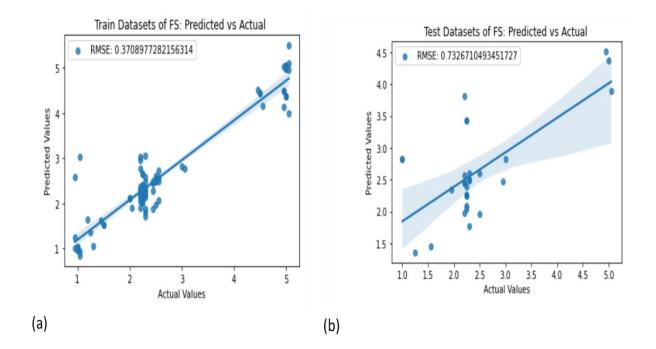


Figure 5.8 (a) Predicted values of FS against actual values for training datasets (b) Predicted values of FS against actual values for test datasets

5.4.6 Protein solubility

The obtained result for protein solubility (PS) is related to the prediction of protein solubility using MLP regressor. The MLP regressor model used has four hidden layers, each with 100 neurons, and the 'LBFGS' solver is used for optimization. The model was trained for a maximum of 50,000 iterations. The result suggests that the MLP regressor model with the given parameters is able to predict the solubility of proteins with high accuracy. The R² values for both training and test sets are 0.954 and 0.810, respectively, indicating that the model explains a high percentage of the variance in the data. The R² value ranges from 0 to 1, with higher values indicating better fit (Muhammed et al., 2022). The mean absolute error (MAE) of the training set is 1.89, which means that on average, the model's predictions deviate from the actual solubility by 1.89 units. The test set MAE is 4.461, which is higher than the training set MAE, indicating that the model may be overfitting to the training data. However, the test set MAE is still relatively low, suggesting that the model is generalizing well to new data. The mean squared error (MSE) and root mean squared error (RMSE) are also provided. The MSE of the training set was 7.26, and the test set MSE was 28.63. The RMSE is the square root of MSE, which indicates the average difference between the predicted and actual solubility values.

training set is 1.378, while the test set RMSE is 5.350 (Figure 5.9). Overall, the results suggest that the MLP regressor model is a highly accurate predictor of protein solubility.

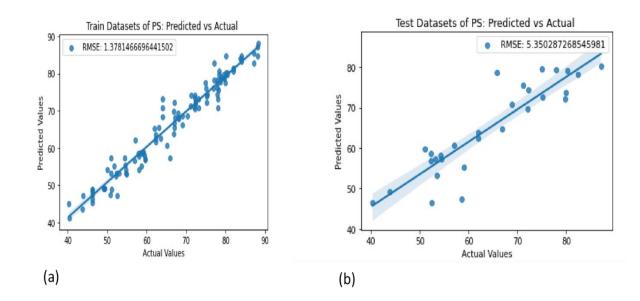


Figure 5.9: (a) Predicted values of PS against actual values for training datasets (b) Predicted values of PS against actual values for test datasets

5.4.7 Emulsifying capacity

The result obtained for emulsifying capacity (EC) shows the performance of a multi-layer perceptron (MLP) regression model in predicting the emulsifying capacity. The MLP regressor is a type of neural network that has 4 hidden layers, each having 100 neurons, and is trained with the 'LBFGS' solver for a maximum of 50,000 iterations. The R^2 values for the training and testing datasets are 0.333 and 0.313, respectively, indicating that the model explains approximately 33% of the variance in the training dataset and 31% of the variance in the testing dataset. Although the R^2 values are not very high, they are still significant and indicate that the model has some predictive power. The MAE values for the training and testing datasets are 1.705 and 1.859, respectively. The MAE measures the average magnitude of the errors in the predictions, and a lower MAE indicates better performance. The MAE values in this case are relatively high, suggesting that the model's predictions are not very accurate. The MSE values for the training and testing datasets are 5.133 and 5.958, respectively. The MSE measures the average of the squared errors in the predictions, and a lower MSE indicates better performance. The MSE values in this case are not very accurate. The MSE walues for the training and testing datasets are 5.133 and 5.958, respectively. The MSE measures the average of the squared errors in the predictions, and a lower MSE indicates better performance.

very accurate. Finally, the RMSE values for the training and testing datasets are 1.306 and 2.441, respectively (Figure 5.10). The RMSE is the square root of the MSE, and a lower RMSE indicates better performance. The RMSE values, in this case, are also relatively high, indicating that the model's predictions are not very accurate. Therefore, the MLP regression model has moderate predictive power but is not very accurate in predicting the emulsifying capacity. Further model optimization may be required to improve its performance.

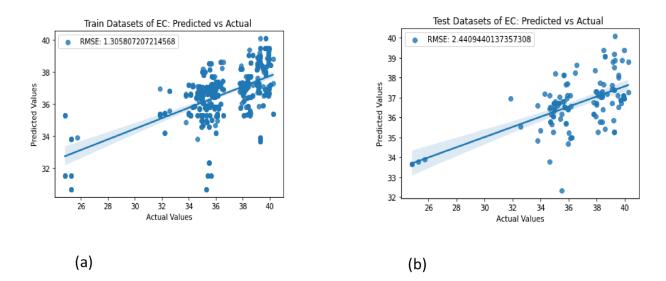


Figure 5.10: (a) Predicted values of EC against actual values for training datasets (b) Predicted values of EC against actual values for test datasets

5.5. ANN for Regression analysis of Thermal properties of Pea protein products

Table 5.2 shows the optimum ANN-machine learning parameters for the thermal properties of pea protein products.

5.5.1. Onset temperature (OS)

The onset temperature (OS) model was developed using a Multi-Layer Perceptron (MLP) with 3 hidden layers of 200 neurons each, trained using LBFGS and for a maximum of 25000 iterations. R^2 values were obtained for the training and testing datasets respectively. The score for the training dataset is 0.8286, which indicates that the model explains 82.86% of the variance in the training data. The score for the testing dataset is 0.53, which suggests that the model generalizes less well to new data than to the data it was trained on.

Pea Protein	Neurons	Iterations	Hidden	R^2_t	MSE _t	MA	RMSE _t	R^2_{tr}	MSE _{tr}	MAE _t	RMSE _{tr}
Properties			layers			E_t				r	
Onset temp	200	25000	3	0.82	107.57	7.24	10.37	0.5	368.34	12.55	19.19
								3			
Peak temp	200	50000	3	0.93	20.32	2.25	4.51	0.7	86.99	6.37	9.32
								3			
Enthalpy	200	10000	2	0.97	65.35	4.63	8.08	0.7	591.27	16.73	24.31
1.4								9			

Table 5.2: Optimum ANN-machine learning parameters for thermal properties

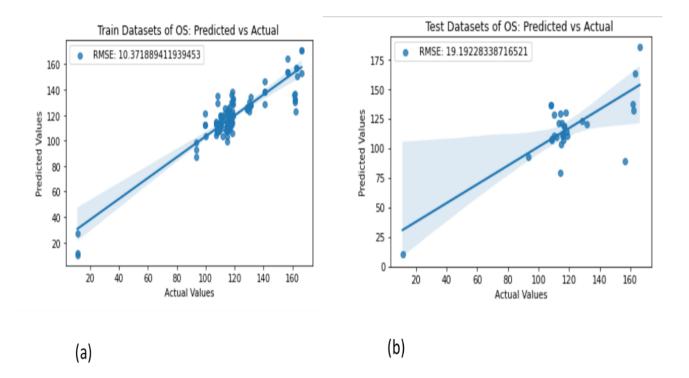


Figure 5.11: (a) Predicted values of onset temperature (OS) against actual values for training datasets (b) Predicted values of onset temperature (OS) against actual values for test datasets

This could be due to overfitting, which occurs when the model becomes too complex and starts to fit the noise in the training data instead of the underlying patterns. The mean absolute error (MAE), mean-squared error (MSE), and root mean-squared error (RMSE) for both the training and testing datasets with lower values indicate better performance. For the training dataset, the MAE is 7.24, the MSE is 107.58, and the RMSE is 10.37. For the testing dataset, the MAE is 12.55, the MSE is 368.34, and the RMSE is 19.19 (Figure 5.11). These values suggest that the model performs better on the training dataset than on the testing dataset, as the errors are smaller for the former.

5.5.2 Peak temperature

The MLP model for peak temperature created the neural network model with 3 hidden layers, each with 200 neurons, and the 'LBFGS' solver is used to optimize the weights of the neural network during training. The model is trained for a maximum of 50,000 iterations. The R² training score is 0.93, while the testing score is 0.73. The training score suggests that the model is performing well on the training dataset and is able to capture the patterns in the data. However, the testing score suggests that the model is not generalizing well to new data. The MAE for the training dataset is 20.33, while for the testing dataset, it is 87.00. Finally, the RMSE for the training dataset is 4.51, while for the testing dataset, it is 9.33 (Figure 5.12). These evaluation metrics suggest that while the model is performing well on the training dataset, it is not generalizing well to new data. This could be due to overfitting, where the model is too complex and is fitting too closely to the noise in the training data.

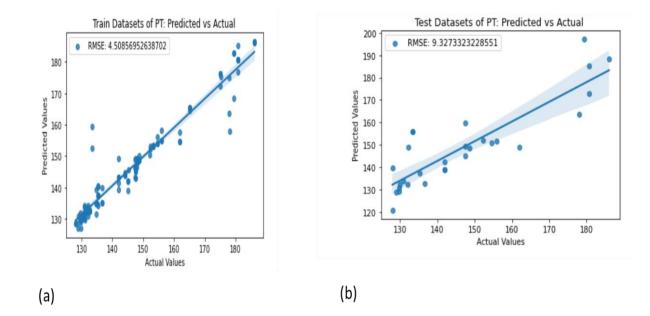


Figure 5.12: (a) Predicted values of onset temperature (OS) against actual values for training datasets (b) Predicted values of onset temperature (OS) against actual values for test datasets

5.5.3 Enthalpy

The MLP model created for enthalpy has 2 hidden layers, each with 200 neurons, and the 'LBFGS' solver was used to optimize the weights of the neural network during training. The model was trained for a maximum of 10,000 iterations. The R² training score is 0.97, while the testing score is 0.80. The training score suggests that the model is performing well on the training dataset and can capture the patterns in the data. The testing score also suggests that the model is performing well on the testing dataset and can generalize well to new data. The MAE for the training dataset is 4.64, while for the testing dataset, it is 16.73. The MSE for the training dataset is 65.35, while for the testing dataset, it is 591.27. Finally, the RMSE for the training dataset is 8.08, while for the testing dataset, it is 24.32 (Figure 5.13). These evaluation metrics suggest that the model is performing well on both the training and testing datasets. The R-squared values are high, indicating that the model is able to explain a significant portion of the variance in the target variable. The MAE and RMSE values are also reasonable, indicating that the model is making reasonably accurate predictions. However, the testing MAE and RMSE values are significantly higher than the training MAE and RMSE values, suggesting that the model may be overfitting to the training data to some extent.

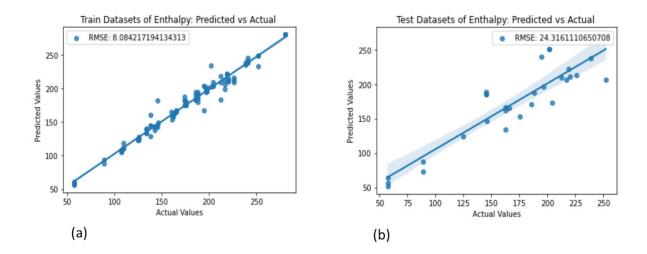


Figure 5.13. (a) Predicted values of onset temperature (OS) against actual values for training datasets (b) Predicted values of onset temperature (OS) against actual values for test datasets

5.6 Building ANN for Regression analysis of Rheological Properties

Table 5.3 shows the optimum ANN-machine learning parameters for the rheological properties of pea protein products.

5.6.1 Storage modulus (SM)

The MLP Regressor model with two hidden layer and 100 neurons, maximum iteration 10000, and solver'lbfgs' was used to predict the storage modulus values. The model achieved high accuracy on the training dataset, with an R²value of 0.99, indicating that 99.99% of the variance in the training data can be explained by the model. However, the model's performance on the test dataset was not as good as the training dataset, with an R² value of 0.97. This indicates that 97% of the variance in the test data can be explained by the model, which is still a good result. The mean absolute error (MAE) for the training data was 17.53, which means that on average, the model's predictions were off by 17.54 units from the actual values in the training data. The root mean squared error (RMSE) was 30.32, which is the standard deviation of the residuals. A low RMSE indicates that the model's predictions are close to the actual values (Figure 5.14). On the test dataset, the model had a higher MAE of 218.29, which means that the model's predictions were off by 218.30 units on average. The RMSE for the test data was 707.29, which is higher than the training dataset's RMSE, indicating that the model's predictions were further from the

Pea	Neurons	Iterations	Hidden	R^2_t	MSE	MAE	RMSEt	R ² tr	MSEt	MAE _{tr}	RMSE _{tr}
Protein			layers		t	t			r		
Properties											
Storage	100	10000	2	0.99	919.	17.53	30.32	0.96	5002	218.29	707.28
modulus	100	10000	_	0.77	2	1,100	20.22	0.70	57.46	210.2	101120
Loss	200	20000	2	0.99	160.	7.17	12.68	0.97	2101	114.49	458.43
modulus					80				61.93		
Complex	200	25000	2	0.99	1734	2339.	4164.3	0.96	9055	10651	300923.
viscosity					1480	86	1		5118	2.69	77
					.29				974.6		
									7		
Loss	200	25000	2	0.95	0.00	0.02	0.031	0.43	0.01	0.07	0.09
factor											

 Table 5.3: Optimum ANN-machine learning parameters for rheological properties

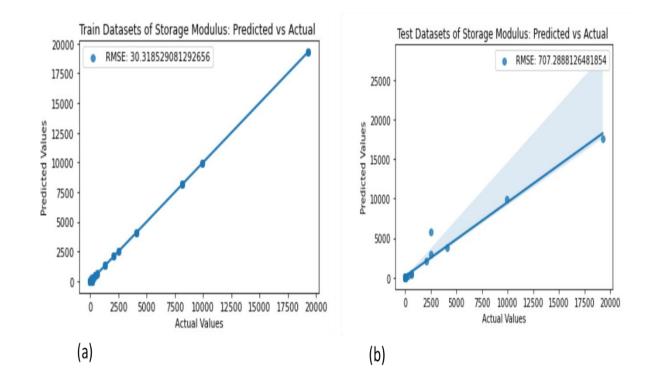


Figure 5.14. (a) Predicted values of storage modulus against actual values for training datasets (b) Predicted values of storage modulus against actual values for test datasets

actual values in the test dataset. The Mean-Squared Error (MSE) and Root Mean Squared Error (RMSE) values are higher in the test set than the training set, indicating that the model's predictions are less accurate in the test set. Overall, the model performed well on both the training and test datasets, with high accuracy and low error metrics.

5.6.2 Complex Viscosity

The given model is a Multilayer Perceptron (MLP) regressor with two hidden layers having 200 neurons each, and a maximum iteration of 25000 using the LBFGS solver. The model is trained and evaluated on complex viscosity data using two sets of data, i.e., training and testing data. The model achieved an excellent performance score of 0.99 on the training dataset and 0.96 on the testing dataset, indicating that the model is very good at predicting the complex viscosity for unseen data. The Mean Absolute Error (MAE) for the training dataset is 2339.86, while for the testing dataset, it is 106512.70.

The high MAE on the testing dataset indicates that there is a considerable difference between the predicted and actual values of complex viscosity, which might suggest that the model is overfitting on the training dataset.

The R^2 value of the training dataset is very close to 1, indicating that the model can explain almost all the variance in the training dataset. On the other hand, the R^2 value of the testing dataset is 0.96, which is still good but lower than that of the training dataset, indicating that the model is less effective at explaining the variance in the testing dataset. The MSE for the training dataset is 17341480.29, while for the testing dataset, it is 90555118974.67. The higher value of MSE on the testing dataset suggests that the model is not able to fit the testing dataset as well as the training dataset. The RMSE for the training dataset is 4164.31, while for the testing dataset, it is 300923.78 (Figure 5.15). The high value of RMSE on the testing dataset also indicates that the model might not generalize well on unseen data. Overall, the model seems to be overfitting on the training dataset, which might reduce its performance on new, unseen data.

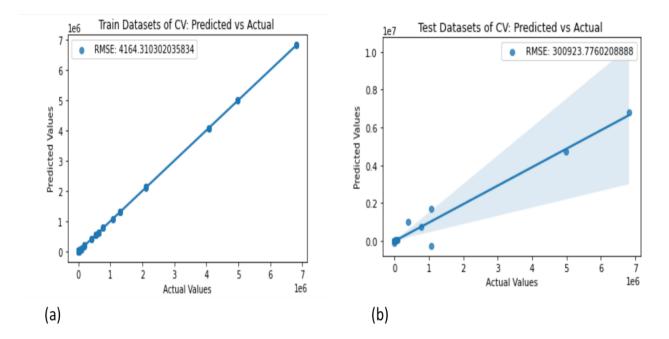


Figure 5.15 (a) Predicted values of complex viscosity (cv) against actual values for training datasets (b) Predicted values of complex viscosity against actual values for test datasets

5.6.3 Loss Modulus

The result in Figure 5.16 shows the prediction of loss modulus using a Multilayer Perceptron Regressor model. The model is trained on a training dataset and then evaluated on a separate testing dataset. The training set results are very good, with a high R^2 value of 0.99, indicating that the model explains 99.99% of the variability in the data. The Mean Absolute Error (MAE) is 7.17, which indicates that on average, the model's prediction of the Loss Modulus is off by about 7.17. The Root Mean Squared Error (RMSE) is 12.68, indicating that the model's predictions are off by an average of 12.68. These training set metrics suggest that the model has learned to fit the training data quite well. However, the testing set results are also as good as the training set results. The R^2 value is 0.97, which is lower than the training set value, indicating that the model's predictive power is reduced on new data. The MAE is 114.49, which is quite large, indicating that on average, the model's prediction of the Loss Modulus is off by about 114.50. The RMSE is 458.43, which is also quite large, indicating that the model's predictions are off by an average of 458.43. Therefore, MLP regressor model with the given hyperparameters appears to perform well in predicting the loss modulus.

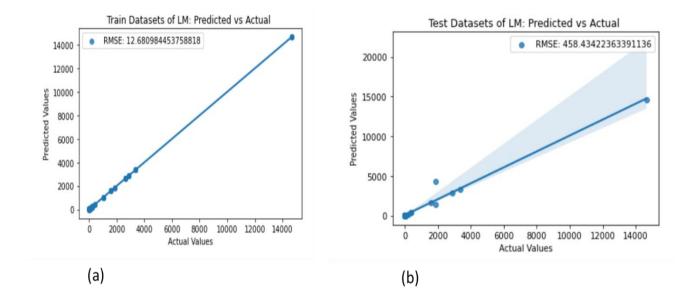


Figure 5.16 (a) Predicted values of loss modulus against actual values for training datasets (b) Predicted values of loss modulus against actual values for test datasets

5.6.4 Loss factor

The MLP model for predicting the loss factor has 2 hidden layers, each with 200 neurons, and the 'lbfgs' solver is used to optimize the weights of the neural network during training. The model is trained for a maximum of 25,000 iterations. The R^2 for training score is 0.95, while the testing score is 0.43. The training score suggests that the model is performing well on the training dataset and can capture the patterns in the data. However, the testing score suggests that the model is not generalizing well to new data. The MAE for the training dataset is 0.02, while for the testing dataset, it is 0.07. The R-squared value for the training dataset is 0.95, while for the testing dataset, it is 0.43. The MSE for the training dataset is 0.001, while for the testing dataset, it is 0.08. Finally, the RMSE for the training dataset is 0.03, while for the testing dataset, it is 0.09 (Figure 5.17). These evaluation metrics suggest that while the model is performing well on the training dataset, it is not generalizing well to new data. This could be due to overfitting, where the model is too complex and is fitting too closely to the noise in the training dataset in such cases, reducing the complexity of the model or introducing regularization techniques may help to improve the model is not able to explain the variance in the testing data accurately.

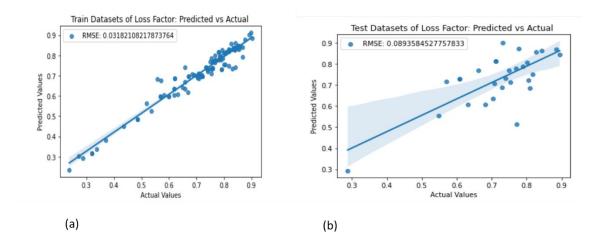


Figure 5.17. (a) Predicted values of loss factor against actual values for training datasets (b) Predicted values of loss factor against actual values for test datasets

5.7. Conclusions

The use of a machine learning artificial neural network algorithm to predict the technofunctional, thermal, and rheological properties of PPP has shown promising results. However, there is a significant variation in the optimum neurons, iterations, and hidden layers required to predict different properties accurately. The mean absolute error, coefficient of determination, mean-squared error, and rmse values also vary for each property, indicating that the model's performance is dependent on the specific property being predicted. Additionally, the slight variation between the training and test data shows that the model's performance needs further improvement. Overall, this study provides valuable insights into the use of machine learning algorithms for predicting food properties, which could potentially have significant implications in the food industry.

CHAPTER SIX

6. GENERAL SUMMARY, CONCLUSION AND FUTURE RESEARCH

6.1 Summary and Conclusions

This study provides valuable insights into the techno-functional, thermal, and rheological properties of pea protein products (flour, concentrate and isolate) from different varieties of peas. The results indicate that protein content has a significant influence on various properties such as water absorption capacity, oil absorption index, forming capacity, peak temperature, and enthalpy. Moreover, this study found that increasing the protein concentration led to a significant rise in storage modulus, loss modulus, and loss factor, while the complex viscosity decreased. Additionally, the machine learning artificial neural network algorithm results showed variations in the optimal number of neurons, iterations, and hidden layers for different properties, as well as differences in Mean Absolute Error, coefficient of determination, Mean-Squared Error, and RMSE for training and test datasets. This study's findings can assist in the selection of the most suitable pea protein product for use in product development and formulation, identifying key composition factors that affect the properties of pea protein products. It also provides insights into the processing behavior and quality control of pea protein products. Moreover, the use of machine learning algorithms to predict the properties of pea protein products is a promising approach that can significantly reduce the time and cost required for product development and formulation. However, further studies are required to improve the accuracy and reliability of the predictions. In conclusion, the study provides essential information for the food industry, researchers, and developers on the techno-functional, thermal, and rheological properties of pea protein products. The findings can contribute to the development of innovative pea protein-based food products with improved functionality and nutritional value. Additionally, the study highlights the potential of machine learning algorithms in predicting the properties of pea protein products and opens up new avenues for future research.

The following conclusions were drawn from this research:

• Pea protein products exhibit a wide range of techno-functional, thermal, and rheological properties, which are affected by the composition of the product.

- Pareto analysis was successfully used to identify the most significant composition on the of techno-functional, thermal, and rheological properties of pea protein products.
- Protein content has the most significant influence on water absorption capacity, oil absorption index, forming capacity, peak temperature, and enthalpy.
- Oil content has the most significant influence on forming stability and onset temperature, while the interaction of oil and protein contents influences water solubility index, water absorption index, and protein solubility the most.
- Principal component analysis and cluster analysis can be used to identify unique varieties based on the cluster of techno-functional and thermal properties of the pea protein products.
- Increasing protein concentration leads to a rise in storage modulus, loss modulus, and loss factor, while complex viscosity decreases.
- An increase in oil causes a decrease in storage modulus, loss factor, and complex viscosity, while a rise in starch content leads to a significant increase in the complex viscosity of the pea protein products.
- The machine learning artificial neural network algorithm can predict the properties of pea protein products with variations in the optimal number of neurons, iterations, and hidden layers for different properties.
- This study provides valuable information for identifying key composition factors that affect the techno-functional, thermal, and rheological properties of pea protein products and assists in product development and formulation.

6.2 Recommendation for Future Studies

- 1. A comparative study could be conducted to evaluate the techno-functional, thermal, and rheological properties of pea protein products with other plant-based proteins, such as soy, wheat, and rice.
- 2. Future studies could explore the potential of using other machine learning algorithms to optimize the processing conditions and predict the properties of pea protein products.

3. Further investigation is needed to explore the potential of using pea protein products as a functional ingredient in food formulations to enhance their nutritional and functional properties.

CHAPTER SEVEN

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