Production of texturized vegetable proteins (TVPs) from pulses and their application in meat products

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ABSTRACT

The overall goal of this thesis was to examine the impact of extrusion parameters on the functional attributes of faba bean and lentil protein extrudates, and their utilization in vegan or hybrid burger applications. Such parameters include screw speed (300 to 450 rpm), die temperature (110ºC to 140ºC), and feed moisture (30, 35, and 40 g water/100 g feed). A lab scale twin-screw extruder was used to produce lentil and faba bean-based TVPs with a wide range of physical and functional properties. The extrusion conditions did not affect the protein content of the TVPs (~84% dry basis) compared to the raw material. For both TVPs the physical properties including color, showed a decrease in brightness with an increase in redness and yellowness attributed to the Maillard reaction. For the specific mechanical energy, there was a trend observed due to the changes in the viscoelastic properties of the raw material affected by all the extrusion variables.

For the functional properties, an increase in moisture showed a significant change in the bulk density of TVPs and in consequence in an increase in the rehydration ratio (~214% for lentil-based TVPs and ~308% for faba bean-based TVPs with 40% MC). A significant effect was observed with changes in moisture content and screw speed with no effect of temperature for water and oil holding capacity for lentil-based TVPs. In contrast, for faba bean-based TVPs the functional properties showed a significant effect with the increase of screw speed for water holding capacity and changes in oil holding capacity (<1 g of oil per g of protein) with the changes in temperature and moisture content. The texture profile showed that the changes in moisture content affected properties including hardness, gumminess, and chewiness, while the changes in screw speed affected springiness, cohesiveness and resilience.

Finally, the addition of 10% TVP to a traditional meat patty formulation showed a significant increase in cooking yield with a significant decrease in thickness and diameter changes. Overall, the findings suggested that the combination of extrusion variables can affect the functional and physical properties of lentil and faba bean-based TVPs. Also, the data collected suggested that lentil and faba bean-based TVPs are a suitable partial or complete replacement for animal meat in terms of functionality and yield, but future studies are needed to investigate the sensory and nutritional aspects of the final product.

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LIST OF ABBREVIATIONS AND SYMBOLS

AACC	American Association of Cereal Chemists
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
a_w	Water activity
BD	Bulk density
CTVP	Commercial pea TVP
FTIR	Fourier transform infrared spectroscopy
%MC	Moisture content
OHC	Oil holding capacity
PA	Protein aggregates
PDCAAS	Protein digestibility-corrected amino acid score
TVP	Texturized vegetable proteins
$T^{o}C$	Temperature
RPM	Revolutions per minute
RR	Rehydration ratio
SEM	Scanning electron microscopy
SME	Specific Mechanical Energy
WHC	Water holding capacity

1. PROJECT OVERVIEW

1.1 Overview

The consumption of meat as a source of protein has been changing in recent years due to multiple concerns related to health, religion and sustainability (Bakhsh et al., 2021). Studies showed that there is a shift in human dietary patterns where protein-based diets have a higher consumption than carbohydrate-based diets. This shift in patterns affected the availability of certain proteins, especially for animal meat (Gravely and Fraser, 2018). The major issue with animal meat production is the excessive use of land and water as well as the emission of greenhouse gases making proteins from plant sources more attractive (Bakhsh et al., 2022). Pulses are one of the biggest sources of plant-based protein with almost double the content than cereals. Pulses have been consumed around the world as an important source of nutrition due to the high source of protein, dietary fiber, minerals and vitamins (Gravely and Fraser, 2018).

The consumption of pulses is encouraged to populations due to their health benefits such as prevention of some chronic diseases including diabetes, hypertension, cardiovascular disease, and others (Havemeier et al., 2017). Protein is the main macronutrient to increase satiety since when consumed it enhances the secretion of specific hormones (i.e. cholecystokinin) in the small intestine that are responsible for satiety in the human body (Havemeier et al., 2017). Also, the high content of fiber in pulses creates an increase in satiety, reducing gastric emptying into the intestine and in consequence managing body weight. Finally, pulses have low fat content which can improve the lipid profile by reducing triglycerides and cholesterol and consequently reduce the risk of any cardiovascular disease. Pulses are a rich source of macronutrients, making them an ideal alternative source of protein (Havemeier et al., 2017).

Lentils (*Lens culinaris*) are part of the legume family, which are a nutrient-dense crop consumed around the world (Oduro-Yeboah et al., 2022). Lentils originated in Mesopotamia with high production in India, Pakistan, and Bangladesh however, this crop was introduced to Canada in the earlies 1970s (Bhatty, 1988). Nowadays, Canada ranks first in lentil production with an average of 2.87 million metric tons produced in 2020 (Oduro-Yeboah et al., 2022). In terms of nutrition, lentils are a source of protein, fiber, vitamins, and minerals making them superior to other legumes like chickpeas (Oduro-Yeboah et al., 2022). On the other hand, faba bean (*Vicia faba*) is part of the leguminous family with the ability to grow in different climatic zones, being a staple food for the Mediterranean area (Crépon et al., 20120). The world production of faba beans reached 5.43 million metric tons in 2019 with Asia being the lead producer (Dhull et al., 2021). Previous research showed that there are several bioactive compounds in faba bean with high content of phenolic compounds, flavonoids, and lignans (Dhull et al., 2021).

Pulses can be consumed as is but can also be processed into flours, concentrates and isolates to extend the range of products. Meat analogs are considered products that resemble meat in multiple aspects including appearance, taste and nutritional value used as partial or total substitution in many products. Texturized vegetable proteins (TVPs) are widely accepted by industry as it is regarded as healthy option due to the low-fat content, cholesterol-free claim and low in calories (Bakhsh et al., 2021). TVPs are produced by an extrusion processing where heat, shear and pressure are applied to obtain a dry and bulky material that later is rehydrated and added to products. The overall goal of this thesis was to examine the impact of extrusion parameters (i.e., die temperature, feed moisture content and screw speed) on the functional attributes of faba bean and lentil-based protein extrudates, and their utilization in vegan or hybrid burger applications.

1.2 Objectives

- To examine the effect of extrusion variables (moisture content, screw speed, and temperature) on the physical and functional properties of lentil and faba bean based TVP.
- To examine the performance of TVP in a hybrid and vegan burger patty application, as it relates to its processing and functionality.

1.3 Hypotheses

The following hypotheses were tested:

(a) <u>The functional properties of TVPs</u>

  - Water holding capacity was expected to increase as barrel temperature and screw speed increase but decrease with higher moisture content.
  - Oil holding capacity was expected to increase after extrusion but decrease when temperature and screw speed increased.

- The rehydration ratio was expected to increase as feed moisture content and be directly related to the bulk density of each TVP.
- Textural quality attributes of rehydrated TVP where springiness, resilience chewiness, and cohesiveness were expected to increase as moisture content increases but decrease as temperature increases and with no significant effect with changes in screw speed. Hardness was expected to increase as feed moisture content and screw speed increases with no effect noticed with temperature changes.

(b) The physical properties of TVPs:

- Color parameters for TVP were expected to become darker with the extrusion process.
- Bulk density was expected to increase as moisture content increase, but decrease with an increase of screw speed and temperature.

(c) For the extruder response:

- The Specific Mechanical Energy (SME) was expected to increase with an increase in screw speed but decrease with an increase in moisture and temperature

(d) For vegan and hybrid burger applications:

- Cooking yield, thickness and diameter change were expected to decrease with the use of TVPs to the burger formulation
- Moisture retention was expected to decrease with the higher use of TVP in the formulation
- Textural quality attributes including hardness, cohesiveness, and gumminess were expected to decrease while springiness and chewiness were expected to increase

2. LITERATURE REVIEW

2.1 Background

The global demand for protein is constantly increasing; expected to have a demand of 176 million pounds by 2050 creating challenges for the food industry (Sha & Xiong, 2020). The main sources of protein come from plants and animals, however, there are some concerns associated with the nutrition, high cost and sustainability of those from animal-derived sources. Greater attention has been directed towards plant-based options with pulses emerging as the main interest, especially because of the health and nutritional benefits, are cheaper, easy to process, and are considered environmentally friendly. Customers' expectations for new products have been shaped by health concerns and new trends. The consumption of meat and meat products is increasingly seen as an elevated risk of chronic diseases such as obesity, stroke and cancer (Weiss et al., 2010). Therefore, the demand for products high in protein but low in fat and with restricted calories has increased and vegan/vegetarian options are becoming popular. That demand of customers for meat and even non-meat or hybrid processed meat products would promote the use of modern processing technologies and new ingredients to formulate many meat-like products (Hidayat et al., 2018).

Many health organizations have recommended the partial replacement of red meat with pulses as a source of protein once a week to reduce the risk of developing coronary heart disease by 22% and cardiovascular diseases by 11% (Padhi and Ramdath, 2017). Pulses are leguminous seeds consumed as dry grains that are considered to have health benefits and a source of protein, dietary fiber, gluten-free, vitamins and minerals. Some of the most consumed pulses and most used in the food industry are pea, faba beans, lentils and chickpeas. The protein content in pulses varies from 17% to 30% with high content of essential amino acid (lysine) and low in cysteine and methionine (Cotacallapa-Sucapuca et al., 2021). The focus of this research is directed towards faba bean and lentil proteins. Faba bean (*Vicia faba L*) contains 31-34% protein, 44-47% starch, 8% dietary fiber, 0.5-3% fat and 3.5-4% ash. The high protein makes it especially attractive for value-added fractionation where they can obtain higher protein ingredients and yields than other pulses (e.g., pea). Faba beans also contain polyphenolic compounds that will act as an antioxidants, as well as the presence of isoflavones that prevents the risk of chronic disease including diabetes (Rahate et al., 2021). In contrast, lentil (*Lens culinaris*) contains 19-23% protein, 46-51% starch, 10-15% dietary fiber, 1-2% fat and 2-4% ash (Ek et al., 2021).

2.2 Texturized vegetable proteins (TVPs)

One of the many uses of pulses as a raw material is in extruded products such as cereals, puffed snacks, pasta, meat analogs and others. Texturized vegetable proteins (TVP) are part of these products and are defined as a dry bulky extrudate emerging from an extruder, that are usually made from high protein flour (50% or higher), concentrates or isolates to replace meat. The production of TVP can be through a low moisture extrusion process, which are then rehydrated to obtain a sponge-like texture to mimic a meat-like product (Osen et al., 2014). There are many benefits that TVP can bring beginning with reducing the use of saturated fat, free of cholesterol, increasing water holding capacity, and increasing the protein content of the final product (Bakhsh et al, 2022). Usually, TVPs are used as a partial substitution since it cannot hold the sensory and quality attributes of a meat patty on its own, however, there are benefits including the reduction in cost of production and sustainability. In 2019, the overall market size of TVP was estimated at ~ US$ 1.1 billion, where by 2029 it is expected to grow to US$ 2 billion (Transparency Market Research, 2020). Many factors affect the quality of TVP including molecular interactions between proteins, carbohydrates, and lipids, temperature, shear force, solubility, emulsifying properties, and unwanted sensory attributes (Samard et al., 2021).

2.3 Extrusion

Extrusion is a thermal-mechanical process where pressure is applied to obtain a fully cooked product in a short period and since shear is applied during the process, there are changes in the structure of the raw materials. This technology has been studied over the last 50 years to produce a wide variety of foods such as protein supplements, meat replacements, cereals, and many others. Some of the benefits that extrusion cooking has are high product quality, high capacity, continuous operation, high productivity, and low cost of production that opens new options for food product development. Also, extrusion eliminates the anti-nutritional compounds such as tanins or trypsin inhibitors to increase protein digestibility. Finally, extrusion is a process that does not involve any chemical or enzymatic change in the raw material, only a physical one, helping to make a clean label final product (Alam et al., 2016).

Many parameters can be measured or calculated to characterize the extrusion process such as specific mechanical energy (SME) that will establish a relationship between the processing variables and the properties of the expanded extrudates. To monitor the general process, some standard values can be measured including protein solubility, free sulfhydryl content, particle size, intrinsic fluoresce, water holding capacity, bulk density, and texture profiles. Having these parameters will allow making changes during the process, or in the raw material to obtain the best results according to the objective of the final product (Zhang et al., 2022).

As mentioned before, low moisture extrusion will affect the structure of the protein since shear and heat is applied in consequence, unfolding and cross-linking occurs having changes in the secondary, tertiary and quaternary structure affecting functional properties, for example protein solubility and water holding capacity. Protein unfolding is characterized by the changes in ß-sheets and α-helix structures that tend to break due to high temperatures leading to changes in the protein functional and physical properties (Guerrero et al., 2014).

Studies show that extrusion conditions have an effect on the functional and physical properties of TVPs. For instance, when moisture content decreases more friction is generated meaning that there will be an increase in the product temperature leading to a change in physical and functional properties (Wang et al., 1999). On the other hand, an increase in temperature during extrusion, will denature the protein meaning that it will unfold the structure and elongate the protein molecules which tend to aggregate and crosslink resulting in texturization. Finally, a variation in screw speed will have an impact in the process of the unfolded protein crosslinking and will have an increase in pressure, reducing heat exposure but with a rise in the torque and viscosity values (Maurice & Stanley, 2013).

Wang et al. (1999) used air classified pea protein (55.4% protein) under different extrusion conditions to analyze how moisture content, screw speed and temperature values have a direct impact over characteristics including bulk density, color, water holding capacity and others. First, an increase in moisture content showed an increase in bulk density, nitrogen solubility index and brightness, but it also showed a decrease in yellowness, redness, and water holding capacity. Second, an increase in screw speed showed a decrease in bulk density, nitrogen solubility and brightness while redness increased. However, this study showed that there is no direct relationship between temperature and yellowness of the product. For example, with a moisture content of 30.5%, screw speed of 135 rpm, and a barrel temperature of 130ºC results showed a higher value for bulk density and brightness (L value), but the sample had the lowest value for redness (a value), yellowness (b value) and water holding capacity. The sample with a moisture content of 24%, screw speed of 245 rpm and barrel temperature of 170ºC showed the lowest values for bulk density and nitrogen solubility index, with a higher value for redness, yellowness and water holding capacity. Finally, according to Beck et al. (2017) the extrusion process for pea protein isolates reduced the solubility and favored the formation of aggregates due to the changes in the protein secondary structure.

Ghumman et al. (2016) showed that the different combinations of temperature and moisture content have an effect over color, expansion rate, protein solubility and others for extrudates made of lentil grit (22% of protein) with a constant the screw speed of 300 rpm. The sample with a moisture content of 15% and barrel temperature of 150ºC that had the highest expansion rate, yellowness (b value) and brightness (L value) but the lowest water solubility index and low redness (a value). The results showed that the variation in moisture content has a direct effect on the color parameters values (L, a and b) under the same temperature profile. On the other hand, Ek et al. (2021) used different combinations of screw speed and barrel temperature to determine the effect on functional properties (i.e expansion rate, density and water solubility index). The results determined that the combination of 250 rpm for screw speed and 125ºC for barrel temperature showed the higher expansion rates for different varieties of lentil while for the highest water solubility index the combination of 110ºC for barrel temperature and 250 rpm for screw speed worked for 2 of the 3 varieties analyzed (Ek et al., 2021).

Finally, for faba bean, Abd & Habiba (2003) observed that for a constant screw speed of 250 rpm with changes in moisture content and barrel temperature during extrusion had an effect over the content of trypsin inhibitors, *in vitro* protein digestibility and total phenols. The sample that had the higher *in vitro* protein digestibility was processed under a 22% moisture content and barrel temperature of 180ºC. Regarding the trypsin inhibitors values, it was shown that any combination of conditions eliminated this antinutritional factor by having as a result no value detected after the extrusion process.

2.4 Faba bean

Different studies have shown that the particle size and screw speed have an impact on the optimization of the extrusion process (Rokey et al., 2010). For instance, Smith and Hardacre (2011) examined the extrusion conditions for faba beans of different varieties (Disco and Melodie) with particle sizes ranging from 0.5-2.5 mm. Disco and Melodie varieties had protein levels of 29% and 26%, respectively. Texture analysis showed that hardness and crispiness were related to the level of expansion and amount of air bubbles present, where extrudates prepared with higher screw speeds and flours with lower protein levels were softer. Particle size was found to have no effect of extrudate texture over the size range examined. On the other hand, screw speed had an effect on the expansion rate of the samples were values from 200-300 rpm where used having as a result that at 300 rpm expansion was higher for both varieties (Smith& Hardacre, 2011).

During extrusion, unraveling of the protein structure occurs due to denaturation to expose buried polar sites. These sites can lead to increased interactions with water to increase their water binding abilities. Saldanha do Carmo et al. (2021) observed that non-extruded faba bean flour had lower water-binding capacity than the extruded form. Saldanha do Carmo et al. (2021) reported the denaturation temperature for faba bean is approximately 100.5ºC. Finally, all these data combined showed that is possible to obtain meat analogs with both high and low moisture extrusion solely from a protein concentrate of faba bean flour with protein content from 55-65% produced by dry fractionation with optimum parameters of temperature between 130-140ºC and SME between 145-172 kJ/kg (Saldanha do Carmo et al., 2021).

2.5 Lentil

Lentil is a pulse widely grown in Western Canada. There are different varieties of lentils depending on the region it grows and some of them includes Brewer, Crimson and Richler that will have a different chemical composition with a significant difference in the protein content (varying from 20.4-to 22.7%). The expansion ratio is the diameter measurement of extrudates compared to the die diameter used during extrusion which varies from 1.9 to 3.6 depending on the lentil varieties used (Brewer, Crimson and Richlea). The expansion ratio was mostly affected by screw speed and temperature, where higher screw speed and low temperatures created a higher value not only for lentils, but for faba bean, lima bean and others (Ek et al., 2021). Data showed that the pasting profiles of lentil flours had a type C profile (defined as a mixture of type A and B in the same granule) without or small breakdown viscosities meaning that the flour has low swelling and high hot paste stability, whereas the Brewer variety showed the highest gelatinization temperature (~75ºC). On the other hand, Richler has the highest peak viscosity followed by Brewer due to the starch content in the flowers affecting the pasting profiles. Melting peak temperature at 20% of moisture content was around 121-126ºC depending on the variety because of the interactions between starch protein and lipids, meaning that lentil flour melts at a lower temperature during the extrusion process will affect the expansion rate (Ek et al., 2021).

Torque was measured as a extruder response and ten to decrease as the temperature and the screw speed increased independent of the lentil variety. On the other hand, SME is dependent on the screw speed, barrel temperature, moisture content, and feed composition. For the Richlea variety, the SME was the highest and had the highest ER at 110ºC and 250 rpm. Brewer has the lowest SME due to the high fat and total dietary fiber showing that a high expansion index has a positive correlation with SME. All of the varieties had a higher expansion at the lowest temperature (110ºC) and can vary to higher temperatures with no significant differences in the ER value (125ºC) but for other pulses like faba bean, the lowest temperature for extrusion that had a higher expansion rate was 120ºC (Ek et al., 2021).

2.6 Use of TVP

Meat substitutes are known as meat-free products that mimic traditional meat in sensory, nutritional, and functional aspects. TVPs are plant-based products with high content of protein (around 80%) and is used as a partial or complete substitute for red meat, in products including meatballs, sausages, and patty burgers. TVPs are being produced mainly from soy, but greater attention has been directed to other pulses such as chickpeas, lentils, faba bean, and pea due to low allergenicity and health benefits. Currently, the substitution with pulse-based TVPs ranges from 10-100% which shows a positive effect on the sensory acceptance of certain products (Starowicz et al., 2022).

Consumers are looking for specific textural attributes in burgers such as juiciness and tenderness which can be a challenge for the food industry. To develop meat-like products containing TVP many factors need to be taken into consideration including the ingredients used based on their functionality and sensory aspects that can affect the final quality and perception of the final product. Fat is one of the most important ingredients in meat products since it plays a major role in quality, texture attributes such as juiciness, tenderness, and mouthfeel as well as flavor release. The use of canola oil as a source of fat (ranging from 3% to 15%) can also have health benefits including low levels of saturated fatty acids (around 7%) and high levels of monosaturated and polyunsaturated fatty acids (i.e. oleic and linoleic acid) that are considered cardioprotective compounds (Lin et al., 2013). Methylcellulose is a traditional binder used in the food industry especially in meat products due to its high functionality including an improvement in the texture properties of burgers due to the high-water retention (Chen et al., 2023). Finally, there are other ingredients salt (1%) and black pepper (0.3%) are used to enhance the flavor of the burger.

The production of a burger with TVPs needs as a first step the rehydration of TVP to then be mixed with the rest of the ingredients to go through the kneading process before dividing and shaping. Rehydration ratio is measured to obtain a value of water retained by TVPs after rehydration that can be affected by the interactions between proteins and water molecules during the extrusion process as well as the porosity of TVPs. Second, the cooking properties of TVPs are measured by the cooking loss which involves the capability of the burger patty to retain water and other juices after cooking affecting the appearance and texture of the cooked product. To determine the loss of juiciness during cooking measurements including moisture, diameter and thickness are needed where a result of lower loss of juiciness (compared to a meat patty) is expected since most of the TVP structure is protein and since heat is applied in extrusion denaturation occurs enhancing protein-water interaction by exposing hydrophilic groups. The rehydration process use of different ratios of TVPs and water (1:1.5 to 1:3) depending on the type of TVPs that includes protein denaturation, raw material, etc. (Samard et al., 2021). It is recommended to achieve the 50% to 65% of moisture content to achieve the highest quality parameters mentioned before (Riaz, 2001). Textural properties (cohesiveness, chewiness, hardness, and cutting strength) are measured as a reference for consumer's acceptability of the TVP burger since all of these properties tend to be lower than a normal burger because of the stronger network that is formed (Samard et al., 2021)

3. PRODUCTION OF TEXTURIZED VEGETABLE PROTEIN (TVPS) FROM LENTIL PROTEIN ISOLATE AND ITS APPLICATION IN HYBRID AND VEGAN BURGER PATTIES

3.1 Abstract

With an underlying objective of producing sustainable meat alternatives from major Canadian pulses, low-moisture texturized vegetable proteins (TVPs) were developed from lentil protein isolate. A lab scale twin-screw extruder was used at three different die temperatures (120, 130 and 140 °C), feed moisture contents (30, 35 and 40 g water/100 g feed, wet basis) and screw speeds (300, 375 and 450 rpm) to produce lentil protein based TVPs with a wide range of physical and functional properties. Overall, extrusion processing parameters did not change the protein content and produced TVPs with a water activity in the range of 0.39 and 0.44, reflecting their shelf stable nature at room temperatures as it relates to microbial growth. Compared to raw lentil protein, TVPs were darker, more red and more yellow. An increase in die temperature, moisture content and screw speed reduced the torque and specific mechanical energy requirements during extrusion. A decrease in moisture content and an increase in screw speed caused an improvement in the water-holding capacity of the TVPs. For oil-holding capacity, there was a significant increase after extrusion with values <1 g of oil per g of protein. An increase in moisture and screw speed showed an increase in oil-holding capacity with no effect with an increase in temperature. The rehydration ratio improved while the bulk density decreased at higher moisture content and higher screw speed. In terms of texture, all texture profile attributes, except for hardness, chewiness, and gumminess, showed a negative relationship with moisture content while a positive relationship with the screw speed. Overall, the alteration of extrusion processing parameters produced significant modifications in the physical and functional attributes of the TVPs. Based on the functional and physical analysis performed, an optimal treatment was selected (140ºC, 40%MC and 375 rpm) to use it as a partial and complete replacement of beef in a burger patty to measure

Overall, the addition of 10% lentil TVP increased the cooking yield and moisture retention with a significant decrease in thickness and diameter change that affected the texture profile of each sample.

3.2 Introduction

The consumption of red meat is widely done as a source of protein and essential nutrients like zinc, iron, and vitamin B12 which are necessary for nutrition (McAfee et al., 2010). However, increased concerns associated with health (e.g., high cholesterol and cardiovascular disease), and environmental sustainability has led industry and consumers to explore other protein alternatives that mimic the properties of meat in product applications. Some of the main sources of protein now being used as alternatives are from cereals, pulses, insects, fungi, and others to create new products available for consumers taking into consideration the religious, ethnic, and sustainability issues. The use of plant proteins, particularly those from pulses, seems to be leading the way for their nutritional/functional value, environmental sustainability story, low cost, and supply (Frank et al., 2021).

Pulses are crops defined as legumes harvested only for their seeds that are cultivated and consumed worldwide because of the high nutritional benefits including protein content, dietary fiber, minerals, and vitamins (Mudryj et al., 2014). Lentils (*Lens culinaris)* are one of the many pulses that has been gaining strength in the plant-based market, with an increase in production of 57% from 2020 to 2021 (Bard, 2022). Lentils are being cultivated in Canada, mostly in southern Saskatchewan, with around 37% of the world's production representing a total of 2.5 million tons produced in 2021 (FAOSTAT, 2023). Recent studies showed that the health benefits attributed to lentils are because of the presence of phytochemicals compounds (i.e. phenolic compounds), where lentil has the highest value for total phenolic content of 7.53 GAE/g compared to the rest of the pulses, which can act as antioxidants and anti-inflammatories in the human body preventing many cardiovascular diseases (Zhang et al., 2018). Lentils also have agricultural benefits including the reduction of inorganic nitrogen fertilizer requirements which can improve the yield, as well as recycling water that helps with pest control (Warne et al., 2019). Lentils are processed before being consumed creating a different range of products such as flours, concentrates, and isolates that are more attractive to the consumer and to the industry to incorporate into different technologies.

Extrusion is a process widely used by industry for decades to create different types of products such as breakfast cereals, pasta, snacks, texturized vegetable proteins, and high-moisture meat analogs. Extrusion combines shear, temperature, and pressure processes in one unit operation to change the structure of the raw material, meaning it can be tailored according to the function of the final product. This process is considered clean technology since it has many benefits including high yield, low energy consumption, and low waste which also represents lower costs. Also, extrusion increases the shelf life of the product since it inactivates enzymes and anti-nutritional factors without the addition of any chemical compounds (Yi et al., 2022). Low moisture extrusion (less than 50% MC) is used to produce sponge-like texture products, known as texturized vegetable proteins (TVPs), to mimic the sensory and functional aspects of meat including juiciness, high water holding capacity, high rehydration ratio, and other (Vatansever et al., 2020). During the extrusion process, the denaturation (unfolding) of proteins occurs due to shear and temperature being applied in the barrel to later cause protein cross-linking during cool down creating an expansion that attributes the texture and functional properties of the TVPs (Yi et al., 2022).

There are many factors that can affect the quality and functional properties of TVPs including, the composition of the raw material, moisture feed rate, screw speed, and temperature profile since the combination of these variables will create different changes in the protein structure during the process that can affect properties such as bulk density, color, hardness, and others. Previous studies show that the pasting and viscoelastic properties of each pulse also have an effect on the final functional and organoleptic properties of TVPs (Espinosa-Ramírez et al., 2021). TVPs can be applied to a wide range of products including nuggets, sausages, patties, and others to replace animal meat and improve some functional properties like color, moisture retention, firmness, and juiciness (Baune et al., 2022). The health and sustainability benefits associated with TVPs are making consumers demand more products available in the market, increasing the production of TVP reaching 1.1 billion USD in 2020 and continuing to grow expected to reach 2.1 billion USD by 2027 (Baune et al., 2022). The aim of this study was to produce texturized vegetable proteins (TVPs) from lentil isolate through low moisture extrusion under different die temperatures (120,130, and 140ºC), feed moisture contents (30, 35, and 40 g water/100 g feed), and screw speeds (300, 375, and 450 rpm) to observe the changes in physical and functional properties, and then test their applicability in hybrid and vegan burger patty applications.

3.3 Materials and Methods

3.3.1 Materials

Lentil protein isolate with 85.2% protein content (dry basis) was provided by AGT Food and Ingredients (Regina, SK, Canada) (produced using an alkaline extraction-isoelectric precipitation process). Upon arrival, the feed material was stored at 4 °C.

3.3.2 Methods

3.3.2.1Extrusion cooking

The appropriate amount of boiling water was added to the feed material to pre-condition it to 22 ± 0.5 % (wet basis) a day prior to the actual extrusion run. The pre-conditioning was performed by mixing the feed material in a Hobart mixer at a low speed for 5 min while the boiling water was added. Immediately after mixing, the pre-conditioned feed was packed in zipped plastic bags and stored in a climate chamber (HPP 260 IPP plus, Memmert, Schwabach, Germany) at constant temperature (50 °C) until extrusion.

Texturized vegetable proteins (TVPs) were produced on a lab scale, co-rotating, twin-screw extruder (MPF19, APV Baker Ltd., Peterborough, UK) with a screw length to diameter ratio of 25:1 and using a circular die of 2.3 mm diameter. A constant feed rate of 2.75 kg/h (dry basis) was used. The screw configuration was set as: 7D feed screw, 4D lead screw, 2D 60° forward paddle, 4D lead screw, 1.5D 30° forward paddle, 6.5 D lead screw, where D is the screw diameter which is equal to 19 mm. Extrudates were collected at three screws speeds (300, 375 and 450 rpm) under these conditions: at three different feed moisture contents (30, 35, and 40 g water/100 g wet feed) and different die temperatures (120, 130, and 140°C) as showed in table 1. At all extrusion processing conditions, TVPs were collected in triplicates after reaching steady-state conditions.

The torque and die pressure values were recorded in quadruplicates during extrudate collection and the specific mechanical energy (SME) was calculated following Luo and Koksel (2020) using Eq. 1:

$$\mathrm{SME}\ \left(\frac{\mathrm{Wh}}{\mathrm{kg}}\right) = \frac{\frac{actual\ screw\ speed}{max\ screw\ speed} \times \frac{torque}{100} \times motor\ power}{mass\ flow\ rate} \qquad \text{[Eq. 1]}$$

Table 1. Extrusion conditions for lentil-based TVPs

Treatment code	**Barrel temperature profile [ºC]**	**Moisture content [%]**	**Screw speed [rpm]**
Treatment 1 [T1]	90-105-115-125-140	30	375
Treatment 2 [T2]	90-105-115-125-140	35	375
Treatment 3 [T3]	90-105-115-125-140	40	375
Treatment 4 [T4]	90-105-115-125-140	30	300
Treatment 5 [T5]	90-105-115-125-140	30	450
Treatment 6 [T6]	70-85-95-105-120	35	375
Treatment 7 [T7]	80-95-105-115-130	35	375

After extrusion, the TVPs were dried for up to 24 h in ambient temperature followed by drying in an air oven (Isotemp 180 L Oven Gravity, Thermo Scientific, Langenselbold, Germany) up to 72 h at 40 °C to reduce their moisture content below 10% for long term storage. The drying time varies between 24 to 72 h for TVPs collected at different processing conditions due to difference in their initial moisture contents. After the moisture content of the TVPs reached below 10%, TVPs were placed in zipped plastic bags and stored at 4 °C for further analysis.

3.3.2.2 Physical properties

Bulk density of the TVPs was measured by adding the TVPs into a graduated cylinder with a defined volume. Bulk density was calculated as the mass of the TVPs divided by the sample bulk volume at the cylinder following the method described by Lisiecka et al. (2021). All measurements were performed in triplicate. Results are reported as the mean ± one standard deviation.

Protein content of the protein isolates and TVPs was determined through measuring the nitrogen contents of the samples by using the Dumas combustion method with a Nitrogen/Protein analyzer (CN628, LECO Corporation, St. Joseph, MI, U.S.A). Protein contents were calculated by multiplying the nitrogen contents with a conversion factor of 6.25 according to AACC International Method 46-30.01 (AACC, 2000). All measurements were performed in triplicate. Results are reported as the mean ± one standard deviation.

Moisture content of the protein ingredients and TVPs was determined according to the Association of Official Analytical Chemists (AOAC) methods 925.10 moisture (AOAC, 2003).

Water activity (a_w) of the samples was conducted with an AquaLab 4TE water activity meter (Decagon Devices, Inc., Pullman, WA, USA) at 22 °C. The color of the lentil isolate and TVPs were measured using a Hunter Colorimeter (ColorFlex EZ 45/0, Hunter Associates Laboratory, Inc., Reston, VA, USA). The $L*$ (lightness), $a*$ (redness), and $b*$ (yellowness) values are reported. All measurements were performed in triplicate. Results are reported as the mean ± one standard deviation.

Fourier transform infrared spectroscopy (FTIR) was used to analyze the changes to the secondary structure of proteins using the method described by Guldiken et al. (2021) with some modifications. For this project, FTIR was performed for the raw material and TVPs (milled) using Renishaw inVia™ Reflex Raman Microscope (Renishaw, Gloucestershire, UK) with an IlluminatIR II FTIR microscope accessory (Smith's Detection, Danbury, CT) with a 36X ATR objective lens within the wavelength range of 4000-650 cm^{-1} with 4 cm^{-1}resolution. The sample was loaded on a glass slide and placed under the ATR lens then an average of 518 spectra were collected. Measurements were made on triplicates for each sample and the measurement with less contamination was analyzed. Second- derivative spectra of the amid region (1525-1725 cm^{-1}) and the identification of the buried peaks (corresponding to secondary structures) were fitted inside the original amide I curve (curve fitting process) and smoothed using the Gaussian fit using WiRE 3.3 software. The percentage of the each secondary structure was obtained and reported as the mean ± one standard deviation.

3.3.2.3 Functional properties

Water and oil holding capacity were measured by weighing 0.5 g of sample (on *protein basis)* into a 50-mL centrifuge tube, and 5 g of distilled deionized water/canola oil was added and vortexed for 10 s every 5 min for 30 min at maximum speed 10 (Analog Vortex Mixer, VWR International, Mississauga, ON, Canada) and then centrifuged at 1,000 x g for 15 min (Sorvall ST8 Centrifuge, Thermo Fisher Scientific Inc., Waltman, MA, USA). The supernatant was drained, and the mass of the resulting pellet was weighed. The amount of water/ oil absorbed was determined based on differences in mass prior to and after analysis (report as g water or oil /g sample). All measurements were made in triplicate. WHC/ OHC were calculated through Eq (2):

$$WHC/OHC = \frac{Wet\ sample\ wt - dry\ sample\ wt}{dry\ sample\ wt} \qquad \text{[Eq.2]}$$

Rehydration ratio (RR) was conducted according to Brishti et al. (2021) and Yu et al. (2012) with some modifications. About 40 g (W_1) of TVPs (and 15 g for commercial TVPs) were placed in 1000 mL of distilled water at 80℃ for 30 min. Water was drained through a 60-mesh screen for 10 min, and the weight of the rehydrated samples was recorded (W_2). All measurements were made in triplicate. Rehydration ratio was calculated through Eq. (3):

$$RR\ (\%) = \frac{W_2 - W_1}{W_1} \times 100 \qquad \text{[Eq. 3]}$$

Texture profile of the TVPs was measured using a texture analyzer (Texture Technologies Corp., South Hamilton, MA, U.S.A.) with Probe TA-4 (contact area 1140 mm^2) according to Brishti et al. (2021) with some modifications. TVPs were prepared similarly as in rehydration ratio, and the rehydrated and drained TVPs were then filled into a petri dish with the height of 15.6 mm. TVPs were then pressed to 50% of the original thickness as a speed of 1 mm/s. Hardness, springiness, cohesiveness, gumminess, chewiness, and resilience are reported. Measurements are performed in triplicate, and six measurements were made in each replicate.

Scanning electron microscopy (SEM) is a tool used to analyze the morphological information of polymers and was used to examine the materials according to the method of Koksel & Masatcioglu (2018). The sample was placed on aluminum stubs with silver paste and coated with Au-Pd alloy at a thickness of 20nm using a cold sputter coater (Denton Vacuum, Desk II, NJ, USA). The images of the TVP sample were obtained using the SEM (Quanta FEG 650, FEI, OR, USA) with an acceleration voltage of 3kV at 500x magnification for the TVPs and a range of 250x to 900x for the raw material.

3.3.2.4 Burger testing

To determine the functionality of TVPs in a hybrid and vegan burger patty formulations were developed at the Saskatchewan Food Industry Development Centre (Saskatoon, SK, Canada) (formulations/process variables not shown) to allow the partial and complete substitution of red meat with TVPs. For partial replacement, 10% of lean ground beef was replaced with TVPs.

The texture profile was analyzed using a texture analyzer (Texture Technologies Corp., South Hamilton, MA, U.S.A.) with Probe TA-4 (contact area 1140 mm^2). Each burger was pressed to 25% of the original thickness at a speed of 1 mm/s. Hardness, springiness, cohesiveness, gumminess, chewiness, and resilience are reported. Measurements are performed in triplicate, and six measurements were made in each replicate.

Cooking properties were measured beginning with cooking yield, which measures the capability of the patty to retain water and other juices before and after cooking. The determination uses the weights between the raw and cooked patty as a percentage according to the method of Crowe & Johnson (2001). Measurements were made on triplicates for each sample. The cooking loss of the patty was calculated through Eq (4):

$$Cooking\ yield\ (\%) = \frac{cooked\ patty\ weight\ (g)}{raw\ patty\ weight\ (g)} x100 \qquad \text{[Eq.4]}$$

The patty diameter change measures the loss of moisture and fat during cooking due to protein denaturation (Ramadhan, et al., 2011). The method used measured the diameter before and after cooking to obtain the difference as a percentage according to (Kovácsné, et al., 2005) with some modifications. Measurement were made on triplicates for each sample and it was calculated through Eq(5):

$$Diameter\ change\ (\%) = \frac{raw\ patty\ diameter\ (mm) - cooked\ patty\ diameter\ (mm)}{raw\ patty\ diameter\ (mm)} x100 \qquad \text{[Eq.5]}$$

Thickness change was measured according to Bakhsh et al. (2022) with some modifications. The method used measured the thickness in four different locations before and after cooking for each sample and calculated through Eq(6). Measurements were made in quadruplicates.

$$Thickness\ change\ (\%) = \frac{raw\ patty\ thickness\ (mm) - cooked\ patty\ thickness\ (mm)}{raw\ patty\ thickness\ (mm)} x100 \qquad \text{[Eq.6]}$$

Finally, the moisture content for the raw and cooked patties was measured based on the AOAC procedure at 105ºC for 18 hours. The moisture content value was used to calculate the moisture retention of each burger through Eq (7) according to Heywood et al. (2002). All measurements were done in triplicates for each sample.

$$Moisture\ retention\ (\%) = \frac{cooking\ yield\ (\%) * moisture\ of\ cooked\ patty\ (\%)}{100} \qquad [Eq.7]$$

3.3.3 Statistical analysis

The data were analyzed using Minitab statistical software (MINITAB Inc., USA) with an analysis of variance (ANOVA) and Tukey test with a significance level of 0.05. A probability of $p<0.05$ was used to determine a significant result. The data were reported as mean values with standard deviations.

3.4 Results and discussion

3.4.1 Specific Mechanical Energy

Specific mechanical energy (SME) is defined as the amount of work that the extruder motor needs to produce the extruded material (TVPs); and is affected by torque, temperature, screw speed and mass flow rate (Fang et al., 2014). Studies have shown that SME influences the final quality of the TVP including bulk density, protein solubility, color, hardness and chewiness (Chen et al., 2010). In the present study, SME ranged from 66.9 to 125.9 Wh/kg to produce lentil-based TVPs, depending on the extrusion conditions used. As shown in Table 2, there is a tendency for SME to decrease as moisture content increases (140°C, 375 rpm), where conditions with the highest moisture (T3, 40% MC) had the lowest SME (66.9 Wh/kg) and that with the lowest moisture (T1, 30% MC) having a higher value (103.6 Wh/kg). This change occurs since the added moisture acts as a plasticizer to create a decrease in viscosity and friction to reduce the SME (Singh et al., 2015). These results were also noticed by Kaur et al. (2022) who observed that SME decreased with an increase in moisture content for maize extrusion.

On the other hand, higher temperatures help to create a viscoelastic flow since it reduces the melt viscosity of the material resulting in a decrease in SME (Guha et al., 1997). As shown in Table 2, an increases in temperature from 120°C (T6) to 140°C (T2) (at 35% MC, 375 rpm) resulted in an increase in SME from 79.9 Wh/kg to 99.5 Wh/kg. Similar results were reported by Ali et al. (2016) where a quadratic effect was noted with temperature and SME values for corn extrudates. According to Zhang et al. (2018) temperature is a critical factor for extrusion because of the melting of the raw material that will influence texturization of the protein. Temperature above 100ºC ensures the melting and denaturation of the raw material to be able to pass through the die and have a successful elongated structure (Akdogan, 1996). For screw speed, research shows that an increase in this parameter creates higher shear, less residence time, and higher friction having, as a result, an increase in SME (Zhuang et al., 2010). In the present study, as screw speed increased from 300 (T4) to 450 (T5) (140ºC, 30% M), SME was found to increase from 96.3 Wh/kg to 125.9 Wh/kg (Table 2). Singh et al. (2015) also had similar results when finding the optimization of potato-based extruded snacks.

3.4.2 Fourier transform infrared spectroscopy

Secondary structures of proteins change due to the denaturation of the protein during the extrusion process affecting physical and functional properties such as water holding capacity, and digestibility (Cabonaro et al., 2012). For protein aggregates (PA1) there is a slight increase for most of the treatments (around 2%) compared to the raw material, but a decrease when the temperature is increased from 120 ºC to 140ºC (Table 4; 35% MC, 375 rpm). No effect was observed when moisture content increased from 30% (T1) to 40% (T3) and when screw speed increased from 300 rom (T4) to 450 rpm (T5) for PA1. Similar results were seen for a-helix, where there was a slight increase after extrusion whit values ranging from 17 to 22% with no impact with changes in moisture content, screw speed, or temperature. For ß -sheets, the reduction of the value represents the denaturation of the protein where the lowest value of 24.8% was for T5 (30% MC, 140ºC, 450 rpm) which can be attributed to the harsh conditions during extrusion that can also be corroborated with the highest SME value of 125.9 Wh/kg (Table 2). The screw speed had little effect, where an increase from 300 rpm (T4) to 450 rpm (T5) (Table 3; 140ºC, 30% MC) created a decrease of 2% in the ß -sheet. As moisture content decreased from 40% (T3, 140ºC, 375 rpm) to 35% (T2, 140ºC, 375 rpm) there was a decrease in ß -sheet from 29.5% (T3) to 24.9 % (T2) (Table 3). However, with an increase in temperature from 120ºC (T6) to 140ºC (T2) there was no change that can be attributed to the thermal stability of the secondary structures in lentil. Zhang et al (2022) reported similar results for pea isolates where ß -sheets decreased with lower moisture content with the lowest value at 30% MC.

Table 2. The effect of die temperature, screw speed and moisture content on response variable within the extruder for lentil-based TVPs

	Die pressure (kPa)	**Torque (%)**	**SME (Wh/kg)**
T1	6516.7 ± 460.9^{ab}	24.7 ± 0.4^{bc}	103.6 ± 1.6^{b}
T2	4541.7 ± 347.6^{d}	20.5 ± 0.7^{d}	79.9 ± 2.6^{e}
T3	2833.3 ± 290.9^{e}	18.6 ± 0.1^{e}	66.9 ± 0.5^{f}
T4	6775.0 ± 374.5^{a}	28.7 ± 0.7^{a}	96.3 ± 2.4^{cd}
T5	6633.3 ± 834.8^{ab}	25.0 ± 0.7^{b}	125.9 ± 3.8^{a}
T6	6133.3 ± 296.4^{b}	25.5 ± 0.4^{b}	99.5 ± 1.7^{bc}
T7	5125.0 ± 263.3^{c}	23.7 ± 0.2^{c}	92.6 ± 1.0^{d}

Notes:

Treatments are as follow: T1-140ºC, 30%MC, 375 rpm, T2-140ºC 35%MC, 375 rpm, T3-140ºC 40%MC, 375 rpm, T4-140ºC 30%MC, 300 rpm, T5-140ºC 30%MC, 450 rpm, T6-120ºC 35%MC, 375 rpm and T7-130ºC 35%MC, 375 rpm. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different (p>0.05)

For ß -turn structures, there was an increase in the extrusion process ranging from 13.4% to 15.8% compared to the raw material with a value of 12.5% that can be attributed to the extrusion process changing ß -sheet and a-helix structures to ß -turns and protein aggregates (PA1) (Beck et al., 2017). Similar results were obtained by (Beck et al., 2017) where pea isolate was used to obtain TVPs through low moisture extrusion (26% MC) at 400 rpm with an increase of ß -turn structures from 9.7 to 13.1 for pea isolate and TVP respectively. However, the change in moisture content from 30% to 40% (Table 3; 140ºC, 375 rpm), screw speed from 300 rpm to 450 rpm and (Table 3; 140ºC, 30% MC), and temperature from 120ºC to 140ºC (Table 3; 35% MC, 375 rpm) had no significant effect on ß -turns, anti-parallel- ß -sheet structures and protein aggregates (PA2). Beck et al (2017) also found that there is no significant change in anti-parallel- ß -sheet structures and protein aggregates in pea isolate when the temperatures change from 130ºC to 170ºC, and screw speed increased from 450 rpm to 700 rpm at a constant moisture content of 26%. There was a decrease of Anti-parallel- ß -sheet-structures of 3% after extrusion which can be attributed to the formation of protein aggregates (PA1) (Zhang et al., 2022). Finally, for protein aggregates (PA2) there was a decrease after extrusion from 9.9% for the raw material to values ranging from 4.1% to 6.1 % for the lentil-based TVPs. Changing the barrel temperature, moisture content and screw speed did not significantly affect the secondary structure of lentil-based TVPs compared to the raw material despite the differences in physical and functional properties, which indicates the thermal stability of lentil isolate within the range of these variables used during the study.

3.4.3 Moisture content and water activity

Moisture content is defined as the total amount of water by weight present in the product that influences the process of the raw material, quality and texture (Ahmand et al., 2017). Common dry products such as flour, concentrates and isolates have a moisture value ranging from 6-13% to ensure the safety and quality of the product over time (Chandra et al., 2014). As shown in table 4 all the treatments showed a maximum value of 7% which is attributed to the extrusion and drying process. This moisture value ensures the preservation of the TVPs over time with a lower risk of contamination by mold or spoilage creating a longer shelf life. Water activity represents the available water for microorganisms and mold to reproduce and create quality and shelf-life

Table 3. The effect of die temperature, screw speed and moisture content on secondary structure for lentil-based TVPs

	Protein aggregates(A1) [%]	**ß-sheets [%]**	**α-helix [%]**	**ß-turn [%]**	**Anti-parallel-ß-sheets [%]**	**Protein aggregates(A2) [%]**
LPI	23.2 ± 0.0^{a}	25.9 ± 0.0^{a}	17.4 ± 0.0^{a}	12.5 ± 0.0^{a}	11.0 ± 0.0^{a}	9.9 ± 0.0^{a}
T1	25.3 ± 0.5^{a}	27.3 ± 0.7^{a}	18.6 ± 0.5^{a}	13.6 ± 1.4^{a}	9.5 ± 0.3^{a}	5.7 ± 1.0^{ab}
T2	21.7 ± 3.0^{a}	24.9 ± 4.3^{a}	22.2 ± 2.4^{a}	15.8 ± 2.2^{a}	9.5 ± 0.6^{a}	5.8 ± 1.7^{ab}
T3	25.9 ± 4.6^{a}	29.5 ± 2.1^{a}	17.2 ± 3.6^{a}	13.2 ± 2.3^{a}	9.4 ± 1.7^{a}	4.8 ± 0.7^{b}
T4	25.0 ± 1.1^{a}	26.3 ± 4.8^{a}	21.2 ± 1.9^{a}	13.9 ± 3.4^{a}	8.4 ± 1.1^{a}	5.2 ± 0.6^{b}
T5	26.3 ± 7.7^{a}	24.8 ± 4.8^{a}	19.2 ± 2.8^{a}	12.9 ± 3.8^{a}	10.8 ± 1.3^{a}	6.1 ± 1.6^{ab}
T6	26.3 ± 1.7^{a}	25.4 ± 1.4^{a}	19.7 ± 1.5^{a}	14.7 ± 0.5^{a}	9.8 ± 0.3^{a}	4.1 ± 0.4^{b}
T7	24.4 ± 3.3^{a}	27.0 ± 3.5^{a}	17.9 ± 2.0^{a}	15.1 ± 2.4^{a}	10.2 ± 1.6^{a}	5.4 ± 1.0^{b}

Notes:

Treatments are as follow: T1-140ºC, 30%MC, 375 rpm, T2-140ºC 35%MC, 375 rpm, T3-140ºC 40%MC, 375 rpm, T4-140ºC 30%MC, 300 rpm, T5-140ºC 30%MC, 450 rpm, T6-120ºC 35%MC, 375 rpm and T7-130ºC 35%MC, 375 rpm. LPI= Lentil protein isolate. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

damage. Generally, food with a water activity value higher than 0.6 is considered microbiologically stable, which will have an effect in the shelf life of the final product. All the extrusion treatments have a value lower than 0.4 (Table 4) which indicates good stability of the final product since less water is available there is a low probability for microorganisms to grow and proliferate (Que et al., 2007). In contrast, the two commercial pea TVP had similar moisture levels, but water activities at 0.1 (Table 4).

3.4.4 Color

Color is a physical property that determines the consumer's acceptability of a product and also how variables such as the presence of carbohydrates, lipids, enzymatic and non-enzymatic reactions, moisture content, and temperature affect the TVPs. First, the L value (brightness) of all TVPs was significantly lower compared to the raw lentil protein isolate (Table 4). This darkening is caused by the Maillard browning reaction occurring in the extruder since the sugar reacts with the free amino acids, lysine, during the elevation of temperature in the extrusion process having as a result dark brown TVPs (Samard et al., 2018).

For the lentil-based TVPs, the effect of temperature [(120°C - T2) vs 140°C - T6 at 30% MC and 375 rpm] on color (L, a, b values) had small impact, probably since the temperature differential was too low to see an effect. Nor did the impact of screw speed have an effect (140°C, 30% MC) when it was increased from 300 rpm (T4) to 450 rpm (T5) within the extruder. However, as moisture increased from 30% (T1, 140°C, 375 rpm) to 40% (T3), there was a lightening of TVP at the higher temperature indicating less of a Mallard reaction was occurring with the higher moisture (i.e., reactants were less concentrated). Here, L values increased from 64.6 to 71.5, a decreased from 7.2 to 4.9, and b values decreased from 30.4-27.5 (Table 4). Ilo & Berghofer (1999) reported a similar brightening effect when moisture in the TVP increased from 13% to 17%, for extruded maize grits, leading to an increase in L value from 73.0 to 86.2. In contrast, commercial pea TVP were brighter than most lentil-based TVPs treatments with L, a and b values of 72.8-76.8, 11.5-11.6, and 27.5-33.3, respectively, which was somewhat similar to the lentil-based TVPs (T4 – 140°C, 30%MC, 300 rpm) sample.

3.4.5 Water holding capacity

Extrusion can affect WHC because the raw material is exposed to temperature and shear which can create a denaturation of the protein that can expose buried sites and create new bonds between amino acids during cool down creating a stronger protein structure (Brishti et al., 2021). For lentil-based TVPs, WHC ranged from 2.9 to 3.5 g of water /g of protein. No effect of increased temperature (35% MC, 375 rpm) from 120ºC (T6) to 140ºC (T2) was seen in WHC that can be attributed to the stability of lentil protein isolate in that range of temperature corroborated by the FTIR results for the secondary structure of lentil-based TVPs in table 3.

Studies by Rueda et al. (2004) showed that temperatures higher than 150ºC increased the WHC of soy flour due to the partial denaturation of the protein, but higher temperatures (>180ºC) decreased the water-holding capacity of navy beans (Verbeek & Van den berg., 2010).On the other hand, an increase in moisture (140ºC, 375 rpm) from 30% (T1) to 40% (T3) showed a significant decrease in WHC values that can be attributed to porosity. Previous studies carried out by Thymi et al. (2005) showed that an increase in moisture content (from 12% to 25%) decreased the porosity of corn extrudates creating a lower WHC. Finally, an increase in screw speed from 300 rpm (T4) to 450 rpm (T5) (Table 5 140ºC, 30% MC) showed a significant increase in WHC for lentil-based TVPs that can be attributed to the time of residence and the porosity. The two-commercial pea TVPs had a higher value for WHC (3.7 and 3.9 g of water/g of protein respectively) that is closer to the lentil isolate WHC.

3.4.6 Oil holding capacity

Oil holding capacity (OHC) is defined as the ability to hold oil during the application of forces which can be directly related to sensory properties (i.e. the juiciness of the product). This physical property is related to the protein’s amino acid composition and conformation. The interaction between lipids and proteins is due to the non-polar sites of the amino acid and the aliphatic chains of the lipid structure, meaning that OHC is affected by the amount of non-polar amino acids exposed in the protein structure (Lam et. al., 2018). For lentil-based TVPs, the values ranged from 1.2 to 1.3 g of oil /g of protein compared to 1.0 g of oil /g of protein for the raw lentil protein isolate.

Table 4. The effect of die temperature, screw speed and moisture content on physical properties for lentil-based TVPs

	Protein content [%]	Moisture [%]	Water Activity	Color		
				L	a	b
LPI	85.2 ± 0.8^{a}	5.9 ± 0.0^{bcd}	0.4 ± 0.0^{abc}	84.2 ± 0.0^{a}	0.3 ± 0.0^{f}	17.8 ± 0.0^{g}
CTVP1*	80.7 ± 0.2^{c}	5.4 ± 0.2^{d}	0.1 ± 0.0^{d}	72.8 ± 0.4^{c}	11.5 ± 0.2^{a}	33.3 ± 0.5^{a}
CTVP2	75.6 ± 0.1^{d}	6.5 ± 0.2^{abc}	0.1 ± 0.0^{d}	76.8 ± 0.1^{b}	11.6 ± 0.0^{a}	27.5 ± 0.1^{ef}
T1	83.9 ± 0.2^{b}	6.7 ± 0.2^{a}	0.4 ± 0.0^{ab}	64.6 ± 1.3^{f}	7.2 ± 0.4^{b}	30.4 ± 0.9^{bc}
T2	84.8 ± 0.1^{a}	6.8 ± 0.2^{a}	0.4 ± 0.0^{bc}	67.7 ± 0.7^{d}	5.9 ± 0.1^{d}	28.7 ± 0.3^{de}
T3	84.7 ± 0.1^{a}	6.7 ± 0.4^{a}	0.4 ± 0.0^{abc}	71.5 ± 0.9^{c}	4.9 ± 0.3^{e}	27.5 ± 0.4^{f}
T4	83.9 ± 0.1^{b}	6.9 ± 0.1^{a}	0.4 ± 0.0^{abc}	65.0 ± 1.5^{ef}	7.0 ± 0.5^{b}	30.7 ± 1.0^{b}
T5	83.8 ± 0.3^{b}	6.5 ± 0.4^{ab}	0.4 ± 0.0^{a}	66.9 ± 0.9^{de}	6.9 ± 0.3^{bc}	29.9 ± 0.6^{bc}
T6	83.8 ± 0.1^{b}	5.8 ± 0.3^{cd}	0.4 ± 0.0^{abc}	66.8 ± 3.5^{def}	6.2 ± 1.1^{cd}	29.3 ± 1.6^{cd}
T7	84.8 ± 0.4^{a}	7.0 ± 0.6^{a}	0.3 ± 0.0^{c}	66.0 ± 1.6^{def}	6.6 ± 0.5^{bc}	29.6 ± 0.8^{bcd}

Notes:

Treatments are as follow: T1-140ºC, 30%MC, 375 rpm, T2-140ºC 35%MC, 375 rpm, T3-140ºC 40%MC, 375 rpm, T4-140ºC 30%MC, 300 rpm, T5-140ºC 30%MC, 450 rpm, T6-120ºC 35%MC, 375 rpm and T7-130ºC 35%MC, 375 rpm. *CTVP= Commercial pea TVP; LPI= Lentil protein isolate. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

Similar results were also obtained by Kaleda et al. (2021) where the mix of oat concentrate, and pea isolate had a lower OHC (~0.8 g of oil per g of protein) than the extrudates with a mean of 1.00 g of oil per gram of protein. No effect of increased temperature (35% MC, 375 rpm) from 120ºC (T6) to 130ºC (T2) was seen in OHC, which can be attributed to the thermal stability of lentil isolate in that range of temperatures. However, there is a significant change when the temperature was increased to 140ºC (T2, 35% MC, 375 rpm) which can be attributed to the exposure of proteins to higher temperatures under the same residence time that causes unfolding and denaturation of the structure exposing a higher number of hydrophobic sites increasing the OHC. Previous research showed the same trend for horse gram extrudates where OHC did not show significant change from 100ºC to 125ºC but increased at 150ºC under the same extrusion conditions (15% MC and 300 rpm) (Ghumman et al., 2016).

An increase in moisture (140ºC, 375 rpm) from 30% (T1) to 35% (T2) showed a significant increase in OHC from 1.2 to 1.3 g oil/ g protein which can be attributed to the dissociation of proteins during extrusion that can expose non-polar amino acids making those TVPs more capable of interacting with the oil molecules (Ghumman et a., 2016). However, when MC was increased to 40% (140ºC, 375 rpm) the OHC decreased due to the lubricant effect of water that decreased the residence time of the raw lentil protein isolate in the extruder barrel creating a decrease in the denaturation process and in consequence, less hydrophobic groups were exposed (Zhang et al., 2022). Similar results were obtained by Ghumman et al. (2016) OHC with the maximum value at 20%MC (125ºC, 300 rpm) followed by 15%MC (125ºC, 300 rpm) and a decrease at 25% MC (125ºC, 300 rpm) for lentil flour extrudates.

An increase in screw speed from 375 rpm (T1) to 450 rpm (T5) (140ºC, 30%MC) increased the OHC for lentil-based TVPs, but no significant change was observed when the screw speed was increased from 300 to 375 rpm (140ºC, 30% MC). Increasing the screw speed results in a shorter residence time but can create an increase in SME and product temperature that can enhance the unfolding of the proteins having as a result a higher OHC (Beck et al., 2017). Previous studies carried out by Alonso et al. (2000) used pea flour for extrusion (25% MC, 148ºC) where a decrease in OHC was observed due to the low screw speed (100 rpm) that can be attributed to protein aggregation during the extrusion process that leads to a decrease in functional properties. Protein aggregates are shear sensitive meaning that higher shear can break the bonds, exposing buried sites and in consequence increase OHC (Beck et al., 2017).

3.4.8 Bulk Density

Bulk density (BD) is a physical property of the final product that determines the expansion considering the porosity produced by the extrusion. This property is mainly affected by the friction generated during the extrusion, where a low moisture content will create a lower viscosity increasing the friction and reducing the bulk density of the extrudate (Ali et al., 2016). For lentil-based TVPs, BD values ranged from 0.38-0.42 g/mL. No effect of increased moisture (140ºC, 375 rpm) on BD (~0.4 g/mL) was seen between 30% (T1) and 40% (T3) moisture for the lentil-based TVPs. Nor was there an effect of temperature seen between 120ºC (T6) and 140ºC (T2,35% MC, 375 rpm), with BD (0.4 g/mL). In the case of screw speed, BD declined from 0.42 to 0.35 g/mL as screw speed increased from 300 (T4) to 450 (T5) rpm (Table 5; 140ºC, 30% MC). Increases in screw speed are thought to increase friction within the screw profile negatively impacting TVP expansion due to bubble collapse (Guha et al., 1997). In contrast, to the lentil TVP, both commercial pea TVP had lower BD values ranging between 0.13-0.20 g/mL. Ali et al. (2016) reported BD data for chickpea and maize extrudates to range from 0.1-0.2 g/mL depending on the extrusion conditions.

3.4.7 Rehydration ratio

The rehydration ratio (RR) measured the matrix porosity and water retention of the lentil-based TVPs that can be directly related to textural attributes because of the juiciness. Studies show that extrusion variables like screw speed, temperature and moisture content can create a change in the barrel pressure that favors the creation of air bubbles improving porosity (Brishti et al., 2021). The data presented (Table 5; 140ºC, 375 rpm) showed that there is an increase of 26% in RR when 40% MC (T3) was used during extrusion compared to 30% MC (T1). Also, when the moisture content was increased by 5% (Table 5; 140ºC, 375 rpm) there was a significant increase in the rehydration ratio from 188% (T1) to 202% (T2). Similar results were found for mung bean extrudates where the highest value of RR was found at 45% MC (Brishti et al., 2021). Bulk density is related to the rehydration ratio because of the porosity since is easier for the water to enter the matrix of the TVP with higher porosity, meaning that with higher bulk density the result is a lower rehydration ratio A slight increase in BD for lentil-based TVPs created a decrease in RR (Table 5,140ºC, 375 rpm). Previous studies using soy protein isolate in extrusion showed that there is a positive correlation between bulk density, expansion rate and rehydration ratio (Tehrani et al.,

Table 5. The effect of die temperature, screw speed and moisture content on water and oil holding capacity, rehydration rate and bulk density of lentil-based TVPs

	Water holding capacity [g water/ g protein]	**Oil holding capacity [g oil/ g protein]**	**Rehydration ratio [%]**	**Bulk density [g/mL]**
LPI	3.5 ± 0.0^{bc}	1.0 ± 0.0^{e}	NA	NA
CTVP1*	3.7 ± 0.0^{ab}	1.9 ± 0.0^{b}	532.9 ± 22.0^{a}	0.20 ± 0.01^{f}
CTVP2	3.9 ± 0.0^{a}	2.7 ± 0.0^{a}	326.9 ± 25.8^{b}	0.13 ± 0.00^{g}
T1	3.5 ± 0.1^{c}	1.2 ± 0.1^{de}	188.4 ± 5.5^{e}	0.40 ± 0.01^{bc}
T2	3.0 ± 0.1^{ef}	1.3 ± 0.0^{c}	202.1 ± 9.1^{cd}	0.40 ± 0.01^{b}
T3	2.9 ± 0.2^{f}	1.2 ± 0.0^{cd}	214.6 ± 4.6^{c}	0.38 ± 0.01^{de}
T4	3.3 ± 0.0^{d}	1.2 ± 0.1^{cd}	182.4 ± 4.4^{e}	0.42 ± 0.03^{a}
T5	3.5 ± 0.1^{bc}	1.3 ± 0.1^{c}	191.9 ± 4.9^{de}	0.35 ± 0.02^{e}
T6	3.1 ± 0.0^{e}	1.2 ± 0.0^{de}	193.4 ± 1.1^{de}	0.38 ± 0.01^{d}
T7	3.1 ± 0.1^{e}	1.2 ± 0.0^{de}	192.9 ± 1.2^{de}	0.38 ± 0.00^{cd}

Notes:

Treatments are as follows: T1-140ºC, 30%MC, 375 rpm, T2-140ºC 35%MC, 375 rpm, T3-140ºC 40%MC, 375 rpm, T4-140ºC 30%MC, 300 rpm, T5-140ºC 30%MC, 450 rpm, T6-120ºC 35%MC, 375 rpm and T7-130ºC 35%MC, 375 rpm. *CTVP= Commercial pea TVP; LPI = Lentil protein isolate. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different (p>0.05)

2017). Finally, rehydration ratio for commercial TVPs are significantly different from lab scale TVPs that can be attributed to the raw material as well as the conditions carried for each TVP. As mentioned before, the conditions of extrusion can affect this property and similar values of bulk density were obtained by (Ali et al., 2016), with extrusion conditions of 18% moisture content, 550 rpm, and 125ºC. No effect was observed with the increase in temperature from 120ºC (T6) to 140 ºC (T2) (Table 5; 35% MC, 375 rpm) or change in screw speed from 300 rpm (T4) to 450 rpm (T5) (Table 5; 140 ºC, 30% MC) for lentil-based TVPs. Finally, both of the commercial pea TVPs had higher rehydration ratio (326.9 and 532.9 %) compared to lentil-based TVPs, which can be attributed to the low value for BD (0.13-0.20 g/mL).

3.4.9 Texture profile

Texture profile analysis measures various mechanical parameters that closely aligns with their sensory attributes. Texturization of lentil protein isolate was produced due to the pores produced due to the friction generated in the extruder barrel combined with the changes in pressure in the shaping die that create an elongated structure in the final product. TVPs were imaged by scanning electron microscopy where no elongated structures were found on the raw material (figure 1) while for all treatments, elongated structures were found as shown in figures 2-4.

The attribute hardness measures the force that is needed to create a deformation, chewiness measures the force that is needed to chew solid food until is ready to swallow, while cohesiveness quantifies the internal resistance of the food structure (Amrut et al., 2022). On the other hand, springiness measures the elasticity based on the recovery between the first and second compression, gumminess measures the sample's cohesive and sticky properties, and resiliencies measure how well the product regains its original height (Ma and Ryu, 2019).

For lentil-based TVPs hardness, gumminess, and chewiness had no change when the moisture content changed from 30% (T1) to 40% (T3) (Table 6; 140ºC, 375 rpm), increase in temperature from 120ºC (T6) to 140 ºC (T2) (Table 6; 35% MC, 375 rpm) or change in screw speed from 300 rpm (T4) to 450 rpm (T5) (Table 6; 140 ºC 30% MC). For springiness, the values range from 67.5% to 83.6% for lentil-based TVPs where an increase in moisture content from 30% (T1) to 40% (T3) created a decrease in this property that is associated with the viscoelastic properties including viscosity (Brishti et al., 2021). Similar results were obtained by Maung et al. (2021) with higher MC (55-65%) for a mix of soy isolate, wheat gluten and corn starch extrudates lowered the

Table 6. The effect of die temperature, screw speed and moisture content on texture profile for lentil-based TVPs.

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
CTVP1	2.9 ± 0.1^{d}	92.2 ± 1.2^{a}	0.77 ± 0.00^{a}	2.3 ± 0.1^{b}	2.1 ± 0.0^{c}	0.42 ± 0.00^{a}
CTVP2	5.5 ± 0.7^{c}	82.7 ± 1.9^{abc}	0.69 ± 0.02^{b}	3.8 ± 0.6^{ab}	3.2 ± 0.5^{abc}	0.40 ± 0.01^{a}
T1	8.3 ± 0.9^{ab}	81.3 ± 3.2^{bc}	0.60 ± 0.01^{c}	4.9 ± 0.5^{a}	3.9 ± 0.3^{a}	0.34 ± 0.01^{bc}
T2	8.5 ± 0.9^{ab}	73.9 ± 3.7^{d}	0.55 ± 0.01^{ef}	4.6 ± 0.4^{a}	3.4 ± 0.4^{ab}	0.30 ± 0.00^{d}
T3	9.2 ± 2.2^{a}	67.5 ± 3.8^{e}	0.52 ± 0.02^{g}	4.7 ± 1.5^{a}	3.3 ± 1.1^{ab}	0.28 ± 0.03^{e}
T4	8.3 ± 0.7^{ab}	77.1 ± 0.2^{cd}	0.57 ± 0.01^{e}	4.7 ± 0.3^{a}	3.6 ± 0.2^{ab}	0.32 ± 0.00^{c}
T5	7.8 ± 0.3^{ab}	83.6 ± 4.6^{b}	0.62 ± 0.01^{c}	4.8 ± 0.3^{a}	3.9 ± 0.2^{a}	0.35 ± 0.01^{b}
T6	8.4 ± 0.5^{ab}	73.3 ± 2.4^{de}	0.54 ± 0.01^{f}	4.6 ± 0.3^{a}	3.4 ± 0.3^{ab}	0.30 ± 0.01^{d}
T7	7.5 ± 0.5^{b}	72.2 ± 1.3^{de}	0.53 ± 0.00^{fg}	4.0 ± 0.2^{a}	2.9 ± 0.2^{bc}	0.30 ± 0.01^{d}

Notes:

Treatments are as follow: T1-140ºC, 30%MC, 375 rpm, T2-140ºC 35%MC, 375 rpm, T3-140ºC 40%MC, 375 rpm, T4-140ºC 30%MC, 300 rpm, T5-140ºC 30%MC, 450 rpm, T6-120ºC 35%MC, 375 rpm and T7-130ºC 35%MC, 375 rpm. *CTVP= Commercial pea TVP. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different (p>0.05)

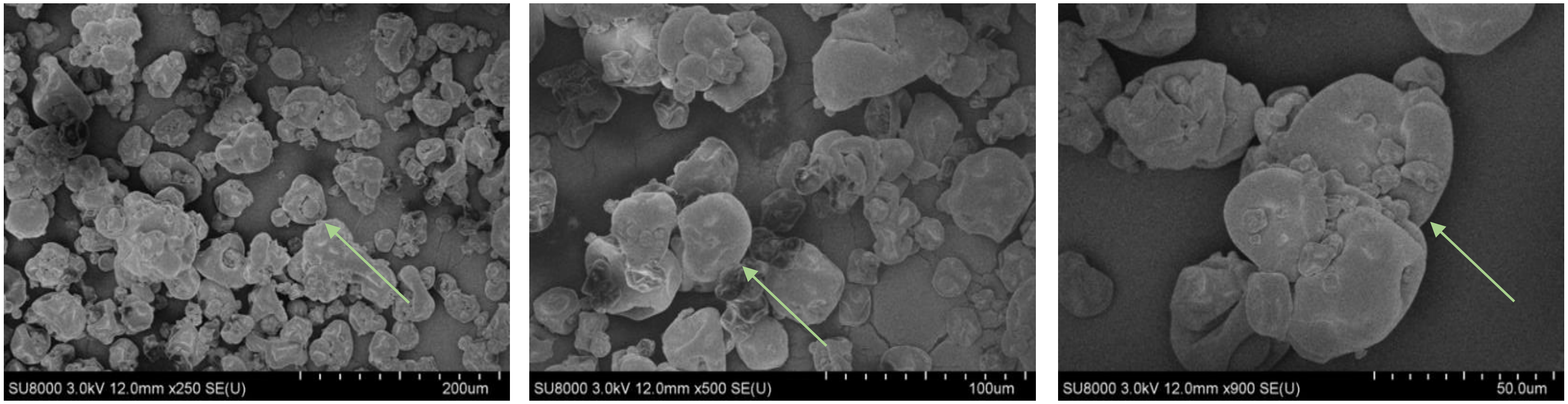

Figure 1. SEM images of raw lentil protein isolate with different magnification, (a) 250x, (b) 500x and (c) 900x. Arrows indicate globular structure.

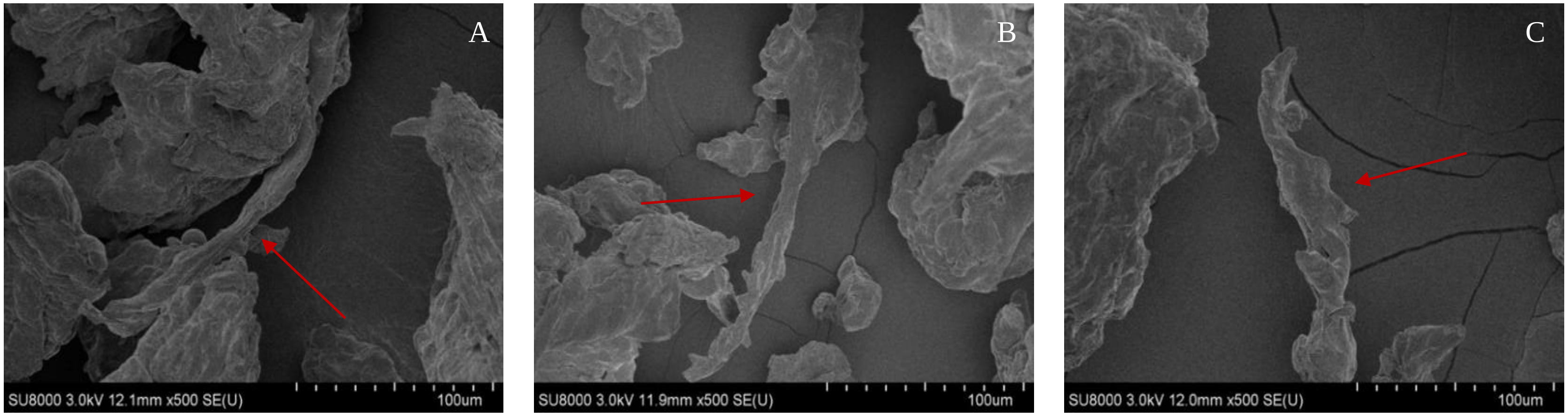

Figure 2. SEM images of lentil TVP as function of feed moisture content, (a) 30%, (b) 35% and (c) 40% at constant temperature of 140ºC. The magnification used for these images was 500x. Arrows indicate the elongated structure.

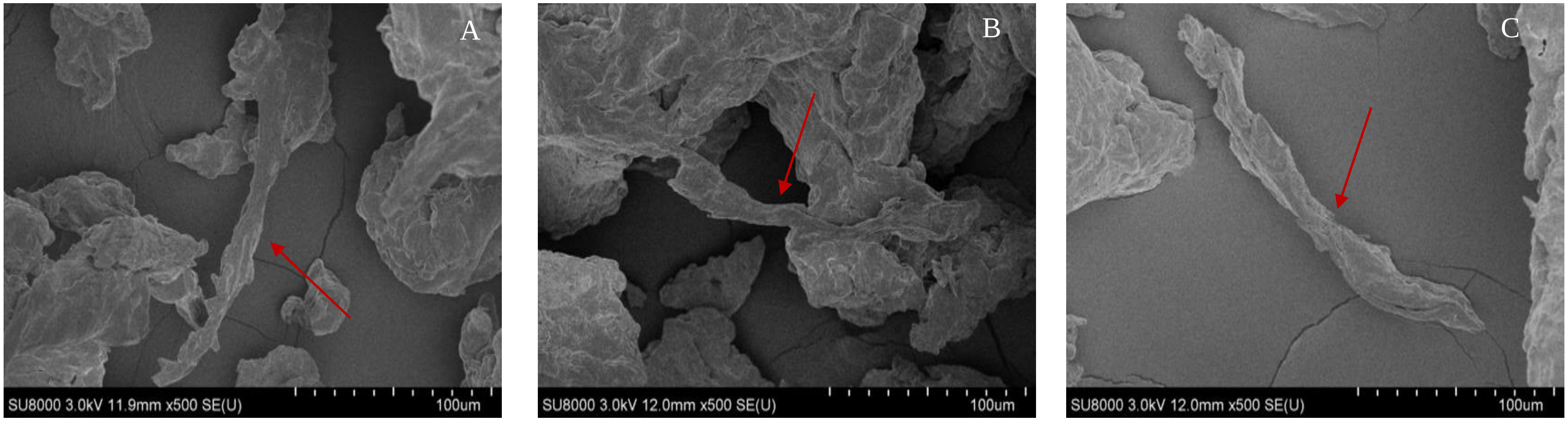

Figure 3. SEM images of lentil TVP as a function of die temperature (a) 120ºC, (b) 130ºC and (c)140ºC at constant feed moisture of 35%. The magnification used for these images was 500x. Arrows indicate the elongated structure.

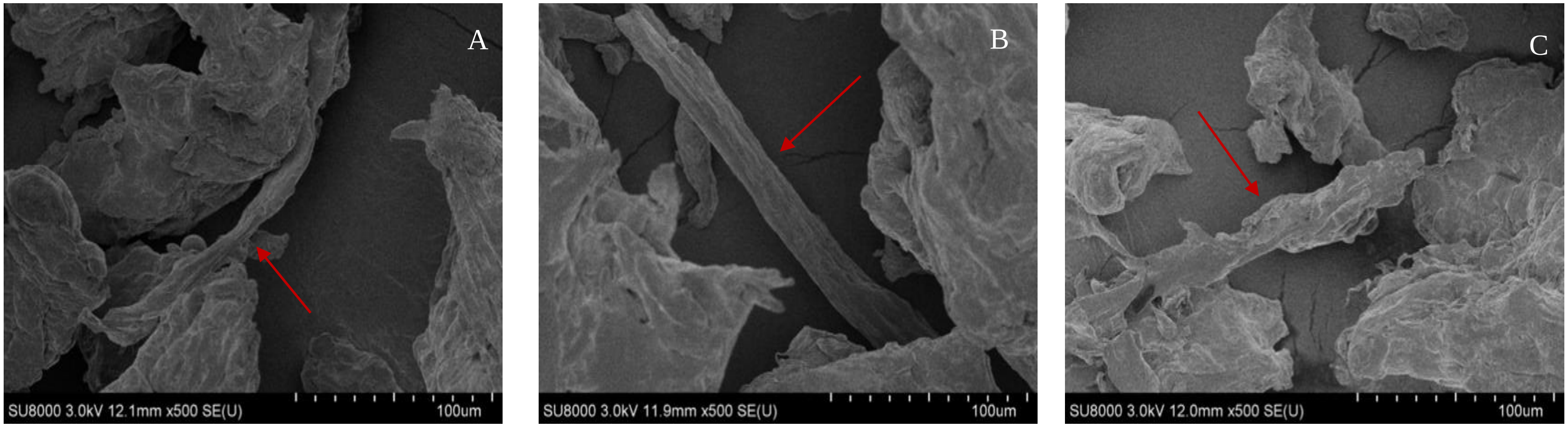

Figure 4. SEM images of lentil TVP as a function of screw speed (a) 300 rpm, (b) 375 rpm and (c) 450 rpm at constant temperature of 140ºC. The magnification used for these images was 500x. Arrows indicate the elongated structure.

viscosity and elasticity of the mix during extrusion creating a softer extrudate. The change in screw speed from 300 rpm (T4) to 450 rpm (T5) (Table 6; 140 ºC 30% MC) showed a significant increase in springiness from 77.1 % to 83.6% that can be attributed to higher friction generated during extrusion creating a decrease in viscosity (Ma& Ryu, 2019). No effect of temperature wad seen between 120ºC (T6) and 140ºC (T2; 35%, 375 rpm). Cohesiveness values for lentil-based TVPs are lower than the results obtained from Brishti et al (2021) for texturized mung bean (0.6 and 0.9 respectively) which is attributed to the difference in the degree of texturization between samples.

When the moisture content changed from 30% (T1) to 40% (T3) (Table 6; 140ºC, 375 rpm) there was a decrease in cohesiveness and resilience. A slight increase for both texture attributes was seen when the screw speed changed from 300rpm (T4) to 450 rpm (T5) (Table 6; 140 ºC 30% MC) for lentil-based TVPs, but no effect was seen with an increase in temperature from 120ºC (T6) to 140 ºC (T2) (Table 6; 35% MC, 375 rpm).

3.4.10 Application of lentil-based TVPs in a meat product

3.4.10.1 Cooking properties for hybrid burgers

To successfully incorporate TVPs to any meat product, there must be a resemblance in functional and sensory aspects close to real meat, meaning that TVPs with higher rehydration ratio need to have a higher consumer acceptance since it would create a decrease in hardness and cohesiveness with an increase in juiciness and mouthfeel. Industry is looking for TVPs as a partial substitution that can reduce the products’ costs while maintaining the burgers' physical and quality properties. Previous studies showed that replacing meat with TVP was successful in a range from 10-20% in terms of quality and sensory aspects (Bakhsh et al., 2021). One of the main characteristics that affect the final sensory aspects of a meat product is the ability to retain liquid after cooking which is directly related to the juiciness and tenderness of the patty. Based on the data collected for lentil-based TVPs, the best treatment selected for the application was T3 (140ºC, 40% MC, 375 rpm) which showed higher functional properties including rehydration ratio.

Cooking yield is a property that measures the changes in weight due to a cooking process that directly affects the appearance and texture of the cooked product (USDA, 2012). In the present study, the incorporation of 10% lentil-based TVPs showed a significant increase in the cooking yield of the burgers compared to the control (i.e., real beef). As shown in Figure 5, the burgers made with a commercial TVPs and the lentil-based TVPs showed a higher yield of 81.3% and

79.2% respectively compared to 68.7% with no substitution. These results can be attributed to the higher capability of TVPs to retain water and oil during cooking which also increased moisture retention from 42.7 to 44.6 and 48.1 for lentil-based and commercial TVPs respectively (Bakhsh et al., 2022). Similar results were observed by Bakhsh et al (2021) with a replacement of 10-40 % of soy TVP in beef burgers that created a linear and quadratic decrease in cooking loss. Finally, the difference observed in the commercial and lentil-based TVPs can be attributed to the extrusion conditions used that affected the functional properties including WHC, OHC, and RR (Table 5). The diameter and thickness change are other properties used to measure the loss of shape during cooking (Samard et al., 2021). The diameter changes of the lentil-based and commercial TVP hybrid burgers are significantly lower than the real beef burger with a decrease of around 8% (Figures 5 & 6).

The change in thickness only showed a significant difference with the lentil-based TVP burger with a value of 11.9% compared to 22.7% for real beef (Figure 5). The changes in the values can be attributed to the denaturation of the protein during the cooking process which can enhance the extra release of water and oil, where the substitution with TVPs tend to decrease that release of fluids due to the functional properties (i.e. OHC and WHC) (Bakhsh et al., 2021). Similar results were obtained by Bakhsh et al (2021) using soy TVP in a burger with a 10% replacement that reduced the thickness change from 35.4% to 24.4%. The changes in diameter changes were not significant for any percentage of replacement (10-40%) however, there was a slight decrease of 3% for 20% and 40% substitution.

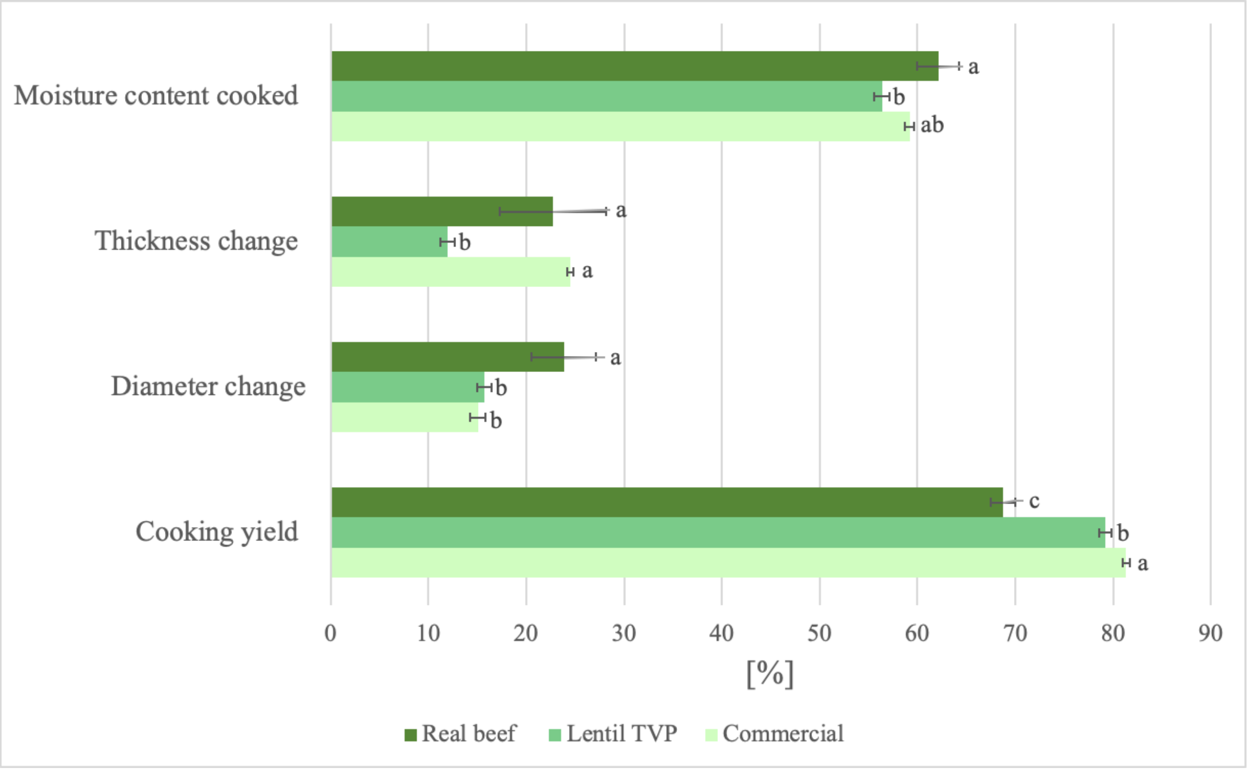

Figure 5. Effect of the addition of 10% lentil TVP in the cooking properties of meat patties. Treatments with the same superscript letter are not significantly different (p>0.05)

Figure 6. Images of hybrid burgers after cooking

3.4.10.2 Texture profile for hybrid burgers

The texture properties of any product are directly related to the mouthfeel that has an impact on the consumer's acceptability (Summo et al., 2016). The different attributes showed significant results for all 3 samples including hardness, cohesiveness, gumminess, and chewiness. First, the hardness, defined as the force required for compression, significantly changed when TVPs were added whereas, for the lentil-based TVP sample, a lower value was obtained compared to the control with a difference of ~2 N that can be attributed to a weaker myofibril protein network that reduces the resistance to an external force (Feng & Xiong, 2002). A similar result was observed by Bakshs et al. (2021) where a 10% replacement of soy TVP decreased the hardness values from 32.9 N to 26.0 N. On the other hand, for the commercial TVPs, the hardness value significantly increased from 16.3 N (control) to 28.5 N which can be related to the crusting properties of the burgers due to evaporation of water which increased the solid matter and in consequence the hardness value (Moll et al., 2023). For cohesiveness, which measures the ability of the sample to deform before breaking, there was no significant change between the 2 formulations with the 10% replacement of TVP but had a significant increase compared to the control. These changes can be attributed to the changes in moisture content for each sample (Figure 5) where less water available tends to increase cohesiveness (Al-Juhaimi et al., 2015). Gumminess and chewiness are related to the force needed to disintegrate the sample before swallowing where significant changes were observed for all 3 samples (Summo et al., 2016). The lentil-based TVP sample showed the lowest value for these attributes followed by the real beef and the commercial TVPs respectively. These changes can be related to the hardness value of each sample since animal meat tends to have a higher hardness and in consequence, require more force to break it down before swallowing compared to a plant-based option (lentil TVP) (Bakshs et al., 2021). Finally, no effect was seen for springiness and resilience values between the samples with 10% replacement and the real beef.

Table 7. Effect of 10% addition of TVP in texture profile of meat patties

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
C-TVP*	28.5 ± 0.8^{a}	117.9 ± 17.9^{a}	0.9 ± 0.0^{a}	26.3 ± 0.8^{a}	30.7 ± 4.9^{a}	0.6 ± 0.0^{a}
L-TVP**	14.5 ± 0.5^{c}	115.2 ± 15.0^{a}	0.9 ± 0.0^{a}	13.4 ± 0.5^{c}	15.5 ± 1.9^{b}	0.6 ± 0.0^{a}
Real beef	16.3 ± 0.2^{b}	88.8 ± 4.6^{a}	0.8 ± 0.0^{b}	15.1 ± 0.2^{b}	21.4 ± 2.7^{b}	0.6 ± 0.0^{a}

Notes:

Treatments with the same superscript letter are not significantly different ($p>0.05$)

*C-TVP=Commercial pea TVP

**L-TVP=Lentil TVP

3.4.10.3 Cooking properties for vegan burgers

Meat is part of the human diet due to its nutritional benefits as well as the taste and organoleptic features that are highly liked by consumers. Meat proteins are responsible for characteristics, for example: texture, juiciness, tenderness, and functionality which can become a challenge for the plant-based industry. A complete vegan product can have some difficulties in terms of functionality, hardness and off-flavors attributed to the legumes used as a protein source to produce them (Bakhsh et al., 2021). The capability of retaining water and other juices of the burger after cooking is directly related to appearance and texture making this property essential for testing TVP functionality. For lentil-based TVP vegan burgers the cooking yield obtained was 70.0% which showed no significant difference from the real beef sample, however, both commercial samples (i.e commercial TVP and beyond meat) showed a higher yield with values ranging from 73. 8% to 76.3% which can be attributed to WHC of the TVPs that reduced the loss in moisture during cooking (Samard et al., 2021). Similar results were observed by Samard et al. (2021) where different conditions of TVP made from soy isolate, wheat, and corn starch were tested on a meatless burger to compare it to a commercial sample where the cooking loss decreased for the lab scale TVPs by around 5-8%. The moisture retention and moisture content showed a direct correlation with the cooking yield for the vegan burgers. The values for moisture retention ranged from 34.4 to 42.1 with the higher value corresponding to the Beyond meat sample. As mentioned before, TVPs show high functional properties including the water and oil binding capacities which can explain the higher moisture retention and in consequence the higher cooking yield (Hale et al., 2002).

Finally, one of the main challenges of the meat alternatives is the texture and appearance since TVPs tend to be softer and have a lower binding ability than animal proteins. Additives are a suitable solution to increase binding abilities and enhance moisture retention and textural properties (Palanisamy et al., 2018). For this study, a binder was used in the vegan burgers which is considered safe for consumption (GRAS) and allowed the usage in meat products by the USDA regulations (Bakhsh et al., 2021). The changes in the diameter of the vegan burgers showed a significant decrease compared to the real beef sample from 23.8% to 10.5% and 8.7% for the lentil TVP and Commercial pea TVP, respectively, and no significant change compared to the Beyond meat sample (9.1%). On the other hand, the changes in thickness had a significant decrease of 16% for both TVPs compared to the real beef sample and a decrease of 8% compared to the Beyond meat sample. These changes observed in figure 6 can be attributed to the addition of the binder in the formulation since it can hold all the ingredients together creating a more homogeneous and cohesive interior, enhancing the shape and firm texture of the product (Bakhsh et al., 2021). Previous studies observed the effect of adding different percentages of a binder (ranging from 1.5% to 4%) in beef, commercial TVP, and soy isolate TVP protein burgers where the diameter change decreased from 22% for the beef to 12% for the commercial TVP and 7% to the soy TVP with the addition of 3% of binder (Bakhsh et al., 2021).

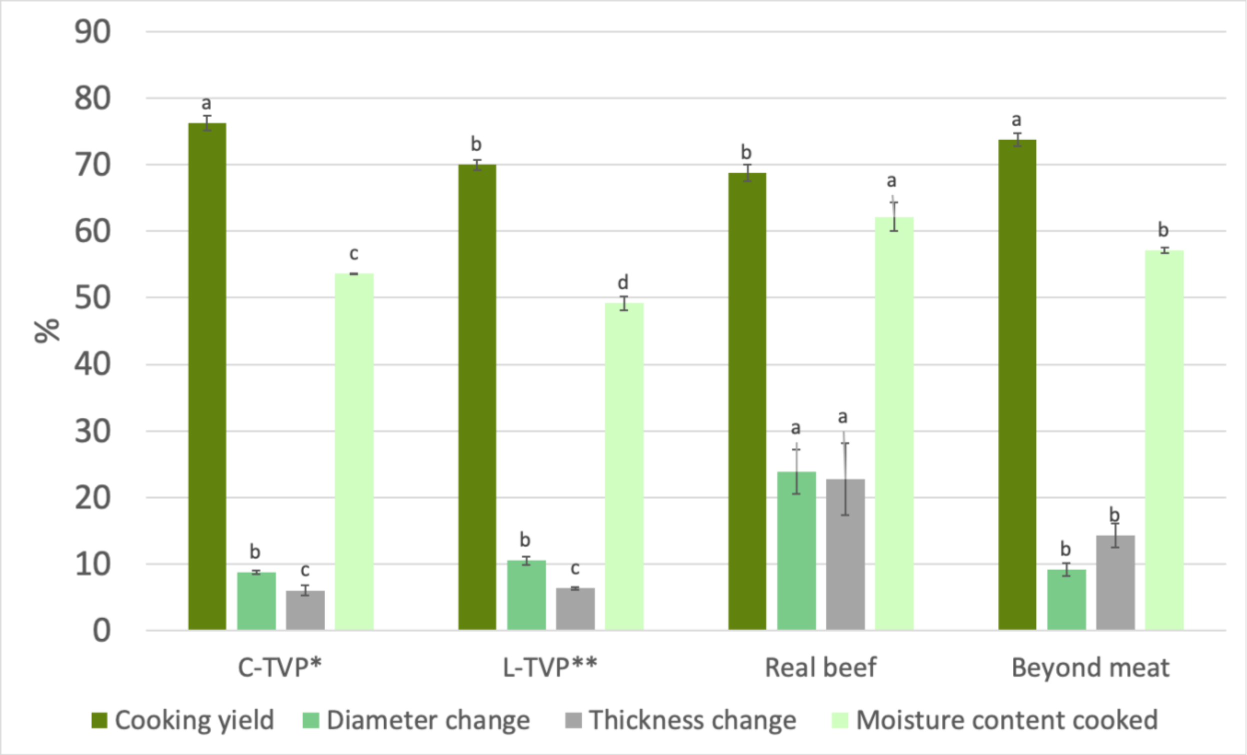

Figure 7. Effect of TVPs in the cooking properties of vegan patties. *C-TVP= Commercial pea TVP; **L-TVP= Lentil-based TVP. Treatments with the same superscript letter are not significantly different (p>0.05).

Figure 8. Images of vegan and real beef burgers after cooking

3.4.10.4 Texture profile for vegan burgers

The main concern for textural properties to develop meatless burgers is to mimic the organoleptic properties of the meat muscle including hardness, chewiness, cohesiveness, and others (Bakhsh et al.,2021). The results of the study are shown in Table 8 where hardness tends to increase for plant-based burgers compared to real meat which can be attributed to the hard structure of TVP even after rehydration and to the crusting properties of the vegan burgers. During cooking, the patties developed a crust due to the water evaporation which increased the solid matter and in consequence, increased the hardness values (Moll et al., 2023). Previous studies showed the influence of the cooking method and the different binders used in plant-based burgers made with TVP. Results showed that frying patties in a pan leads to textural and optical changes in plant-based burgers and were highly influenced by the raw materials used (i.e. TVP) rather than the binder used (Moll et al., 2023). For springiness, all the plant-based samples showed higher values compared to the real beef sample which can be attributed to a stronger network formed during cooking. Previous studies carried out by Bakhsh et al. (2021) showed similar results for vegan burgers made from TVP under different conditions (i.e. MC and TºC) where the springiness value was significantly higher than the real beef sample. A similar trend was observed for cohesiveness and gumminess where there was a significant increase for all the plant-based burgers compared to the real beef which can be attributed to the higher moisture retention and the incorporation of a binder (Al-Juhaimi et al., 2015).

Table 8. Texture profile of TVP-based vegan patties.

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
C-TVP*	51.70 ± 0.61^{a}	102.26 ± 8.72^{b}	0.89 ± 0.00^{ab}	46.26 ± 0.32^{a}	47.08 ± 3.60^{a}	0.57 ± 0.01^{ab}
L-TVP**	30.00 ± 2.66^{b}	92.93 ± 0.85^{b}	0.86 ± 0.02^{b}	25.76 ± 2.56^{b}	23.97 ± 2.46^{b}	0.52 ± 0.02^{b}
Real beef	16.34 ± 0.25^{d}	88.85 ± 4.56^{b}	0.77 ± 0.03^{c}	15.06 ± 0.17^{d}	21.38 ± 2.68^{b}	0.63 ± 0.03^{a}
Beyond meat	24.85 ± 0.70^{c}	139.29 ± 15.41^{a}	0.92 ± 0.02^{a}	19.16 ± 0.62^{c}	17.11 ± 1.47^{b}	0.32 ± 0.01^{c}

Notes:

Treatments with the same superscript letter are not significantly different ($p>0.05$)

*C-TVP=Commercial pea TVP

**L-TVP=Lentil-based TVP

For resilience, there was a significant decrease for all the plant-based samples due to the correlation with moisture content of the samples where the real beef sample showed a higher value (Vu et al., 2022). Finally, for chewiness, there was no significant change for the lentil-based TVPs and the beyond meat compared to the real beef. Similar results were obtained by Vu et al. (2002) where a plant-based burger (Impossible meat made from soy TVP) was compared to a real beef under different cooking conditions with significant changes for cohesiveness, gumminess, and resilience.

3.5 Conclusion

The study produced texturized vegetable proteins (TVPs) from lentil isolate under different extrusion conditions for an evaluation of their functionality and potential use in meat applications. The different treatments used in extrusion with die temperatures (120, 130 and 140 °C), feed moisture contents (30, 35 and 40 g water/100 g feed, wet basis) and screw speeds (300, 375 and 450 rpm) showed a significant impact on the physical and functional properties of lentil-based TVPs. First, specific mechanical energy (SME) showed that was highly affected by all the extrusion variables. The heat used in the process to create a stable product in terms of shelf life, reflected in the water activity values lower than 0.4 and the moisture content lower than 10% for all treatments. The changes in color were mostly affected by the moisture content with a significant increase in brightness (L), and a decrease in redness (a) and yellowness (b) that can be attributed to the Maillard reaction between the reducing sugars and the free amino acids.

The functional properties that were mostly affected by moisture content were WHC with a significant decrease that can be attributed to the porosity of TVPs under the different MC% and the denaturation of the protein, while the OHC showed a significant increase after extrusion because of the buried sites that are exposed during extrusion due to the unfolding of the protein. The rehydration ratio increased with higher moisture content due to the higher number of pores produced during extrusion which also created a decrease in the bulk density for lentil-based TVPs. For the texture profile, no effect was seen with changes in moisture content for most of the attributes, however, springiness showed changes when moisture content increased during extrusion. No significant effect of temperature was observed for most of the physical and functional properties analyzed which can be attributed to the thermal stability of the lentil isolate observed in the FTIR Spectra results. Finally, screw speed showed a significant effect for attributes such as WHC, texture profile and bulk density. Overall, the combination of extrusion variables and raw material used created a significant change in the functional and physical properties of TVPs which can be used as an advantage to tailor the protein structure based on the application.

For this study, the optimal treatment for lentil-based TVPs (140ºC, 40% MC, and 375 rpm) was selected for a meat application of producing a hybrid burger with a 10% replacement and a vegan burger. The results showed that by only adding 10% of lentil-based TVPs the cooking properties had a significant effect in reducing the loss of moisture after cooking which increased the cooking yield of the burgers and decreased the changes in shape (i.e., thickness and diameter). Textural attributes including hardness, gumminess, and chewiness showed a significant decrease with the addition of lentil-based TVPs while springiness and cohesiveness showed a significant increase with no changes in resilience. Future studies should include a sensory evaluation to obtain the overall acceptance of this substitution in terms of texture, flavor, and juiciness. Finally, according to the results for the meatless burger lentil TVP is a suitable replacement for meat in terms of reducing moisture loss during cooking which highly affects the cooking yield, diameter, thickness, and texture.

3.6 Linkage to study 2

The current market for TVPs is dominated by soybeans as a raw material, so research on different sources is necessary to combat different negative aspects such as its allergenicity and tendency to inflammation. There are different plant-based sources to produce TVPs that can compete in the current market that have not been fully studied. In the case of study 1, it was shown that lentil-based TVPs produced with low moisture extrusion is a viable alternative solution. Also, it was shown that lentil-based TVPs can be applied as a partial or complete substitution of animal meat in hamburgers improving the cooking properties that have an effect on the texture and therefore on the sensory aspects. For all the reasons mentioned before, the second study focuses on faba bean as another raw material for extrusion to understand how extrusion affects the functional and physical properties of faba bean-based TVPs.

4. STUDY 2: PRODUCTION OF TEXTURIZED VEGETABLE PROTEIN (TVPS) FROM FABA BEAN AND ITS APPLICATION IN HYBRID AND VEGAN BURGER PATTIES

4.1 Abstract

The aim of this study was to produce texturized vegetable proteins with faba bean isolate and concentrate throughout low moisture extrusion as an alternative protein source that can be used as a complete or partial replacement for animal meat. A lab-scale twin-screw extruder was used at three different die temperatures (110, 125, and 140 °C), feed moisture contents (30, 35, and 40 g water/100 g feed, wet basis), and screw speeds (200, 300 and 400 rpm) to produce faba bean based TVPs with a wide range of physical and functional properties. The protein content of the TVPs did not change compared to the raw mixture and parameters including moisture (<7.5%) and water activity (0.17-0.23) showed that the product is stable at room temperature. All the treatments showed a decrease in brightness, and an increase in redness and yellowness compared to the raw material. An increase in moisture content, temperature, and screw speed reduced the Specific mechanical energy and torque by 40-45% during extrusion. An increase in moisture created a lower value for bulk density and rehydration ratio while an increase in screw speed and temperature increased the bulk density and rehydration ratio of the TVPs. An increase in screw speed caused a decrease in the water-holding capacity of the TVPs with no effect of temperature and moisture content. For oil-holding capacity, there was a significant increase after extrusion compared to the raw material. An increase in moisture showed an increase in oil-holding capacity, but an increase in temperature showed a decrease in oil-holding capacity with no effect with an increase in screw speed. The texture profile showed that an increase in moisture influenced hardness, gumminess, and chewiness with higher values compared to the rest of the treatments with lower moisture content. On the other hand, for springiness, cohesiveness, and resilience were more affected by a change in screw speed with higher values at 200 rpm. All these data showed that the combination of extrusion variables influenced the physical and functional properties of faba bean-based TVPs.

Based on the functional and physical analysis performed, an optimal treatment was selected (125ºC, 40% MC and 300 rpm) to use it as a partial and complete replacement of beef in a burger patty to measure different attributes such as cooking yield, moisture retention, diameter change, thickness change, and texture profile. Overall, the addition of 10% faba bean TVP increased the cooking yield and moisture retention with a significant decrease in thickness and diameter change that affected the texture profile of each sample.

4.2 Introduction

Meat is considered a high-quality protein because it contains enough of all the essential amino acids and has an appearance, texture, and mouthfeel that is highly accepted by consumers (Asgar et al., 2010). However, meat sources have been associated with increased risks of cardiovascular diseases, consumer religious concerns and sustainability (Bakhsh et al., 2021). There are potential factors that have increased health and sustainability concerns like a high content of saturated fats and the limitation of resources including water and land (Beck et al., 2017). As an alternative, the food industry developed plant-based proteins to substitute the consumption of animal-derived products such as milk, meat, eggs, and others. Many consumers are moving towards a more sustainable and healthier lifestyle with high acceptance of vegan or vegetarian products including tofu and tempeh creating a new space for innovation in the plan-based market (Ma et al., 2022). Meat replacements are part of these new products that used plant proteins to mimic the functional, taste, texture, and look of real meat while also having some health and environmental benefits (Bakhsh et al., 2021).

Pulse proteins obtained from leguminous (i.e. soy, pea, chickpea, and faba bean) are emerging as raw material to produce meat alternatives due to the wide range of functionality, low-fat content, and high protein. There are challenges for plant-based products that include bitter and beany flavors that can decrease the consumer’s acceptance (Ma et al., 2021). Faba bean (*Vicia faba*), is a type of pulse, part of the *fabacean* family consumed in the middle east and is mainly cultivated in China and Ethiopia (Augustin et al., 2022). The market for faba bean reached 3.18 billion USD in 2021 and is expected to grow 3.77% annually reaching 3.47 billion USD by 2025 (Bangar & Kajla, 2022). Canada is one of the countries that began with the breeding of faba bean to increase yield, productivity and decrease anti-nutritional factors like tannin and vicine/convicine content (Khazaei et al., 2021). Faba bean is a rich source of nutrients including fiber, high-quality protein (i.e balanced amino acids), and low content of anti-nutritional factors (Bangar & Kajla, 2022). Faba bean is used in the food industry as a plant source of protein (25-40%) and confers health benefits like lowering the risk of diabetes type 2, cardiovascular diseases, and colon cancer (Jarpa-Parra, 2018).

Low moisture extrusion technology allows to produce plant-based extrudates that have sponge-like structures, that give good functional properties including high water-holding capacity, rehydration ratio, and textural attributes (Zhang et al., 2019). Extrusion improves the native protein structure through denaturation that exposes certain amino acids influencing the functional properties including higher rehydration ratio, water holding capacity, and mimic animal tissue texture. With this technology, it is possible to produce higher-quality products that maintain or increase the nutritional benefits of pulses while also removing the anti-nutritional factors (Petrat-Melin et al., 2022). The food industry uses the combination of different variables during extrusion such as moisture content, barrel temperature, and screw speed, to produce texturized vegetable proteins (TVPs) that are later rehydrated and used as a partial or complete substitution for meat in products such as snacks, burger patty, noodles, sausages, and others (Zhang et al., 2019). TVPs can be used as a partial substitution of meat in products such as sausages, burgers, meatballs, and others to increase yield, water-holding capacity, and protein content while also decreasing the costs of production. The addition of TVPs in burgers can also have a positive impact on textural properties (hardness and cohesiveness) while also improving appearance attributes including color and shape (Samard et al., 2021). Consumers are trying to reduce their meat consumption due to the awareness of health and sustainability that makes TVP a suitable source of protein to fulfill the protein demand. The interest in plant-based alternatives is growing creating an increase in research studies to develop new meat analogs to increase food diversification (Yuliarti et al., 2021). Based on the results obtained, the food industry is looking for new products in the plant-based market which creates an increase in growth each year and is expected to reach 14.32 billion USD by 2025 (Choi et al., 2022).

The aim of this study was to produce texturized vegetable proteins (TVPs) from faba bean isolate and concentrate through low moisture extrusion under different die temperatures (110,125, and 140ºC), feed moisture contents (30, 35, and 40 g water/100 g feed), and screw speeds (200, 300, and 400 rpm) to observe the changes in physical and functional properties to later be used as a substitution in a burger patty.

4.3 Materials and methods

4.3.1 Materials

Faba bean protein isolate with 92.4% protein (dry basis) and concentrate with 60.5% protein (dry basis) were provided by AGT Food and Ingredients (Regina, SK, Canada). Upon arrival, the feed material was stored at 4 °C. Based on preliminary extrusion trial runs of the raw material the faba bean ingredient mix used for the extrusion was determined as 70% faba protein isolate and 30% faba bean protein concentrate with 82.8% protein (dry basis).

4.3.2 Methods

4.3.2.1 Extrusion

The appropriate amount of boiling water was added to the feed material to pre-condition it to 26.5 ± 0.5 % (wet basis) a day prior to the actual extrusion run. The pre-conditioning was performed by mixing the feed material in a Hobart mixer at a low speed for 5 min while the boiling water was added. Immediately after mixing, the pre-conditioned feed was packed in zipped plastic bags and stored in a climate chamber (HPP 260 IPP plus, Memmert, Schwabach, Germany) at constant temperature (50 °C) until extrusion.

Texturized vegetable proteins (TVPs) were produced as mentioned in section 3.3.2.1. Extrudates were collected at three screws speeds (200, 300 and 400 rpm) under these conditions: at three different feed moisture contents (30, 35, and 40 g water/100 g wet feed) and different die temperatures (110, 125, and 140°C) as showed in Table 9.

At all extrusion processing conditions, TVPs were collected in triplicates after reaching steady-state conditions. After extrusion samples were dried and stored with the same conditions mentioned in section 3.3.2.1. Values for torque and die pressure were collected in quadruplicates. Specific mechanical energy (SME) was calculated as mentioned in section 3.3.2.1.

Table 9. Extrusion conditions for faba bean-based TVPs.

Treatment code	Barrel temperature profile [ºC]	Moisture content [%]	Screw speed [rpm]
Treatment 1 [T1]	75-95-115-120-125	35	200
Treatment 2 [T2]	75-95-115-120-125	35	300
Treatment 3 [T3]	75-95-115-120-125	35	400
Treatment 4 [T4]	75-95-115-120-125	30	300
Treatment 5 [T5]	75-95-115-120-125	40	300
Treatment 6 [T6]	90-110-130-135-140	35	300
Treatment 7 [T7]	60-80-100-105-110	35	300

4.3.2.2 Physical properties

Bulk density, protein content, and moisture content for faba bean-based TVPs were measured based on the methods described in section 3.3.2.2. All measurements were performed in triplicate. Results are reported as the mean ± one standard deviation.

4.3.2.3 Functional properties

Water holding capacity (WHC), oil holding capacity (OHC), rehydration ratio (RR), scanning electron microscopy (SEM) and texture profile of faba bean-based TVPs were measured based on the methods described in section 3.3.2.3. All measurements were performed in triplicate and results are reported as the mean ± one standard deviation.

4.3.2.4 Burger testing

Burger testing was prepared under the same conditions mentioned in section 3.3.2.4. The texture profile, cooking properties, diameter change, thickness change, and moisture retention were measured and calculated based on section 3.3.2.4. Measurements were made on triplicates for each sample and results are reported as the mean ± one standard deviation.

4.3.3 Statistical analysis

The data were analyzed using Minitab statistical software (MINITAB Inc., USA) with an analysis of variance (ANOVA) and Tukey test with a significance level of 0.05. A probability of

$p<0.05$ was used to determine a significant result. The data were reported as mean values with standard deviations.

4.4 Results and Discussion

4.4.1 Specific mechanical energy

Specific mechanical energy (SME) was calculated based on the torque, screw speed, motor power and mass flow rate for each treatment to produce faba bean-based TVPs where values ranged from 72.0 to 118.4 Wh/kg. Torque values ranged from 23.3% to 56.9%, where there was a tendency to have lower values with higher temperatures as shown in Table 10 for 140ºC (T6) (35% MC, 300 rpm) the torque value decreased by 10% compared to 110ºC (T7) (35% MC, 300 rpm) that is attributed to the change in viscoelastic properties of the material associated with higher mobility. The increase in temperature had a significant impact on the SME observed in Table 10 where 140ºC (T6) (35%, 300 rpm) had a significant decrease of 31.9 Wh/kg compared to 110ºC (T7) (35%, 300 rpm). Similar results were obtained by Saldanha do Carmo et al. (2023) where SME values decreased from 557.7 to 520.4 Wh/kg when the temperature was increased from 140ºC to 160ºC with 30.8%MC and 900 rpm for faba bean and oat extrudates. As shown in Table 10, there is a decrease in SME from 109.16 to 72.0 Wh/kg when moisture content increases from 30% (T4) (125ºC, 300 rpm) to 40% (T5) (125ºC, 300 rpm) which is attributed to the decrease in viscosity. Finally, screw speed had an impact on SME where an increase from 300 rpm (T2) to 400 rpm (T3, table 10, 35%MC, 125ºC) created an increase in the energy attributed to higher friction generated, but similar results were observed when a 200 rpm was used (35%MC, 125ºC) that can be attributed to lower mobility of the material with a longer residence time. Previous studies on faba, lima, pinto, and red kidney beans showed that a screw speed lower than 250 rpm increased the SME and torque for all the pulses (Gu et al., 2008). One of the desirable features of TVP is a network matrix with voids to obtain higher functional benefits. The combination of pressure, moisture, temperature, and shear creates a mass that traps water where gaps or air bubbles are generated, due to the change in pressure, resulting in porous elongated structures (Brishti et al., 2017). Raw faba bean and TVPs were imaged by scanning electron microscopy where no elongated structures were found on the raw material (Figure 9) while for all treatments, elongated structures were found as shown in Figures 10-12.

Table 10. The effect of die temperature, screw speed and moisture content on response variable within the extruder for faba bean-based TVPs.

	Die pressure [kPa]	**Torque [%]**	**SME [Wh/Kg]**
T1	3234.7 ± 126.2^{d}	56.9 ± 3.1^{a}	118.4 ± 8.3^{a}
T2	3558.1 ± 394.6^{d}	28.8 ± 1.0^{c}	89.9 ± 3.5^{c}
T3	3962.5 ± 175.1^{c}	25.2 ± 1.0^{d}	105.1 ± 6.4^{b}
T4	5337.2 ± 315.1^{b}	32.5 ± 1.8^{b}	109.2 ± 6.6^{b}
T5	2163.2 ± 92.6^{f}	25.0 ± 1.0^{d}	72.0 ± 3.7^{d}
T6	2628.2 ± 194.9^{e}	23.3 ± 0.3^{d}	72.8 ± 3.3^{d}
T7	5761.8 ± 264.4^{a}	33.6 ± 0.6^{b}	104.8 ± 7.2^{b}

Notes:

Treatments are as follow: T1-125ºC, 35%MC, 200 rpm, T2-125ºC, 35%MC, 300 rpm, T3-125ºC 35%MC, 400 rpm, T4-125ºC 30%MC, 300 rpm, T5-125ºC 40%MC, 300 rpm, T6-140ºC 35%MC, 300 rpm and T7-110ºC 35%MC, 300 rpm. *CTVP= Commercial pea TVP. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

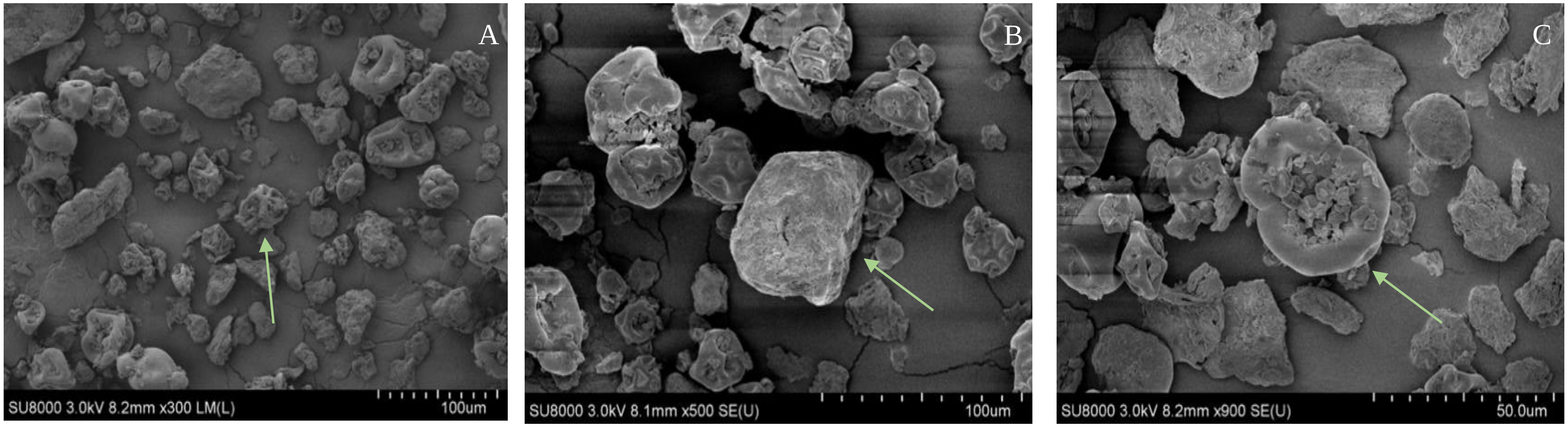

Figure 9. SEM images of faba bean isolate and concentrate with different magnification, (a) 300x, (b) 500x and (c) 900x. Arrows indicate the globular structure.

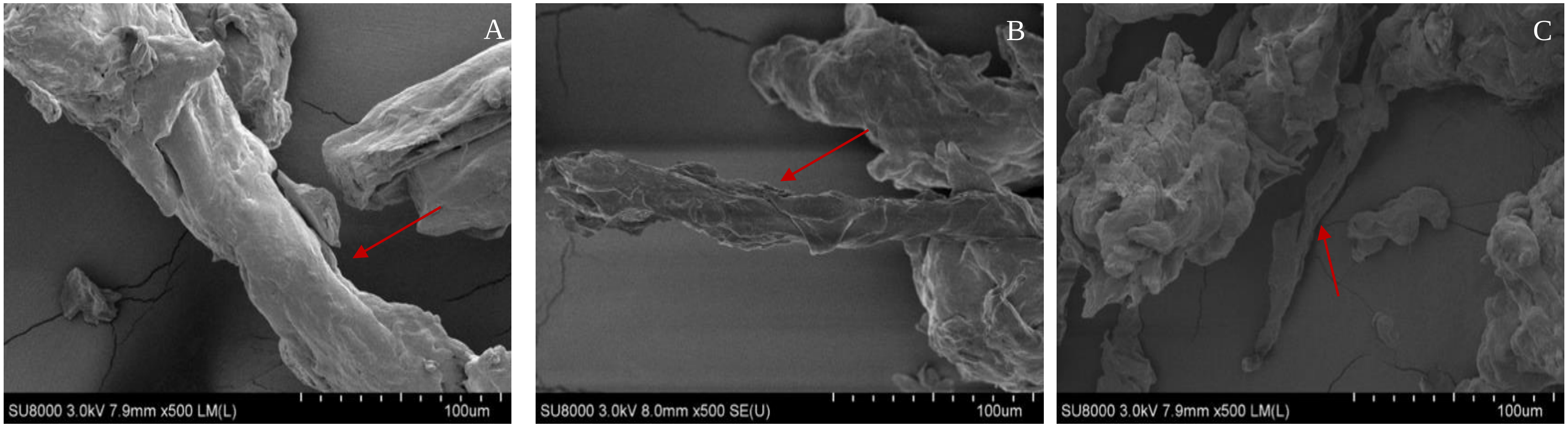

Figure 10. SEM images of faba bean-based TVP as function of fee moisture content, (a) 30% (b) 35% and (c) 40% at constant temperature of 125ºC. The magnification used for these images was 500x. Arrows indicate the elongated structure.

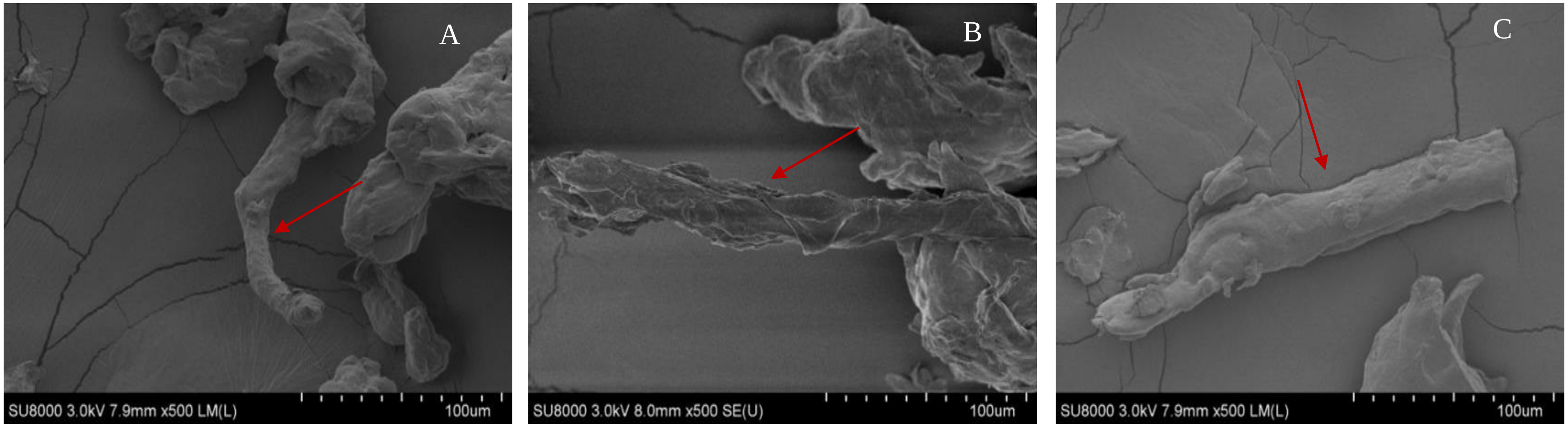

Figure 11. SEM images of faba bean-based TVPS as a function of die temperature (a) 110ºC (b) 125ºC and (c) 140ºC at constant feed moisture of 35%. The magnification used for these images was 500x. Arrows indicate the elongated structure.

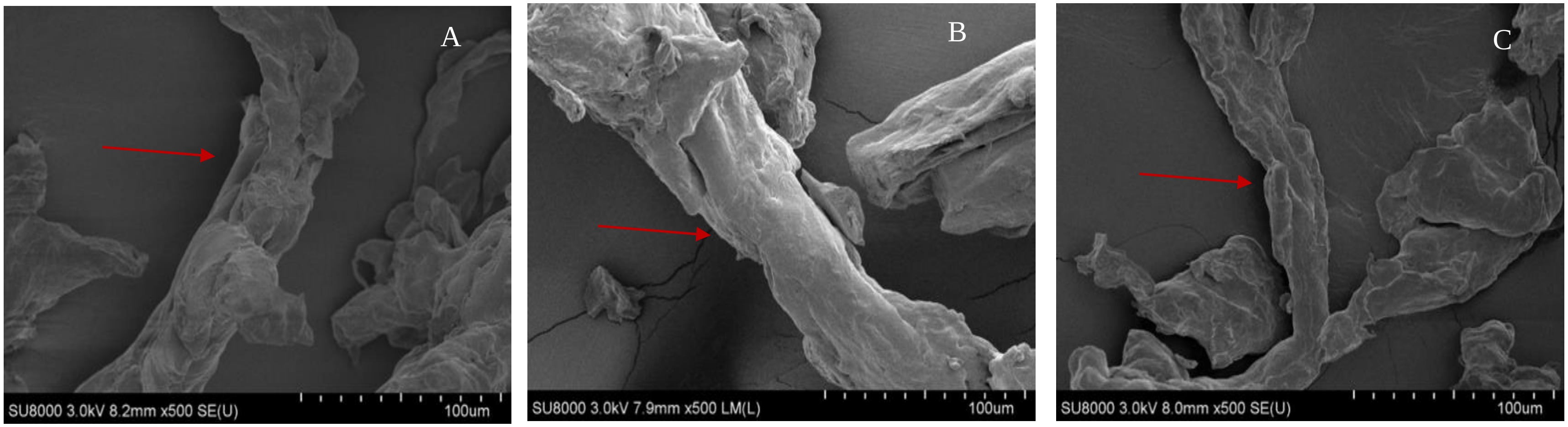

Figure 12. SEM images of faba bean-based TVP as a function of screw speed (a) 200 rpm (b) 300 rpm and (c) 400 rpm at constant temperature of 125ºC. The magnification used for these images was 500x. Arrows indicate the elongated structure.

4.4.2 Moisture content and water activity

Water plays a key role in the quality and stability of foods since it can act as reaction medium for other molecules that could create unwanted flavors or odors that decrease the product's shelf life (Li et al., 2011). For faba bean-based TVPs, moisture content varied from 5.9 to 7.3% because of the extrusion and drying process. For pulses, a wide range of moisture content levels (typically lower than 16%) are considered safe for storage and avoid the risk of mycotoxins development, where all faba bean-based TVPs meet the criteria (Manitoba government, 2023). Water activity can be related to the microbial stability of the product since it determines the growth of microorganisms related to spoilage (Maltine et al., 2003). In general, bacteria grow faster when water activity is higher than 0.91, and yeast or molds can survive in values higher than 0.85 (Adams & Moss, 2000). All the TVPs showed values lower than 0.23 (Table 11) creating a stable product where the possibility of growing molds, yeast or bacteria is low making it safe for human consumption. Finally, for commercial pea TVPs moisture content were similar to the lab scale TVPs, but water activity was significantly lower with a value of 0.15 that can be attributed to the extrusion conditions used.

4.4.3 Color

The Maillard reaction is a non-enzymatic reaction that leads to browning pigments. The reaction involves a free amino acid (Lysine) from proteins and a carbonyl group from the reducing sugars present in carbohydrates to create the changes in color, in the presence of higher temperatures. The use of high barrel temperatures and low feed moisture favors the Maillard reaction because of the denaturation of the protein that can lead to free lysine with the hydrolysis of starch having as a result reducing sugars available. As shown in Table 11 there was a change in the color for faba bean-based TVPs where brightness values (68.2-71.6) for all treatments were significantly lower compared to the raw material. The changes in moisture from 30% (T4) to 40% (T5) (Table 4, 125ºC, 300 rpm) showed a slight increase in brightness (L) that is attributed to the loss of lysine, meaning that there is more lysine available to react with free reducing sugars creating a darker color with lower moisture contents. Similar results were obtained by Beaufrand et al. (1978) where extrudates made from a mix of cereals and corn flour with 10-14% MC at 170ºC showed a loss of 32-80% of lysine that led to a darker product. The increase in temperature also leads to a darker product as shown in Table 11 where there is a slight decrease in brightness

Table 11. The effect of die temperature, screw speed and moisture content on physical properties for faba bean-based TVPs.

	Protein content [%]	**Moisture [%]**	**Water Activity**	**Color**		
				L	**a**	**b**
Raw faba bean*	82.8 ± 0.1^{de}	6.4 ± 0.1^{bcde}	0.3 ± 0.00^{a}	86.5 ± 0.0^{a}	4.2 ± 0.0^{f}	16.9 ± 0.3^{g}
CTVP 1**	80.6 ± 0.1^{f}	5.4 ± 0.1^{f}	0.15 ± 0.00^{g}	73.6 ± 0.2^{c}	12.8 ± 0.1^{a}	35.8 ± 0.2^{a}
CTVP 2	75.2 ± 0.3^{g}	6.3 ± 0.2^{cdef}	0.15 ± 0.01^{fg}	77.0 ± 0.3^{b}	11.9 ± 0.3^{b}	27.2 ± 0.8^{f}
T1	83.0 ± 0.1^{de}	5.9 ± 0.2^{def}	0.19 ± 0.01^{de}	71.5 ± 0.9^{d}	9.4 ± 0.1^{e}	30.40 ± 0.2^{e}
T2	84.1 ± 0.3^{a}	6.5 ± 0.2^{cd}	0.20 ± 0.02^{cd}	71.0 ± 0.9^{de}	9.9 ± 0.3^{d}	32.2 ± 0.8^{d}
T3	82.7 ± 0.3^{e}	5.9 ± 0.2^{ef}	0.19 ± 0.00^{de}	70.6 ± 0.7^{de}	10.2 ± 0.1^{d}	32.7 ± 0.2^{cd}
T4	83.2 ± 0.3^{cd}	6.2 ± 0.2^{cde}	0.18 ± 0.01^{ef}	68.6 ± 1.2^{f}	10.7 ± 0.3^{c}	33.4 ± 0.5^{bc}
T5	83.3 ± 0.4^{cd}	7.3 ± 0.2^{a}	0.21 ± 0.00^{bc}	71.2 ± 0.3^{de}	9.4 ± 0.6^{e}	31.0 ± 0.9^{e}
T6	83.9 ± 0.2^{ab}	6.7 ± 0.2^{bc}	0.21 ± 0.01^{cd}	68.2 ± 0.3^{f}	10.8 ± 0.1^{c}	33.9 ± 0.2^{b}
T7	83.5 ± 0.2^{bc}	7.2 ± 0.1^{ab}	0.23 ± 0.00^{b}	70.4 ± 0.3^{e}	10.0 ± 0.1^{d}	32.8 ± 0.1^{cd}

Notes:

Treatments are as follow: T1-125ºC, 35%MC, 200 rpm, T2-125ºC, 35%MC, 300 rpm, T3-125ºC 35%MC, 400 rpm, T4-125ºC 30%MC, 300 rpm, T5-125ºC 40%MC, 300 rpm, T6-140ºC 35%MC, 300 rpm and T7-110ºC 35%MC, 300 rpm. *Raw faba= 70% of faba bean isolate +30% of faba bean concentrate; **CTVP= Commercial pea TVP. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

when the temperature was increased from 110ºC (T7) (35% MC, 300 rpm) to 140ºC (T6) (35% MC, 300 rpm), but no change with an increase in screw speed. Previous studies showed that the browning index was affected by higher temperatures (140ºC) and lower moisture content (46%) that promoted the browning reactions for faba bean concentrate meat analogs (Saldanha do Carmo et al., 2021). For redness (a) and yellowness (b) the values doubled after extrusion for all the faba bean-based TVPs after extrusion which can be attributed to the pigments created by the non-enzymatic reaction. Finally, the commercial pea TVPs showed similar values for brightness (L), redness (a) and yellowness (b) compared to lab scales faba bean-based TVPs.

4.4.4 Water holding capacity

Water holding capacity (WHC) is associated with the protein matrix and relates to how much water the ingredient can hold (Brishti et al., 2017). Faba bean-based TVPs showed WHC values ranging from 2.9 to 3.8 g water/ g of protein, which significant changed with screw speed. As shown in Table 12 there was a decrease in WHC when screw speed increased from 200 (T1) (125ºC, 35% MC) to 400 rpm (T3) (125ºC, 35% MC), hypothesized to be caused by a) shear induced protein aggregation where hydrophilic sites became more positioned in the interior; and b) increased protein-starch interactions, resulting in less starch binding to water (Li & Swanson., 2013). Previous studies show extrudates produced from pea and oat protein with low moisture extrusion (25-35% MC, 135-160ºC and 200-1200 rpm) ranged from 1.6 to 2.5 g water/ g of sample (Kaleda et al., 2021). No effect was seen with changes in moisture from 30% (T4) to 40% (T5) (Table 12, 125ºC, 300 rpm) or increase in temperature from 110ºC (T7) to 140ºC (T6) (Table 12, 35% MC, 300 rpm) which can be attributed to the stability due to high content of globulins (69-78%) present in the faba beans (Vatansever et al., 2020). Similar results were obtained by Wei et al. (2009) where an increase from 60-180 rpm decreased gradually the WHC of texturized soy protein. Finally, commercial pea TVPs had a higher value for WHC from 3.9 to 4.1 g water/ g of protein that can be attributed to the structure and amino acid profile of pea and the extrusion conditions used.

4.4.5 Oil holding capacity

The oil holding capacity (OHC) is defined as the interactions between hydrophobic amino acids on the surface and oil particles. In general, the OHC of any extrudate tends to increase due to the partial unfolding of the protein structure caused by denaturation. The value for the raw material (i.e. mix of faba bean concentrate and isolate) was significantly lower (1.0 g of oil per gram of protein) compared to the values for all treatments of TVP that ranged from 1.32 to 1.69 g of oil per gram of protein. The changes in moisture content from 30% (T4, 125ºC, 300 rpm) to 35% (T2, 125ºC, 300 rpm) showed a significant increase in OHC that can be attributed to the higher heat transfer attributed to the higher MC that created an increase in denaturation and decrease in protein aggregates. On the other hand, the change in moisture from 30% to 40% MC (125ºC, 300 rpm) showed a significant decrease which can be attributed to the changes in viscosity, since more moisture is available the material moves through the barrel in a shorter period that reduces the exposure to heat and in consequence the denaturation of the protein (Carmo et al., 2023). Previous studies showed that the use of faba bean concentrates with higher moisture contents (<50%) decreased the OHC by around 20% compared to the native material due to the aggregation of proteins that decreases the surface hydrophobicity (Carmo et al., 2021).

Temperature changes also affected the OHC for faba bean-based TVPs with a significant decrease from 1.6 to 1.5 g of oil per gram of protein when temperature increased from 125ºC to 140ºC under the same conditions (35%MC, 300 rpm). The changes in OHC can be attributed to the protein denaturation, which is enhanced by temperature, that can expose hydrophobic amino acids to the surface (Osen et al., 2014). Similar results were observed by Wang et al. (2019) for chickpea flour where a change from 120ºC to 150ºC (24% MC, 317 rpm) created a decrease in OHC from 1.3 to 1.2 g of oil per gram of flour. No changes were observed for an increase from 110ºC to 125ºC (35%MC, 300 rpm) due to the thermal stability of the storage proteins (i.e. vicilin and legumin) present in the faba bean mix with denaturation temperatures higher than 105ºC (Arntfield et al., 1986). No effect was seen with changes in screw speed from 200 rpm (T1) to 300 rpm (T2) and 400 rpm (T3) (Table 12, 125ºC, 35% MC) which can be attributed to the large number of entanglements present that reduces the degradation caused by shear (Day et al., 2013). Finally, there significant changes between the faba bean-based TVPs and commercial pea TVPs can be attributed to the different methods of extraction of the protein, the thermal stability of each storage protein, and also to the difference in extrusion variables.

4.4.6 Bulk Density

Bulk density (BD) is a physical property that considered the expansion in all directions caused by extrusion. There are many factors that can affect BD including, raw material composition, particle size, moisture content, extrusion conditions, and others. For faba bean-based TVPs, BD values ranged from 0.30 to 0.42 g/mL. One of the extrusion variables that affect bulk density is moisture content, where when moisture content increases there is less friction generated in the barrel due to changes in viscosity having as a result higher values for bulk density (Sun & Muthukumarappan, 2007). Badire & Mellowes (1991) showed that for cassava flour an increase in moisture content from 10 to 16% higher BD values where observed. For faba bean-based TVPs, this effect is the opposite where higher moisture content lowered the values for BD. Table 12 shows the results where an increase from 30% (T4) (125ºC, 300 rpm) to 40% (T5) (125ºC, 300 rpm) created a decrease in BD from 0.41 to 0.30 g/mL that can be attributed to an excess of moisture, reducing the melt viscosity which decreases the number air bubbles (Day& Swanson, 2013). A possible reason for the opposite effect is that the cassava flour has very little starch. Higher moisture content can reduce the mobility of the proteins with higher cross-linking that will cause a reduction in BD (Holay & Harper 1982). On the other hand, the increase in temperature from 110 ºC (T7) (35%MC, 300 rpm) to 140ºC (T6) (35%MC, 300 rpm) created an increase in BD. That can be attributed to an increase in the vapor pressure that favors expansion (Li& Swanson, 2013). There was also a slight increase in BD when the screw speed changed from 200 rpm to 400 rpm (Table 12, 125ºC, 35% MC) because a higher number of pores are forming during extrusion because of the high shear. Rajendra et al. (2022) reported similar results for hempseed TVP where an inverse relationship was found between BD and increased shearing. Finally, for the commercial pea TVPs, the values were significantly lower than the faba bean-based TVPs that can be attributed to the raw material used (lower content of starch, and globulins), and different conditions of extrusion.

4.4.7 Rehydration ratio

The rehydration ratio (RR) can be defined as the amount of water that can be held by the TVP structure after rehydration which can affect the texture, juiciness and other sensory properties.

Table 12. The effect of die temperature, screw speed and moisture content on functional properties for faba bean-based TVPs.

	Water holding capacity [g water/ g protein]	**Oil holding capacity [g oil/ g protein]**	**Rehydration ratio [%]**	**Bulk density [g/mL]**
Raw faba bean*	3.2 ± 0.0^{cdef}	1.0 ± 0.0^{g}	--	--
CTVP1**	3.9 ± 0.0^{ab}	1.7 ± 0.0^{bc}	545.9 ± 19.6^{a}	0.20 ± 0.00^{f}
CTVP2	4.1 ± 0.0^{a}	2.8 ± 0.1^{a}	390.1 ± 14.1^{b}	0.11 ± 0.00^{g}
T1	3.8 ± 0.1^{ab}	1.7 ± 0.1^{bc}	296.9 ± 11.2^{cd}	0.36 ± 0.01^{c}
T2	3.5 ± 0.1^{bc}	1.6 ± 0.1^{bc}	279.7 ± 13.7^{d}	0.38 ± 0.01^{b}
T3	3.3 ± 0.0^{cde}	1.6 ± 0.0^{cd}	290.3 ± 6.8^{d}	0.39 ± 0.02^{b}
T4	3.0 ± 0.1^{ef}	1.4 ± 0.1^{e}	308.9 ± 9.4^{c}	0.41 ± 0.00^{a}
T5	2.9 ± 0.1^{f}	1.3 ± 0.0^{f}	155.1 ± 15.4^{f}	0.30 ± 0.01^{e}
T6	3.4 ± 0.1^{cd}	1.5 ± 0.0^{de}	237.5 ± 3.9^{e}	0.42 ± 0.01^{a}
T7	3.1 ± 0.1^{def}	1.6 ± 0.0^{bcd}	235.2 ± 8.0^{e}	0.34 ± 0.00^{d}

Notes:

Treatments are as follow: T1-125ºC, 35%MC, 200 rpm, T2-125ºC, 35%MC, 300 rpm, T3-125ºC 35%MC, 400 rpm, T4-125ºC 30%MC, 300 rpm, T5-125ºC 40%MC, 300 rpm, T6-140ºC 35%MC, 300 rpm and T7-110ºC 35%MC, 300 rpm. *Raw faba bean= 70% of faba bean isolate +30% of faba bean concentrate; **CTVP= Commercial pea TVP. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

This functional property is affected by the interactions between protein and water, bulk density, and the porous structure created by the different extrusion variables (Samard et al., 2019).For faba bean-based TVPs, the values ranged from 155.1 to 308.9% with a significant difference for changes in moisture content. As shown in Table 12, an increase in MC% from 30% (T4) to 35% (T2) and to 40% (T5) (125ºC, 300 rpm) created a decrease in RR from 308.9% to 279.7% and then to 155.12% respectively that can be attributed to the porosity of each treatment, and the interaction between protein-water during denaturation. Similar results were observed by Samard et al (2019) where the RR values ranged from 217.4 to 366.6% with the lowest RR obtained at 40%MC and 130ºC for soy, wheat, and starch TVPs. No effect was observed with an increase in temperature from 110ºC (T7) to 140ºC (T6) (Table 12; 35%MC, 300 rpm) or a change in screw speed from 200 rpm (T1) to 400 rpm (T3) (Table 12; 125ºC, 35% MC) for faba bean-based TVPs. Finally, the values of RR for commercial pea TVPs are 100-250% higher than lab-scale faba bean-based TVPs which can be attributed to the higher porosity of the sample with a significantly lower BD value of 0.11-0.20 g/mL.

4.4.8 Texture profile

Protein unfolding is enhanced in the barrel due to high temperature and shear followed by cross-linking that takes place at the die since proteins are aligned by shear and changes in pressure which attributes to the desired texture of TVPs (Li & Swanson, 2013). Hardness is part of the texture profile which is related to the maximum force required to compress the sample. Cohesiveness measures the strength of the internal bonds while springiness measures how much the sample recovers after deformation. Chewiness measures the force that is needed to chew solid food until is ready to swallow, while gumminess measures the viscoelastic properties (Amrut et al., 2022). Resilience measures how fast and strong the sample recovers after compression (Chandra &Shamasundar, 2015). In Table 13, hardness, chewiness, and gumminess values showed an increase when moisture content increased from 30% (T4) (125ºC, 300 rpm) to 40% (T5) (125ºC, 300 rpm) related to water content that created changes in viscosity and elasticity inside the barrel during extrusion. Previous results for soy extrudates showed that moisture contents higher than 70% reduced significantly hardness and gumminess (Lin et al., 2000). No effect was observed with an increase in temperature from 110ºC (T7) to 140ºC (T6) (Table 13; 35% MC, 300 rpm) or a change in screw speed from 200 rpm (T1) to 400 rpm (T3) (Table 13; 125ºC, 35% MC) for faba bean-based TVPs. An opposite effect was seen for springiness, when moisture content increased from 30% (T4) (125ºC, 300 rpm) to 40% (T5) (125ºC, 300 rpm) created a decrease from 73.1 to 65.1% that can be attributed to the change in the density of the material related to the porosity (Kaleda et al., 2021). The cohesiveness values ranged from 0.54 to 0.63 for faba bean-based TVPs, which is related to the protein-protein interactions where an increase in the value represents a higher number of protein-protein interactions. Kaleda et al. (2021) showed a positive correlation between protein concentration and springiness for a blend of oat and pea protein blends where values decreased when protein content decreased. Finally, for resilience, there is a decrease when screw speed increased from 200 rpm (T1) to 400 rpm (T3) (Table 13; 125ºC, 35%MC) which can be attributed to the bulk density of the samples.

4.4.9 Application in meat products

4.4.9.1 Cooking properties for hybrid burgers

Substituting meat with any other ingredient can affect the nutritional value, functional properties of the product and reduce the protein intake of consumers. Looking for a suitable replacement for meat lead to the development of TVP that present high functional properties and high consumers acceptance (Petra-Melin et al., 2023). Previous studies showed that replacing meat with TVP was successful in a range from 30-50% in terms of quality and sensory aspects (Hale et al., 2002). One of the main characteristics that affect the final sensory aspects of a meat product is the ability to retain liquid after cooking which is directly related to the juiciness and tenderness of the patty. Based on the data collected for faba bean-based TVPs, the best treatment selected for the application was T4 (125ºC, 30% MC, 300 rpm) which showed higher functional properties including rehydration ratio.

Cooking yield is a property that has been correlated with water uptake during cooking based on the differences in weight (Palanisamy et al., 2018). The addition of 10% TVP showed a significant increase of cooking yield from 68.1% to <80% for commercial and lab-scale faba bean-based TVPs. Loss of moisture during cooking can be associated with the contraction of collagen due to heat that enhances liquid expel, meaning that a substitution of 10% will reduce the content of meat

Table 13. The effect of die temperature, screw speed and moisture content on texture profile for faba bean-based TVPs.

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
CTVP1*	2.6 ± 0.0^{e}	88.7 ± 1.7^{a}	0.76 ± 0.00^{a}	2.0 ± 0.0^{cd}	1.8 ± 0.1^{bc}	0.41 ± 0.01^{a}
CTVP2	3.6 ± 0.1^{de}	82.2 ± 1.8^{ab}	0.66 ± 0.03^{b}	2.4 ± 0.2^{cd}	1.9 ± 0.2^{c}	0.36 ± 0.01^{ab}
T1	5.7 ± 0.6^{bcd}	76.8 ± 3.4^{bc}	0.63 ± 0.02^{b}	3.6 ± 0.4^{bc}	2.8 ± 0.4^{ab}	0.34 ± 0.02^{bc}
T2	3.7 ± 0.9^{e}	70.1 ± 2.8^{de}	0.56 ± 0.02^{de}	2.3 ± 0.9^{d}	1.7 ± 0.7^{c}	0.29 ± 0.02^{e}
T3	4.3 ± 0.5^{cde}	70.4 ± 1.5^{cde}	0.58 ± 0.01^{d}	2.5 ± 0.3^{cd}	1.8 ± 0.3^{c}	0.30 ± 0.01^{de}
T4	4.5 ± 0.2^{cde}	73.1 ± 1.5^{cd}	0.63 ± 0.02^{bc}	2.8 ± 0.1^{cd}	2.1 ± 0.1^{bc}	0.34 ± 0.01^{b}
T5	9.9 ± 0.6^{a}	65.1 ± 2.1^{e}	0.54 ± 0.02^{e}	5.4 ± 0.2^{a}	3.6 ± 0.2^{a}	0.33 ± 0.01^{bcd}
T6	7.2 ± 0.6^{b}	68.4 ± 1.6^{de}	0.58 ± 0.01^{cd}	4.2 ± 0.5^{ab}	2.9 ± 0.4^{ab}	0.33 ± 0.00^{bcd}
T7	5.9 ± 0.4^{bc}	67.9 ± 4.8^{de}	0.56 ± 0.01^{de}	3.3 ± 0.3^{bcd}	2.7 ± 0.2^{bc}	0.31± 0.01^{cde}

Notes:

Treatments are as follow: T1-125ºC, 35%MC, 200 rpm, T2-125ºC, 35%MC, 300 rpm, T3-125ºC 35%MC, 400 rpm, T4-125ºC 30%MC, 300 rpm, T5-125ºC 40%MC, 300 rpm, T6-140ºC 35%MC, 300 rpm and T7-110ºC 35%MC, 300 rpm. * CTVP= Commercial pea TVP. RPM= Revolutions per minute. MC%= Moisture content

Treatments with the same superscript letter are not significantly different ($p>0.05$)

and in consequence reduce the loss in moisture (Petra-Melin et al., 2023). Previous studies showed similar results where the addition of 30% whey TVP to a beef burger increased the cooking yield by 6% and the higher substitution showed higher cooking yield (Hale et al., 2002).

Shape retention (i.e. diameter and thickness changes) during cooking is desirable in terms of the mechanization of a process and the general appearance of the product (Hale et al., 2002). Changes in shape including shrinkage is a normal process during cooking due to the denaturation of the meat proteins leads to moisture loss and in consequence changes in thickness and diameter (Gujral et al., 2002). As shown in Figure 13 and Figure 14 the diameter change showed a significant decrease for both TVP replacement (14.7% and 15.1%) compared to the real beef sample (24.8%). Similar results were obtained by Gujral et al. (2002) where adding 20% of soy TVP created a proportional decrease in the shrinkage of the patties from 24.9 % (100% beef) to 8.9%. For thickness change, there was a significant decrease of 7% from the real beef sample compared to the faba bean TVP, but no significant change between the commercial TVP and real beef. The difference between both TVPs can be attributed to the denaturation temperature of each pulse whereas faba bean has a slightly higher temperature ranging from 95ºC to 100ºC and pea 85ºC to 95ºC (Ferawati et al., 2021).

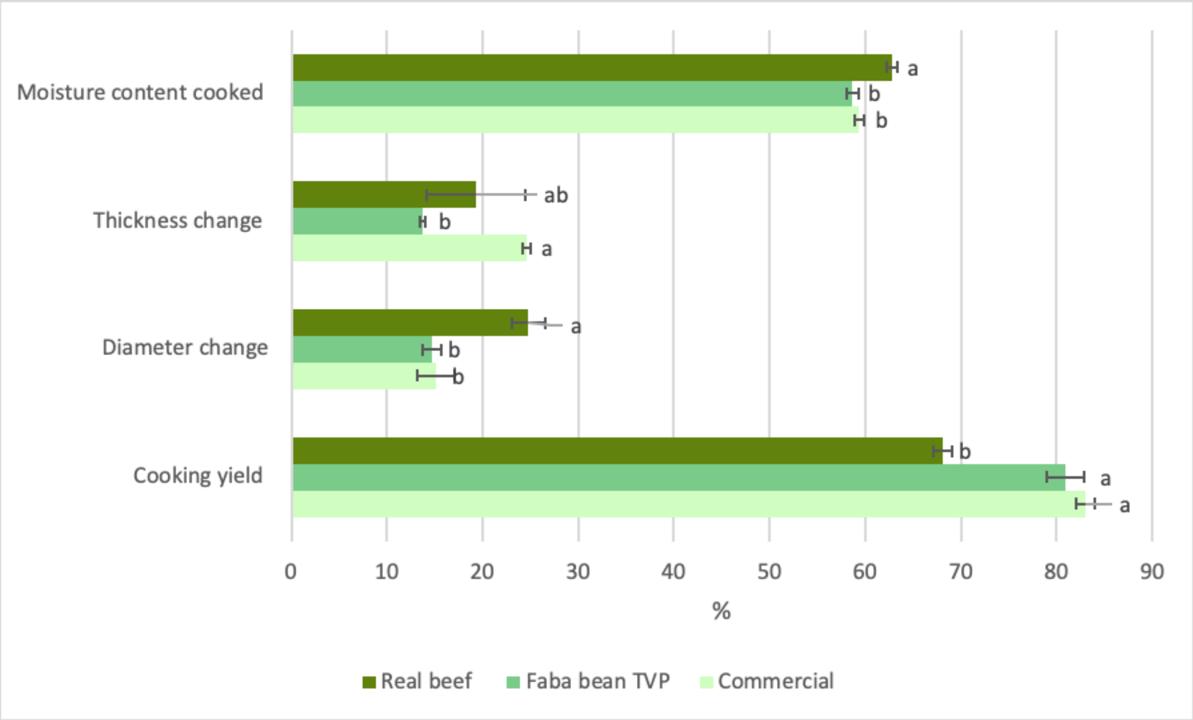

Figure 13. Effect of 10% TVP in cooking properties of meat patties. Treatments with the same superscript letter are not significantly different (p>0.05)

Figure 14. Images of hybrid burgers after cooking

4.4.9.2 Texture profile for hybrid burgers

Moisture plays an important role in terms of texture and sensory attributes in meat products. During mastication, fluid loss is expected since it stimulates the receptors in the mouth which is directly related to the perception of juiciness and other sensory aspects (Godschalk-Broers et al., 2022). The texture profile results showed that the is a slight decrease in hardness, springiness, gumminess, and chewiness for the 10% faba bean-based TVP replacement compared to the real beef sample. This can be attributed to the denaturation of the muscle proteins in animal meat that enhances liquid expel and in consequence increase the textural properties (Carvalho et al., 2017). For the commercial TVP sample, there is a significant increase compared to the faba bean-based TVP which can be attributed to the higher compact and dense structure of the TVP (Godschalk-Broers et al., 2022). A similar result was obtained with the addition of 2% soy TVP with a hardness value of 20.5 N for hardness, 0.64 for cohesiveness, which can be attributed to the water-holding capacity of the TVP and the contractile proteins which increases the cooking loss having an effect on the texture profile(Carvalho et al., 2017). All the results in Table 14 show that is difficult to relate only one variable to the changes in texture, however, there is a higher correlation for some variables including moisture retention, fibrous structures, bulk density, and rehydration ratio of the TVPs.

4.4.9.3 Cooking properties for vegan burgers

The appearance and texture of the cooked patty are highly influenced by the cooking properties shown in Figure 16. All of the properties are related to the juiciness and other sensory aspects of a meatless burger (Samard et al., 2021). The cooking yield increased for the vegan burgers compared to the real beef which can be attributed to the composition of the burger including the high protein (TVP) and the low content of oil that enhances the water retention during cooking (Samard et al., 2021). The differences between the faba bean-based TVP, commercial pea TVP and the beyond meat sample can be attributed to the amino acid composition of each pulse used, and the extrusion parameters use that can affect the hydrophobic and hydrophilic interactions. The results from the meatless burgers showed that there is a high correlation between moisture retention and the cooking yield that will also affect appearance properties and textural attributes. Higher moisture retention was observed for the beyond meat sample (control) compared to the faba bean and commercial pea TVP which can be attributed to the degree of denaturation of each pulse that created a change in the protein structure and in consequence higher WHC and OHC.

The changes in shape can be observed in Figure 15 for all the samples where the thickness and diameter showed a significant decrease for all vegan burgers compared to real beef. Both TVP samples showed higher diameter change than the beyond meat which can be attributed to the higher moisture content and higher thickness of the beyond sample. On the other hand, the change in thickness was significantly lower for all the TVP samples compared to both controls (i.e. real beef and beyond meat) which can also be attributed to the extrusion process and different variables such as temperature that can affect the hydrophobicity (Gujral et al., 2002).

Similar results were observed by Samard et al. (2021) with TVPs produced with soy protein, wheat gluten, and corn starch under different temperatures (130ºC and 150ºC), MC% (40% and 50%), and constant of 200 rpm. The cooking yield and moisture retention increased for all TVP samples compared to the real beef while the changes in shape (i.e. diameter and thickness) showed a significant decrease compared to the real beef sample.

Table 14. Texture profile for hybrid patties.

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
CTVP*	27.1 ± 0.9^{a}	118.2 ± 23.4^{a}	0.9 ± 0.0^{a}	24.6 ± 1.3^{a}	29.6 ± 7.3^{a}	0.7 ± 0.0^{a}
Faba bean-based TVP	18.8 ± 0.7^{b}	119.5 ± 7.5^{a}	0.9 ± 0.0^{a}	17.4 ± 0.6^{b}	20.7 ± 2.1^{a}	0.7 ± 0.0^{a}
Real beef	21.1 ± 4.1^{ab}	123.7 ± 11.1^{a}	0.9 ± 0.0^{a}	19.2 ± 3.8^{ab}	23.5 ± 2.8^{a}	0.6 ± 0.0^{b}

Notes:

Treatments with the same superscript letter are not significantly different ($p>0.05$)

*CTVP= Commercial pea TVP

Real beef Commercial TVP Faba bean TVP Beyond meat

Figure 15. Images of vegan and real beef burgers after cooking

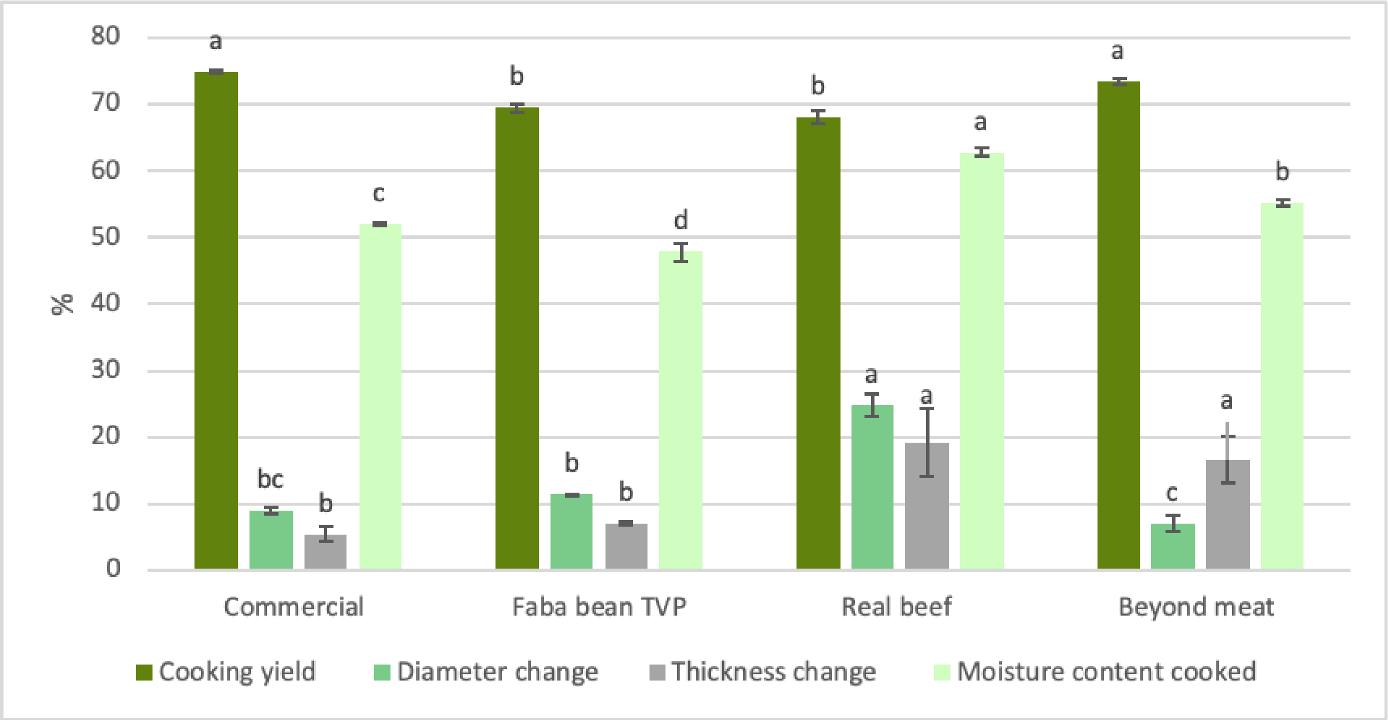

Figure 16. Effect of TVP in cooking properties of vegan patties. Treatments with the same superscript letter are not significantly different (p>0.05.)

4.4.9.4 Texture profile for vegan burgers

The texture properties of all the burgers are shown in Table 15 with significant difference for all attributes. The texture profile of any meat product is directly related to the moisture content of the sample, where a higher moisture content represents a lower hardness (Botella-Martinez et al., 2022). The results in this study showed that the real beef sample showed a higher value of moisture content (62.8%) compared to all vegan burgers which attributed to the lower value of hardness (21.1 N). The difference in hardness for the vegan burgers ranged from 24.3 N to 51.4N which can be attributed to the properties of each TVP like crosslinking (i.e. number strength and type), fiber, and starch content (Botella-Martinez et al., 2022). Springiness, cohesiveness, and resilience showed a decrease for all the plant-based samples which can be attributed to the combination of moisture content and temperature used in the extrusion process to produce TVP.

The changes in extrusion variables showed that a protein structure can be tailored which can create a stronger protein network and in consequence change the textural attributes (Samard et al., 2021). Previous studies had similar results with soy TVPs under different conditions where an increase of MC% during extrusion (from 40% to 50%) increased the springiness, cohesiveness, hardness, and cutting strength of a plant-based burger compared to a beef burger. Also, Samard et al. (2021) found that an increase in die temperature from 130ºC to 150ºC created the opposite effect decreasing the springiness and cohesiveness in the burgers prepared with TVPs.

Table 15. Texture profile of TVP-based vegan patties

	Hardness [N]	**Springiness [%]**	**Cohesiveness**	**Gumminess [N]**	**Chewiness [N]**	**Resilience**
CTVP*	51.4 ± 0.0^{a}	107.2 ± 5.4^{ab}	0.9 ± 0.0^{a}	45.4 ± 0.1^{a}	48.6 ± 2.6^{a}	0.58 ± 0.00^{b}
Faba bean-based TVP	31.8 ± 0.8^{b}	94.1 ± 1.2^{bc}	0.9 ± 0.0^{a}	28.1 ± 0.7^{b}	26.5 ± 1.0^{b}	0.55 ± 0.01^{b}
Real beef	21.1 ± 4.1^{c}	123.7 ± 11.1^{a}	0.9 ± 0.0^{a}	19.2 ± 3.8^{c}	23.5 ± 2.8^{b}	0.64 ± 0.01^{a}
Beyond meat	24.3 ± 0.5^{c}	83.3 ± 5.9^{c}	0.8 ± 0.0^{b}	18.4 ± 0.4^{c}	15.3 ± 1.3^{c}	0.32 ± 0.02^{c}

Notes:

Treatments with the same superscript letter are not significantly different (p>0.05)

*CTVP= Commercial pea TVP

4.5 Conclusion

The study produced texturized vegetable proteins (TVPs) from a mix of faba bean protein isolate and concentrate under different extrusion conditions for an evaluation of their functionality and potential use in meat applications. The different treatments used in extrusion with die temperatures (110, 125, and 140 °C), feed moisture contents (30, 35, and 40 g water/100 g feed, wet basis) and screw speeds (200, 300, and 400 rpm) showed a significant impact on the physical and functional properties of faba bean-based TVPs. First, specific mechanical energy (SME)showed that was highly affected by all the extrusion variables which were also reported in previous literature. The heat used in the process to create a stable product in terms of shelf life, is reflected in the water activity values lower than 0.35 and the moisture content lower than 10% for all treatments. The changes in color were mostly affected by the moisture content and temperature with a significant increase in brightness (L), and a decrease in redness (a) and yellowness (b) that can be attributed to the Maillard reaction and loss of lysine, with no change for screw speed.

On the other hand, the functional properties that were mostly affected by screw speed were WHC with a significant decrease that can be attributed to the protein aggregation due to shear and the denaturation of the protein, while the OHC showed a significant increase after extrusion because of the buried sites that are exposed during extrusion due to the unfolding of the protein. These properties were not affected by the increase in temperature due to the high content of globular proteins that give faba bean a higher thermal stability. The rehydration ratio decreased with higher moisture content due to the density and porosity of the TVPs. For the texture profile, a significant increase was observed when moisture content increased from 30% (T4) (125ºC, 300 rpm) to 40% (T5) (125ºC, 300 rpm) related to water content that created changes in viscosity and elasticity inside the barrel during extrusion. No significant effect of temperature and screw speed was observed for textural attributes. Overall, the combination of extrusion variables and raw material used created a significant change in the functional and physical properties of TVPs which can be used as an advantage to tailor the protein structure based on the application.

For this study, the optimal treatment for faba bean TVP (125ºC, 40%MC and 300 rpm) was selected for a meat application of producing a hybrid burger with a 10% replacement and a vegan burger. The results showed that by only adding 10% of faba bean TVP the cooking properties had a significant effect in reducing the loss of moisture after cooking which increased the cooking yield of the burgers and decreased the changes in shape (i.e. thickness and diameter).

Textural attributes including hardness, gumminess, and chewiness showed a significant increase with the addition of faba bean TVP while springiness, resilience, and cohesiveness showed a significant decrease. Future studies should include a sensory evaluation to obtain the overall acceptance of this substitution in terms of texture, flavor, and juiciness. Finally, according to the results for the meatless burger faba bean-based TVP is a suitable replacement for meat in terms of reducing moisture loss during cooking which highly affects the cooking yield, diameter, thickness, and texture.

5. GENERAL DISCUSSION

5.1 Specific mechanical energy

SME was determined from the torque, screw speed, motor power, and mass flow rate for each treatment to produce TVPs where values ranged from 72.0 to 118.4 Wh/kg for faba bean and 66.9 to 125.9 Wh/kg for lentil-based TVPs. The difference in SME values can be attributed to the different composition of the raw material entering the extruder, which influences the pasting properties. Viscosity is one of the main properties that can induce changes in extruder responses and the final quality attributes of the TVPs (Osen et al., 2014; Ek et al., 2021). Under similar conditions, lentil and faba bean-based TVPs had similar values of SME (79.9 and 72.8 Wh/Kg respectively) at 140ºC and 35%MC and a slight increase in screw speed for lentil (375 rpm) compared to faba bean (300 rpm). Similar results were observed by Gu et al. (2019) where faba bean showed higher resistance to flow with average peak viscosity of 312 mPa*s, whereas lentil values ranged from 157 to 180 mPa*s depending on the variety. The changes in SME can also be attributed to the denaturation temperature of each pulse since is related to the thermal stability depending on the 7 S and 11 S globulins ratio. There are many factors that can change the thermal stability of each pulse including pH, protein aggregates, and salts (Barbana & Boye, 2013). Faba bean has a denaturation temperature ranging from 77ºC to 85ºC, whereas lentils values range from 79ºC to 87ºC (Jiang et al., 2016).

A study carried out by Osen et al (2014) showed that SME values showed a sudden raise with a temperature higher than 80ºC for pea protein isolates explained by the denaturation of proteins at higher temperatures that increased the consumption of energy but decreased when higher temperatures (>120ºC) were reached. Also, when there is a decrease in temperature the protein-protein interactions are favored resulting in higher friction and in consequence a higher demand for energy (Osen et al., 2014).

Similar results were seen for faba bean-based TVPs where temperatures of 110-125ºC (35% MC, 300 rpm) had the highest SME values ranging from 104.8-109.2 Wh/Kg but decreased to 72.8 Wh/Kg when the temperature reached 140ºC (35% MC, 300 rpm). Lentil-based TVPs had a similar effect where temperatures of 120ºC and 130ºC (35% MC, 375 rpm) showed SME of 99.5 and 92.6 Wh/Kg respectively, but when the temperature reached 140ºC (35% MC, 375 rpm) the SME decreased to 79.9 Wh/Kg.

Faba bean-based TVPs also showed higher torque values than lentil-based TVPs due to differences in composition. The faba bean mixture contained concentrate, which was higher in the starch fraction, having an effect on the viscosity during extrusion and also to the higher content of globulins that requires more energy to produce TVPs (Hall et al., 2017).

5.2 Water holding capacity and oil holding capacity

The native proteins tend to have low values for OHC/WHC since the globular protein structure has many buried hydrophilic and hydrophobic sites. During extrusion, denaturation or unfolding happens that breaks bonds to expose buried sites creating an increase in the hydrophobic or hydrophilic moieties on the protein’s surface as well as more micro-channels in structure (Osen et al., 2014). Denaturation of the protein depends on many factors including temperature, moisture content, and others that can affect the functional properties of TVPs (Lefèvre et al., 2022). For example, after the denaturation process, protein aggregates can form reducing the access to certain amino acids and decreasing the OHC and WHC.

Water holding capacity (WHC) is a functional property that depends on the number of polar sites that are available to interact with water. WHC can be influenced by many factors including protein solubility, moisture content, temperature, and particle size. Previous studies carried by Osen et al. (2014) showed a positive correlation between WHC and heat-induced denaturation in pea protein isolates. WHC values ranged from 2.9 to 3.8 g/g for lentil and faba bean-based TVPs. According to Lefèvre et al. (2022) denaturation of pulses occurs between 80-100ºC in excess water, meaning that a higher moisture content will have an impact on the WHC of TVPs. Similar results were seen for faba bean and lentil-based TVPs, where the denaturation process was significantly influenced by moisture content, showing results with lower values for WHC with the highest moisture content used during extrusion (40% MC). No effect was observed with WHC with changes in temperatures or screw speed for both pulses.

Oil holding capacity (OHC) is a functional property that depends on the number of non-polar sites that are available to interact with oil. For faba bean-based TVPs, there was a significant increase in OHC after extrusion with values ranging from 1.3 to 1.7 g /g compared to the raw material with a value of 1.0 g/g. On the other hand, lentil-based TVPs did not show an increase in OHC for T1 (140ºC, 30%MC, 375 rpm), T6 (120ºC, 35%MC, 375 rpm), and T7 (130ºC, 35%MC, 375rpm) which can be attributed to the higher thermal stability of lentil that caused a partial denaturation of the protein. These results can also be linked to the changes in the secondary structure of the lentil-based TVPs where no significant changes were seen for most secondary structure values, only for protein aggregates.

5.3 Bulk density

Bulk density (BD) is a physical property that influences functional properties such as water-holding capacity and rehydration ratio. For lentil-based TVPs, BD values ranged from 0.35-0.42 g/mL while for faba bean-based TVPs values ranged from 0.30 to 0.42 g/mL. An increase in moisture content showed a negative effect on BD for faba bean-based TVPs and no effect over lentil-based TVPs which can be attributed to the viscoelastic properties of each raw material (Sun & Muthukumarappan, 2007). An increase in temperature showed no change for the lentil-based TVPs but created an increase in faba bean-based TVPs that can be attributed to the higher change in temperatures for faba bean-based TVPs (from 110ºC to 140ºC) compared to lentil-based TVPs(from 120ºC to 140ºC). Finally, an increase in screw speed generated more friction inside the barrel during extrusion which tends to increase BD for faba bean-based TVPs (200 rpm to 400 rpm) (125ºC, 35%MC), however for the lentil-based TVPs the opposite effect happened due to the excessive shear that created a collapse in the bubbles, and in consequence a decrease in BD (300 rpm to 450 rpm) (140ºC, 30% MC).

5.4 Rehydration ratio

To successfully use TVP in any application, the product needs to be rehydrated prior to use making the rehydration ratio (RR) one of the most important functional properties when selecting a suitable TVP depending on the product. The amount of water that a TVP can bind has an effect on the consumer’s acceptance since it is directly related to juiciness and hardness (Samard et al., 2019). For lentil-based TVPs, the RR ranged from 182.4 to 214.6% while for faba bean-based

TVPs ranged from 155.12 to 308.9%. The difference can be attributed to the behavior of the raw material during extrusion and the extrusion conditions applied (i.e. moisture content) that can create more air bubbles and in consequence increase the porosity. Previous studies carried out by Ketnawa et al. (2023) that compared a commercial soy TVP and lab TVP (mushroom, wheat flour, soy protein, and others) showed similar results where authors stated that the rehydration ratio is mainly attributed to the product structure rather than the protein-water interactions.

5.5 Texture profile

Texture is one of the most important qualities of plant proteins since the main objective of TVP is to mimic the sensory and functional aspects of real meat. The texture profile showed values for hydrated TVPs including hardness, cohesiveness, springiness, chewiness, gumminess, and resilience for lentil and faba bean samples. The different attributes showed a significant correlation with the changes in moisture content and screw speed during extrusion with no effect of changes in temperature for both TVPs presented in the study. Previous research showed that an increase in hardness may indicate the degree of texturization of the protein attributed to protein cross-linking. (Hong et al., 2022). The hardness for faba bean-based TVPs showed lower values than the lentil-based TVPs which can be attributed to the combination of isolate with concentrate compared to only isolate for lentil-based TVPs, which can cause interference with the protein-protein interactions. Previous studies carried out by Webb et al. (2020) showed that an addition of 10-30% of chickpea flour before extrusion created a decrease in the hardness of the final product. Finally, some of the functional properties including rehydration ratio and bulk density showed a significant effect on resilience and springiness. Previous studies showed that a lower value of bulk density tends to create a loose structure that enhances springiness and resilience (Hong et al., 2022). These results were observed when comparing the lab scale TVPs (i.e. lentil and faba bean) that had a higher bulk density value of ~ 0.32 g/mL with the commercial pea TVP with values around 0.15 g/mL having an influence over resilience and springiness values (higher for commercial pea samples).

5.6 Application in vegan and hybrid meat burger applications

The effect of adding TVP in a burger formulation was measured through cooking yield, moisture content, moisture retention, diameter change, and thickness change for all samples. The attributes mentioned before influence the textural and sensory attributes of meat products related to the juiciness, tenderness, and yield of the final product mainly linked to the loss of moisture during cooking (Hong et al., 2022). For the 10% replacement (hybrid formulation) an increase in cooking yield of 10% was observed for both pulse-based TVP (lentil and faba bean) compared to the real beef sample. On the other hand, the changes in diameter decreased from 23% (real beef) to 15% for both replacements which are related to the functional properties of TVP (i.e. water and oil holding capacity). Previous research carried out by Hong et al. (2022) stated that the changes in the final product could be attributed to the loss of liquid (i.e. fat and water) during cooking and found a positive correlation between the rehydration ratio and cooking loss of different TVPs. Also, previous studies related the cooking loss with the protein viscosities since during denaturation more hydrophobic interactions are formed increasing the viscosity and changing the protein matrix that reduced the water penetration making it easier for water to be lost during cooking (Hong et al., 2022). The vegan formulation showed similar behavior in terms of changes in diameter when compared to the control sample (real beef) and no significant change compared to the vegan control (Beyond meat). The thickness of both TVP formulations showed a decrease compared to both controls which can be attributed to the use of methylcellulose as a binding agent in the formulation that reduces the changes in shape. These parameters and the texture profile were found to be directly correlated with the moisture and fat retention of each TVP rather than the protein source or additional ingredients (Bakhsh et al.,2021).

6. GENERAL CONCLUSION

The overall goal of this thesis was to investigate the utilization of lentil and faba bean proteins as ingredients to produce texturized vegetable proteins through low moisture extrusion to later be used as meat replacements. The present research evaluated how the extrusion variables (moisture content, die temperature, and screw speed) affect the physical and functional properties of TVPs and compared them to commercial pea TVPs. Based on the results obtained, one treatment was selected to be used as a meat replacement (hybrid formulation with 10% TVPs and vegan) in a patty. The research was divided into two studies, where the first study used lentil isolate (85.2% protein content, dry basis) as raw material during extrusion with a lab-scale twin-screw extruder at three different die temperatures (120, 130, and 140 °C), feed moisture contents (30, 35 and 40 g water/100 g feed, wet basis) and screw speeds (300, 375 and 450 rpm) to produce TVPs with a wide range of physical and functional properties.

The protein content of the lentil-based TVPs showed no significant change after extrusion, however, the changes in color showed a significant effect with the changes in moisture content attributed to the Maillard reaction. The functional properties including water and oil holding capacity showed a significant change after extrusion that can be attributed to the changes in the viscosity under different moisture content that enhances the denaturation of the protein and in consequence increased OHC and decreased WHC compared to the raw material. The bulk density showed a correlation with the rehydration ratio which both functional properties were highly affected by the friction generated by the changes in moisture content during extrusion. For all the functional and physical properties no effect of temperature was seen which can be attributed to the thermal stability of lentils corroborated with the results for FTIR that showed no significant changes for all secondary structures except for protein aggregates. Finally, the texture profile showed no effect on the changes in moisture, screw speed, or temperature for all of the attributes except for springiness which could be attributed to the viscoelastic properties.

The main hypothesis, that extrusion variables affect the protein structure of lentil isolates when producing TVPs and in consequence affect functional and physical properties was proven to be true.

The second study investigated the use of a mix of faba bean isolate and concentrate (82.8% of protein dry basis) as a raw material to produce TVPs with a lab-scale twin-screw extruder at three different die temperatures (110,125, and 140ºC), feed moisture contents (30, 35, and 40 g water/100 g feed), and screw speeds (200, 300, and 400 rpm) to measure and compare how the combination of these variables affected the physical a functional properties of the final product. The protein content of all TVPs showed no significant change during extrusion with an average of 83% (dry basis), however, changes were observed in color parameters attributed to the Maillard reaction between free amino acids and reducing sugars mainly affected by changes in moisture content and temperature. The functional properties including water holding capacity and oil holding capacity, showed similar trends to the lentil-based TVPs where screw speed had a significant effect attributed to the aggregation of the proteins during extrusion. The rehydration ratio showed a significant decrease with changes in moisture content which can be attributed to the porosity of TVPs, but no changes with temperature changes due to the high content of globular proteins in faba bean. Finally, the changes in the texture profile were attributed to the higher content of moisture that had an effect on the viscosity and elasticity of the mixture during extrusion.

The optimal conditions for faba bean-based TVPs (125ºC, 40%MC, and 300 rpm) and lentil (140ºC, 40%MC, and 375 rpm) were selected based on the functional properties to use it as a 10% and complete replacement of red meat in burgers to evaluate cooking yield, diameter change, thickness change, and texture profile. The results showed that a substitution of 10% in a burger patty decreased the loss of moisture (i.e., water and oils) during cooking and had an effect on the cooking properties and the texture profile. A complete plant-based burger showed that the TVP tends to hold shape and moisture better than an animal option with slight changes in the texture profile which can be attributed to the denaturation of the proteins during cooking or crusting properties. The findings in both studies suggested that lentil and faba bean-based TVPs produced by low moisture extrusion are promising candidates to mimic the functional characteristics of meat.

7. FUTURE STUDIES

The potential use of pulses as a raw material for extrusion to produce TVPs a plant-based replacement for meat was conducted in this research. Within the two studies, the understanding of the different variables during the extrusion process was analyzed based on the functional and physical characteristics of faba bean and lentil-based TVPs. The resulting TVPs were used to produce 2 different burgers (hybrid and vegan) to determine the cooking properties and compared them to commercial samples and real beef. Based on the results presented in both studies future work can be done to improve the production and utilization of TVPs with attention to the functional and sensory characteristics. Future work should focus on the following aspects:

1) As discussed in previous sections, temperature is a variable that highly affects the functional and physical properties of TVPs related to the viscosity and denaturation of the protein (Osen et al., 2014). To understand the behavior during the heating phase in extrusion the thermal properties and pasting properties of faba bean and lentil can be analyzed for a better understanding of the impact during extrusion. A test known as Rapid Visco Analyzer (RVA) can be performed as a preliminary test to simulate the extrusion process and have a better determination of protein/concentrate ratio as well as the range of temperatures that can be used to produce the TVPs. Finally, RVA can be performed on the grinded final product after extrusion (i.e. TVPs) to understand the effect of each variable on the viscosity properties of each protein which can affect functional properties like WHC and RR (Hong et al., 2022).

2) Based on the results obtained in both studies, a mathematical model can be implemented to predict the experimental behavior of other pulses or other variables that can affect the functionality of TVPs. The model can also create the optimal process conditions for each pulse reducing the preliminary runs and in consequence, increasing the yield of the extrusion runs and raw material.

3) Plant proteins lack some essential amino acids to meet human nutritional needs which can lead to nutritional deficiencies in a plant-based diet. Pulse proteins are known as a rich source of lysine, leucine, and arginine in a native state, however, the different temperature ranges during extrusion can create a change in the amino acid profile of each pulse protein having an effect on the functional properties (i.e. water and oil holding capacity) (Boyce et al., 2010). The amino acid profile of the raw material and the TVPs can be performed to have a better understanding of the denaturation of the protein and how it would affect the functional properties of the TVPs. Protein digestibility-corrected amino acid score (PDCAAS) can also be performed to evaluate the quality of the protein and obtain health claims for the final product (House et al., 2010).

4) Another challenge of the plant-based alternatives is the lower protein digestibility compared to animal proteins. A better knowledge of protein digestibility is needed to understand the nutritional quality of alternative sources of protein and include it as part of the human diet. *In vitro* and *in vivo* protein digestibility assays can be performed to observe how the TVPs and the burger formulation behave in the human body and how the different extrusion conditions, amino acid profiles, and other variables affect these results (Sousa et al., 2023).

5) In both studies, it was shown that the use of TVP in both formulations is possible in terms of functionality. However, the sensory aspect is an important part of launching and testing a new product on the market. For the hybrid formulations, a triangle test can be performed to see if there is a significant difference between two samples (i.e. real beef and TVP substitution). On the other hand, for the vegan burgers a panel can be carried out to describe and obtain the main attributes of each sample based on the current market such as juiciness, hardness, off-flavors, etc. Based on the results obtained from the panel, the formulation and attributes can be adjusted and passed to the next stage that involves consumers with a test, for example, overall acceptance and just about right which can determine the appropriate amount for the majority of the population in terms of salt, hardness, juiciness and others (Meilgaard et al., 2007).

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